

Hierarchic Concept of Condensed Matter:

Role of Water in Biosystems

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Materials, presented in this original article are based on following publications:

- [1]. A. Kaivarainen. Book: **Hierarchic Concept of Matter and Field. Water, biosystems and elementary particles.** New York, NY, 1995 and 2nd edition of this book. (see URL: <http://kftt.karelia.ru/~alexk>).
- [2]. A. Kaivarainen. **New Hierarchic Theory of Matter General for Liquids and Solids: dynamics, thermodynamics and mesoscopic structure of water and ice** (see URL: <http://kftt.karelia.ru/~alexk>).
- [3]. A. Kaivarainen. **Hierarchic Concept of Condensed Matter and its Interaction with Light: New Theories of Light Refraction, Brillouin Scattering and Mössbauer effect** (see URL: <http://kftt.karelia.ru/~alexk>).
- [4]. A. Kaivarainen. **Hierarchic Concept of Condensed Matter : Interrelation between mesoscopic and macroscopic properties** (see URL: <http://kftt.karelia.ru/~alexk>).
- [5]. A. Kaivarainen. **Hierarchic Theory of Complex Systems** (see URL: <http://kftt.karelia.ru/~alexk>).

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Computerized verification of described here new theories are presented, using special computer program, based on Hierarchic Concept of Condensed Matter (copyright, 1997, A. Kaivarainen).

Summary to Part I of book:
Hierarchic Theory of Matter and Field (by: A. Kaivarainen)

A basically new hierarchic quantitative theory, general for solids and liquids, has been developed.

It is assumed, that anharmonic oscillations of particles in any condensed matter lead to emergence of three-dimensional (3D) superposition of standing de Broglie waves of molecules, electromagnetic and acoustic waves. Consequently, any condensed matter could be considered as a gas of 3D standing waves of corresponding nature. Our approach unifies and develops strongly the Einstein's and Debye's models.

Collective excitations, like 3D standing de Broglie waves of molecules, representing at certain conditions the molecular Bose condensate, were analyzed, as a background of hierarchic model of condensed matter.

The most probable de Broglie wave (wave B) length is determined by the ratio of Plank constant to the most probable impulse of molecules, or by ratio of its most probable phase velocity to frequency. The waves B are related to molecular translations (tr) and librations (lb).

As the quantum dynamics of condensed matter does not follow in general case the classical Maxwell-Boltzmann distribution, the real most probable de Broglie wave length can exceed the classical thermal de Broglie wave length and the distance between centers of molecules many times.

This makes possible the atomic and molecular Bose condensation in solids and liquids at temperatures, below boiling point. It is one of the most important results of new theory, which we have confirmed by computer simulations on examples of water and ice.

Four strongly interrelated new types of quasiparticles (collective excitations) were introduced in our hierarchic model:

1. *Effectons* (*tr* and *lb*), existing in "acoustic" (a) and "optic" (b) states represent the coherent clusters in general case;
2. *Convertons*, corresponding to interconversions between *tr* and *lb* types of the effectons (flickering clusters);
3. *Transitons* are the intermediate [$a \rightleftharpoons b$] transition states of the *tr* and *lb* effectons;
4. *Deformons* are the 3D superposition of IR electromagnetic or acoustic waves, activated by *transitons* and *convertons*.

Primary effectons (*tr* and *lb*) are formed by 3D superposition of the **most probable standing de Broglie waves** of the oscillating ions, atoms or molecules. The volume of effectons (*tr* and *lb*) may contain from less than one, to tens and even thousands of molecules. The first condition means validity of **classical** approximation in description of the subsystems of the effectons. The second one points to **quantum properties of coherent clusters due to molecular Bose condensation**.

The liquids are semiclassical systems because their primary (*tr*) effectons contain less than one molecule and primary (*lb*) effectons - more than one molecule. *The solids are quantum systems totally because both kind of their primary effectons (tr and lb) are molecular Bose condensates.*
These consequences of our theory are confirmed by computer calculations.

The 1st order [*gas* → *liquid*] transition is accompanied by strong decreasing of rotational (librational) degrees of freedom due to emergence of primary (*lb*) effectons and [*liquid* → *solid*] transition - by decreasing of translational degrees of freedom due to Bose-condensation of primary (*tr*) effectons.

In the general case the effecton can be approximated by parallelepiped with edges corresponding to de Broglie waves length in three selected directions (1, 2, 3), related to the symmetry of the molecular dynamics. In the case of isotropic molecular motion the effectons' shape may be approximated by cube.

The edge-length of primary effectons (tr and lb) can be considered as the "parameter of order".

The in-phase oscillations of molecules in the effectons correspond to the effecton's (a) - *acoustic* state and the counterphase oscillations correspond to their (b) - *optic* state. States (a) and (b) of the effectons differ in potential energy only, however, their kinetic energies, impulses and spatial dimensions - are the same. The *b*-state of the effectons has a common feature with **Frölich's polar mode**.

The ($a \rightarrow b$) or ($b \rightarrow a$) transition states of the primary effectons (tr and lb), defined as primary transistons, are accompanied by a change in molecule polarizability and dipole moment without density fluctuations. At this case they lead to absorption or radiation of IR photons, respectively.

Superposition (interception) of three internal standing IR photons of different directions (1,2,3) - forms primary electromagnetic deformons (tr and lb).

On the other hand, the [$lb \rightleftharpoons tr$] *convertions* and *secondary transistons* are accompanied by the density fluctuations, leading to *absorption or radiation of phonons*.

Superposition resulting from interception of standing phonons in three directions (1,2,3), forms **secondary acoustic deformons (tr and lb)**.

Correlated collective excitations of primary and secondary effectons and deformons (tr and lb), localized in the volume of primary *tr* and *lb electromagnetic* deformons, lead to origination of **macroeffectons, macrotransistons and macrodeformons** (tr and lb respectively).

Correlated simultaneous excitations of tr and lb macroeffectons in the volume of superimposed *tr* and *lb electromagnetic* deformons lead to origination of **supereffectons**.

In turn, the coherent excitation of *both: tr and lb macrodeformons and macroconvertions* in the same volume means creation of **superdeformons**. Superdeformons are the biggest (cavitational) fluctuations, leading to microbubbles in liquids and to local defects in solids.

Total number of quasiparticles of condensed matter equal to $4!=24$, reflects all of possible combinations of the four basic ones [1-4], introduced above. This set of collective excitations in the form of "gas" of 3D standing waves of three types: de Broglie, acoustic and electromagnetic - is shown to be able to explain virtually all the properties of all condensed matter.

The important positive feature of our hierarchic model of matter is that it does not need the semi-empiric intermolecular potentials for calculations, which are unavoidable in existing theories of many body systems. The potential energy of intermolecular interaction is involved indirectly in dimensions and stability of quasiparticles, introduced in our model.

The main formulae of theory are the same for liquids and solids and include following experimental parameters, which take into account their different properties:

[1]- **Positions of (tr) and (lb) bands in oscillatory spectra;**

[2]- **Sound velocity;**

[3]- **Density;**

[4]- **Refraction index (extrapolated to the infinitive wave length of photon).**

The knowledge of these four basic parameters at the same temperature and pressure makes it possible using our computer program, to evaluate more than 150 important characteristics of

any condensed matter. Among them are such as: total internal energy, kinetic and potential energies, heat-capacity and thermal conductivity, surface tension, vapor pressure, viscosity, coefficient of self-diffusion, osmotic pressure, solvent activity, etc. Most of calculated parameters are hidden, i.e. inaccessible to direct experimental measurement.

The new interpretation and evaluation of Brillouin light scattering and Mössbauer effect parameters may also be done on the basis of hierarchic theory. Mesoscopic scenarios of turbulence, superconductivity and superfluidity are elaborated.

Some original aspects of water in organization and large-scale dynamics of biosystems - such as proteins, DNA, microtubules, membranes and regulative role of water in cytoplasm, cancer development, quantum neurodynamics, etc. have been analyzed in the framework of Hierarchic theory.

Computerized verification of our Hierarchic concept of matter on examples of water and ice is performed, using special computer program: Comprehensive Analyzer of Matter Properties (CAMP, copyright, 1997, Kaivarainen). The new opto-acoustical device (CAMP), based on this program, with possibilities much wider, than that of IR, Raman and Brillouin spectrometers, has been proposed (see URL: <http://www.karelia.ru/~alexk>).

This is the first theory able to predict all known experimental temperature anomalies for water and ice. The conformity between theory and experiment is very good even without any adjustable parameters.

The hierarchic concept creates a bridge between micro- and macro- phenomena, dynamics and thermodynamics, liquids and solids in terms of quantum physics.

1. Role of inter-domain water clusters in large-scale dynamics of proteins

The functioning of proteins, namely antibodies, enzymes, is caused by the physicochemical properties, geometry and dynamics of their active sites. The mobility of an active site is related to the dynamics of the residual part of a protein molecule, its hydration shell and the properties of a free solvent.

The dynamic model of a protein proposed in 1975 and supported nowadays with numerous data (Käiväräinen, 1985, 1989b), is based on the following statements:

1. A protein molecule contains one or more cavities or clefts capable to large scale fluctuations - pulsations between two states: "closed" (A) and "open" (B) with lesser and bigger accessibility to water.

The frequency of pulsations ($v_{A \leftrightarrow B}$):

$$10^4 s^{-1} \leq v_{A \leftrightarrow B} \leq 10^7 s^{-1}$$

depends on the structure of protein, its ligand state, temperature and solvent viscosity. Transitions between A and B states are the result of the relative displacements of protein domains and subunits forming the cavities;

2. The water, interacting with protein, consists of two main fractions.

The 1st major fraction, which solvates the outer surface regions of protein has less apparent cooperative properties than the 2nd minor fraction confined to "open" cavities. Water molecules, interacting with the cavity in the "open" (B)-state form a cooperative cluster, whose lifetime ($\geq 10^{-10} s^{-1}$). Properties of clusters are determined by the geometry, mobility and polarity of the cavity, as well as by temperature and pressure.

It is seen from X-ray structural data that the protein cavities: active sites (AS), other

interdomain clefts, the space between subunits of oligomeric proteins, have a high nonpolar residues content. In contrast to the small intra domain holes isolated from the outer medium, which sometimes contain several H_2O molecules, the interdomain and intersubunit cavities can contain several dozens of molecules (Fig. 1), exchanging with bulk water.

The development of the above dynamic model has lead us to the following classification of dynamics in the native globular proteins.

1. Small-scale (SS) dynamics:

low amplitude ($\leq 1 \text{ \AA}$) thermal fluctuations of atoms, aminoacids residues, and displacements of α -helices and β -structures within domains and subunits, at **which the effective Stokes radius of domains does not change**. This type of motion, related to domain stability, can differ in the content of A and B conformers (Fig. 1, dashed line). The range of characteristic times at SS dynamics is $(10^{-4} - 10^{-11})s$, determined by activation energies of corresponding transitions.

2. Large-scale (LS) dynamics:

is subdivided into LS-pulsations and LS-librations with a character of limited diffusion of domains and subunits of proteins:

LS- pulsations are represented by relative translational-rotational displacements of domains and subunits at distances $\geq 3\text{\AA}$. Thus, the cavities, which are formed by domains, fluctuate between states with less (A) and more (B) water-accessibility. The life-times of these states depending on protein structure and external conditions are in the limits of $(10^{-4} - 10^{-7}) s$.

In accordance to our model, one of contributions to this time is determined by frequency of excitations of $[lb/tr]$ **macroconvertions**. The frequency of macroconvertions excitation at normal conditions is about $10^7 (1/s)$.

The pulsation frequency of big multi-subunit oligomeric proteins of about $10^4 (1/s)$ could be related to stronger fluctuations of water cluster in their central cavity like **macrodeformations** or even **superdeformations** (Fig.3c,d).

The life-times of (A) and (B) conformer markedly exceeds the time of transitions between them $\simeq (10^{-9} - 10^{-11}) s$.

The $(A \leftrightarrow B)$ pulsations of various cavities in proteins could be correlated. The corresponding A and B conformers have different Stokes radii and effective volume.

The geometrical deformation of the inter-subunits large central cavity of oligomeric proteins and the destabilization of the water cluster located in it lead to relaxational change of $(A \leftrightarrow B)$ equilibrium constant:

$$K_{A \leftrightarrow B} = \exp\left(-\frac{G_A - G_B}{RT}\right).$$

The dashed line means that the stability and the small-scale dynamics of domains and subunits in the content of A and B conformers can differ from each other. The $[A \leftrightarrow B]$ pulsations are accompanied by reversible sorption-desorption of $(20 - 50)H_2O$ molecules from the cavities.

Structural domains are space-separated formations with a mass of $(1 - 2) \cdot 10^3 D$. Protein subunits ($MM \geq 2 \cdot 10^3 D$), as a rule, consist of 2 or more domains. The domains can consist only of α or only of β -structure or have no like secondary structure at all (Schulz, Schirmer, 1979).

The shift of $A \leftrightarrow B$ equilibrium of central cavity of oligomeric proteins determines their cooperative properties during consecutive ligand binding in the active sites. Signal transmission from the active sites to the remote regions of macromolecules is also dependent on

$(A \leftrightarrow B)$ equilibrium.

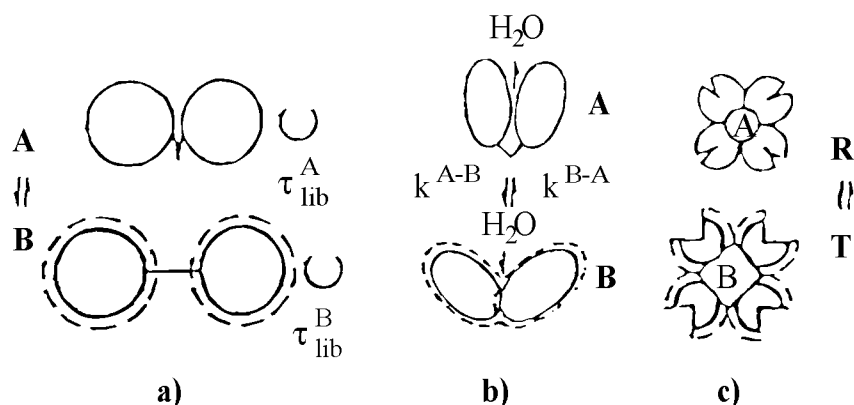


Fig. 1. Examples of large-scale (LS) protein dynamics: $A \leftrightarrow B$ pulsations and librations with correlation times ($\tau_{lb}^B < \tau_{lb}^A$) (Käiväräinen, 1985, 1989):

a) mobility of domains connected by flexible hinge or contact region, like in the light chains of immunoglobulins;

b) mobility of domains that form the active sites of proteins, like in hexokinase, papain, pepsin, lysozyme etc. due to flexibility of contacts;

c) mobility of subunits forming the oligomeric proteins like hemoglobin. Besides transitions of the active sites of each subunit, the ($A \leftrightarrow B$) pulsations with frequencies of $(10^4 - 10^6) s^{-1}$ are pertinent to the common central cavity.

b) *librations* represent the relative rotational - translational motions of domains and subunits in composition of A and B conformers with correlation times $\tau_M \simeq (1 - 5) \cdot 10^{-8}s$.

LS - librations of domains are accompanied by "flickering" of water cluster in the open cavity between domains or subunits. The process of water cluster "flickering", i.e. [dissociation \rightleftharpoons association] is close to the reversible first-order phase transition, when:

$$\Delta G_{H_2O} = \Delta H_{H_2O} - T\Delta S_{H_2O} \approx 0$$

Such type of transitions in water-macromolecular systems could be responsible for so called "enthalpy-entropy compensation effects" (Lumry and Biltonen, 1969).

The "flickering clusters" means excitation of [lb/tr] conversions between librational and translational primary water effectons, accompanied by [association/dissociation] of coherent water cluster (see difference in dimensions of lb and tr effectons on Fig. 18a, b of [1]).

The water cluster (primary lb effecton) association and dissociation in protein cavities in terms of mesoscopic model represent the (ac) - convertons or (bc) - convertons. These excitations stimulate the LS- librations of domains in composition of B-conformer. The frequencies of (ac) and (bc) convertons, has the order of about $10^8 c^{-1}$. This value coincides well with experimental characteristic times for protein domains librations.

The (ac) and (bc) convertons represent transitions between similar states of primary librational and translational effectons: [$a_{lb} \rightleftharpoons a_{tr}$] and [$b_{lb} \rightleftharpoons b_{tr}$] (see Introduction to [1, 2]).

For the other hand, the Macroconvertons, representing simultaneous excitation of (ac + bc) convertons, are responsible for [$B \rightleftharpoons A$] large-scale pulsations of proteins.

The librational mobility of domains and subunits is revealed by the fact that the experimental value of τ_M is less than the theoretical one (τ_M^t) calculated on the Stokes-Einstein formula:

$$\tau_M^t = (V/k) \cdot \eta/T$$

This formula is based on the assumption that the whole protein can be approximated by a rigid sphere. It means, that the large-scale dynamics can be characterized by the "flexibility factor", in the absence of aggregation equal to ratio:

$$fl = (\tau_M/\tau_M^t) \leq 1$$

Antonchenko (1986) has demonstrated, using the Monte-Carlo method for simulations, that the **disjoining pressure** of a liquid in the pores onto the walls changes periodically depending on the distance (**L**) between the limiting surfaces. If the water molecules are approximated by rigid globes, then the maxima of the wedging pressure lie on the values of distance **L**: 9.8; 7; and 3.3Å. It points, that small changes in the geometry of cavities can lead to significant changes in their $A \leftrightarrow B$ equilibrium constant ($K_{A \leftrightarrow B}$).

According to our model the large-scale transition of the protein cavity from the "open" B-state to the "closed" A-state consists of the following stages:

1. Small reorientation (libration) of domains or subunits, which form an "open" cavity (B-state). This process is induced by (*ac*) or (*bc*) convertions of water librational effecton, localized in cavity (flickering of water cluster);
2. Cavitation fluctuation of water cluster, containing (20 – 50) H_2O molecules and the destabilization of the B-state of cavity as a result of [*lb*→*tr*] **macroconvertion** excitation;
3. Collapsing of a cavity during the time about 10^{-10} s, dependent on previous stage and concomitant rapid structural change in the hinge region of interdomain and intersubunit contacts: [*B* → *A*] transition.

The b → a transition of one of the protein cavities can be followed by similar or the opposite A → B transition of the other cavity in the macromolecule.

It should be noted that the collapsing time of a cavitation bubble with the radius: $r \simeq (10 - 15)\text{Å}$ in **bulk** water and collapsing time of interdomain cavity are of the same order: $\Delta t \sim 10^{-10}$ s under normal conditions (Shutilov, 1980).

If configurational changes of macromolecules at $B \rightarrow A$ and $A \rightarrow B$ transitions are sufficiently quick and occur as a jumps of the effective volume, they accompanied by *appearance of the shock acoustic waves in the bulk medium*.

When the cavitation fluctuation of water in the "open" cavity does not occur, then (*b* → *a*) or (*B* → *A*) transitions are slower processes, determined by continuous diffusion of domains and subunits. This happens when [*lb* → *tr*] macroconvertions are not excited.

In their review, Karplus and McCammon (1986) analyzed data on alcoholdehydrogenase, myoglobin and ribonuclease, which have been obtained using *molecular dynamics* approach. It has been shown that large-scale reorientation of domains occur together with their deformation and motions of α and β structures.

It has been shown also (Karplus and McCammon, 1986) that activation free energies, necessary for [$A \leftrightarrow B$] transitions and the reorganization of hinge region between domains, do not exceed (3-4) kcal/mole. Such low values were obtained for proteins with even rather dense interdomain region, as seen from X-ray data. The authors explain such low values of activation energy by the fact that the displacement of atoms, necessary for such transition, does not exceed 0.5 Å, i.e. they are comparable with the usual amplitudes of atomic oscillation at temperatures 20 – 30°C. It means that they occur very quickly within times of 10^{-12} s, i.e. much less than the times of [$A \rightarrow B$] or [$B \rightarrow A$] domain displacements ($10^{-9} - 10^{-10}$ s). Therefore, the high frequency small-scale dynamics of hinge is responsible for the quick adaptation of hinge geometry to the changing distance between the domains and for decreasing the total activation energy of [$A \leftrightarrow B$] pulsations of proteins,.

Recent calculations by means of molecular dynamics reveal that the oscillations in proteins are harmonic at the low temperature ($T < 220K$) only. **At the physiological temperatures the oscillations are strongly anharmonic, collective, global and their amplitude increases with**

hydration (Steinback et al., 1996). Water is a "catalyzer" of protein anharmonic dynamics.

It is obvious, that both small-scale (SS) and large-scale (LS) dynamics, introduced in our model, are necessary for protein function. To characterize quantitatively the LS dynamics of proteins, we proposed the unified Stokes-Einstein and Eyring-Polany equation.

2. Description of large-scale dynamics of proteins, based on generalized Stokes-Einstein and Eyring-Polany equation

In the case of the **continuous** Brownian diffusion of a particle, the rate constant of diffusion is determined by the Stokes-Einstein law:

$$k = \frac{1}{\tau} = \frac{k_B T}{V\eta} \quad 1$$

where: τ is correlation time, i.e. the time, necessary for rotation of a particle by the mean angle determined as $\bar{\varphi} \approx 0.5$ of the turn or the characteristic time for the translational movement of a particle with the radius (a) on the distance $(\bar{\Delta}_x)^{1/2} \approx 0.6a$ (Einstein, 1965);

$V = 4\pi a^3/3$ is the volume of the spherical particle; k_B is the Boltzmann constant, T and η are the absolute temperature and bulk viscosity of the solvent.

On the other hand, the rate constant of $[A \rightarrow B]$ reaction for a molecule in gas phase, which is related to passing through the activation barrier $G^{A \rightarrow B}$, is described with the Eyring-Polany equation:

$$k^{A \rightarrow B} = \frac{k_B T}{h} \exp\left(-\frac{G^{A \rightarrow B}}{RT}\right) \quad 2$$

To describe the large-scale dynamics of macromolecules in solution related to fluctuations of domains and subunits (librations and pulsations), an equation is needed which takes into account the diffusion and activation processes simultaneously.

The rate constant for the rotational- translational diffusion of the particle (k_c) forming a macromolecule (continuous LS-dynamics) is expressed with the generalized Stokes-Einstein and Eyring-Polany equation (Käiväräinen and Goryunov, 1987):

$$K_c = \frac{k_B T}{\eta V} \exp\left(-\frac{G_{st}}{RT}\right) = \tau_c^{-1} \quad 3$$

where: V is the effective volume of domain or subunit, which are capable to the Brownian mobility independently from the rest part of the macromolecule, with the probability:

$$P_{lb} = \exp\left(-\frac{G_{st}}{RT}\right), \quad 4$$

where: G_{st} is the activation energy of structural change in the contact (hinge) region of a macromolecule, necessary for independent mobility of domain or subunit; τ_c is the effective correlation time for the continuous diffusion of this relatively independent particle.

The effective volume V can be changed under the influence of temperature, perturbants and ligands.

The generalized Stokes-Einstein and Eyring-Polany equation (3) is applicable also to describing the diffusion of the whole (integer) particle, dependent on the surrounding medium fluctuations with activation energy (G_a). The ligand diffusion in the active site cavity of proteins is such a type of processes.

To describe noncontinuous process, the formula for rate constant (k_{jump}) of the jump-like translations of particle, related to emergency of cavitation fluctuations (holes) near the particle

was proposed (Käiväräinen and Goryunov, 1987):

$$k_{\text{jump}} = \frac{1}{\tau_{\text{jump}}^{\text{min}}} \exp\left(-\frac{W}{RT}\right) = \frac{1}{\tau_{\text{jump}}}, \quad 5$$

where:

$$W = \sigma S + n_s(\mu_{\text{out}} - \mu_{\text{in}}) \quad 6$$

is the work of *cavitation fluctuation* with the cavity surface S , at which n_s molecules of the solvent (water) change its effective chemical potential from μ_{in} to μ_{out} .

The dimensions of cavity fluctuation near particle must be comparable to corresponding particles.

In a homogeneous phase (i.e. pure water) under equilibrium conditions we have: $\mu_{\text{in}} = \mu_{\text{out}}$. With an increase of particle sizes, surface of cavitation fluctuation (S) and its work (W), the corresponding probability of cavitation fluctuations:

$$P_{\text{jump}} = \exp(-W/RT)$$

will fall.

The notion of the surface energy (σ) retains its meaning even at very small "holes" because of its molecular nature (see Section 11.4 of [1] and [4]).

$\tau_{\text{jump}}^{\text{min}}$ in eq. (5) is the minimal possible jump-time of a particle with mass (m) over the distance λ with the mean velocity:

$$v_{\text{max}} = (2kT/m)^{1/2} \quad 7$$

Hence, we derive for the maximal jump-rate at $W=0$:

$$k_{\text{jump}}^{\text{max}} = \frac{1}{\tau_{\text{jump}}^{\text{min}}} = \frac{v_{\text{max}}}{\lambda} = \frac{1}{\lambda} \left(\frac{2kT}{m} \right)^{1/2} \quad 8$$

In the case of hinged domains, forming macromolecules their relative $A \rightleftharpoons B$ displacements (pulsations) are related not only to possible holes forming in the interdomain (intersubunit) cavities or near their outer surfaces, but to the structural change of hinge regions as well.

If the activation energy of necessary structure changes is equal to $G_{st}^{A \rightleftharpoons B}$, then eq. (5), with regard for (8), is transformed into

$$k_{\text{jump}}^{A \rightleftharpoons B} = \frac{1}{\lambda} \left(\frac{2kT}{m} \right)^{1/2} \exp\left(-\frac{W_{A,B} + G_{st}^{A \rightleftharpoons B}}{RT}\right) \quad 9$$

where: $W_{A,B}$ is the work required for cavitation fluctuations of water; this work can be different in two directions: (W_B) is necessary for nonmonotonic $B \rightarrow A$ transition; (W_A) is necessary for jump-way $A \rightarrow B$ transition.

Under certain conditions $A \rightleftharpoons B$ transitions between protein conformers (LS- pulsations) can be realized owing to the *jump-way and continuous* types of relative diffusion of domains or subunits as two stage reaction. In this case, the resulting rate constant of the process will be expressed through (9) and (3) as:

$$k_{\text{res}}^{A \rightleftharpoons B} = k_{\text{jump}}^{A \rightleftharpoons B} = k_c^{A \rightleftharpoons B} = \frac{1}{\lambda} \left(\frac{2kT}{m} \right)^{1/2} \exp\left(-\frac{W_{A,B} + G_{st}^{A \rightleftharpoons B}}{RT}\right) + \frac{kT}{\lambda V} \exp\left(-\frac{G_{st}^{A \rightleftharpoons B}}{RT}\right) \quad 10$$

The interaction between two domains in *A-conformer* can be described using microscopic Hamaker - de Bour theory. One of the contributions into $G_{st}^{A \rightleftharpoons B}$ is the energy of dispersion interactions between domains of the *radius* (a) (Käiväräinen, 1989b):

$$[G_{st} \sim U_H \approx -A^* a/12H]_{A,B} \quad 11$$

where

$$A^* \approx (A_s^{1/2} - A_c^{1/2})^2 \approx \frac{3}{2} \pi h v_0^s [\alpha_s N_s - \alpha_c N_c]^2 \quad 12$$

is the complex Hamaker constant; H is the slit thickness between domains in *A-state*; A_c and A_s are simple Hamaker constants, characterizing the properties of water in the *A-state* of the cavity and in the bulk solvent, correspondingly. They depend on the concentration of water molecules ($N_c \approx N_s$) and their polarizability ($\alpha_c \neq \alpha_s$):

$$A_c = \frac{3}{2}\pi h\nu_0^c \alpha_c^2 N_c^2 \quad \text{and} \quad A_s = \frac{3}{2}\pi h\nu_0^s \alpha_s^2 N_s^2;$$

where: $h\nu_0^c \approx h\nu_0^s$ are the ionization potentials of H_2O molecules in a cavity and in a free solvent.

In the "closed" A-state of a cavity the water layer between domains has a more compact packing as compared with the ice-like structure of a water cluster in the B-state of a cavity, or with a free solvent. As far $H_A < H_B$ the dispersion interaction (11) between domains in A- state of cavity is stronger, than that in B-state: $U_H^A > U_H^B$.

Disjoining pressure of water in the cavities

$$\Pi = -A^*/6\pi H^3 \quad 12a$$

decreases with the increase of the complex Hamaker constant (A^*) that corresponds to the increase of the attraction energy (U_H) between domains.

Cooperative properties of clusters in open (B)-states of the cavities are more pronounced as compared to that in bulk water. That results in the greater changes of $\alpha_c N_c$ than that of $\alpha_s N_s$, induced by temperature. The elevation of temperature decreasing the dimensions of interdomain water clusters leads to the strengthening of interdomain interaction, while the lowering temperature leads to opposite effect.

We can judge about the changes of $\alpha_s N_s$ in the experiment on measuring the solvent refraction index, as far from our theory of refraction index (eq. 8.14 of [1] or paper [3]):

$$(n_s^2 - 1)/n_s^2 = \frac{4}{3}\pi\alpha_s N_s \quad \text{or:} \quad \alpha_s N_s = \frac{3}{4}\pi \cdot \frac{n_s^2 - 1}{n_s^2} \quad 12b$$

In the **closed cavities** the effect of temperature on water properties is lower as compared to that in bulk water. **It follows that thermoinduced nonmonotonic transition in the solvent refraction index must be accompanied by in-phase nonmonotonic changes of the $[A \leftrightarrow B]$ equilibrium constant ($K_{A \leftrightarrow B}$).** As far (A) and (B) conformers usually have different stability and flexibility, the changes of $K_{A \leftrightarrow B}$ will be manifested in the changes of protein large-scale and small-scale dynamics. It has been shown before that viscosity itself has nonmonotonic temperature dependence due to the nonmonotonic dependence of $n^2(t)$ (eq.11.44, 11.45 and 11.48 of [1] or paper [4]).

Thus, thermoinduced non-denaturational transitions of macromolecules and supramolecular systems located in the aqueous environment are caused by nonmonotonic changes in solvent properties, including its refraction index.

The influence of D_2O and other perturbants on protein dynamics is explained in a similar way. The effect of deuterium oxide (D_2O) is a result of substitution of H_2O from protein cavities and corresponding change of complex Hamaker constant (12).

Generalized equation (3) is applicable not only for evaluating the frequency of macromolecules transition between A and B conformers but also for the frequency of the dumped librations of domains and subunits within A and B conformers. Judging by various data (Käiväräinen, 1985, 1989b), the interval of $A \rightleftharpoons B$ pulsation frequency is:

$$\nu_{A \leftrightarrow B} = \frac{1}{t_A + t_B} \approx k^{A \rightarrow B} = (10^4 - 10^7) s^{-1} \quad 13$$

where: t_A and t_B are the lifetimes of A and B conformers.

The corresponding interval of the total activation energy of the jump-way $A \leftrightarrow B$ pulsations can be evaluated from the eq. (9). We assume for this end that the pre-exponential multiplier is about $10^{10} s^{-1}$ as a frequency of cavitation fluctuations in water with the radius $\sim(10-15) \text{ \AA}$.

Taking a logarithm of (9) we derive:

$$G_{\text{res}}^{A \leftrightarrow B} = (W_{A,B} + G_{st}^{A \leftrightarrow B}) \approx RT(\ln 10^{10} - \ln \nu_{A \leftrightarrow B}) \quad 14$$

At physiological temperatures the following region of energy corresponds to the frequency range of pulsations (13)

$$G_{\text{res}}^{A \leftrightarrow B} \approx (4 - 8) \text{ kcal/mole} \quad 15$$

Such a region of energies is pertinent to a wide range of biochemical processes.

The quick jump-way pulsations of macromolecules can cause acoustic shock- waves in the solvent and its structure destabilization. Concomitant increase in water activity leads to distant interaction between different proteins as well as proteins and cells. Such solvent-mediated phenomena were discovered and studied in our laboratory by set of specially elaborated methods (Käiväräinen, 1985, 1986, 1987; Käiväräinen et al., 1990, Käiväräinen et al., 1993).

When the $[A \leftrightarrow B]$ transitions in proteins are related to *continuous* diffusion only, then the G_{st} values calculated using eq.(3) for the same frequency interval ($10^4 - 10^7$) s, is about (3 - 7) kcal/mole.

The *Kramers equation* (1940), which has earlier been widely used for describing diffusion processes, has the form:

$$k = \frac{A}{\eta} \exp\left(-\frac{H^*}{RT}\right) \quad 16$$

where A is a constant and η - solvent viscosity.

The pre-exponential factor in our generalized equation (3) contains not only the viscosity, but also the temperature and the effective volume of a particle. It was shown in our experiments that eq. (3) describes the dynamic processes, which occur in macromolecules, solutions much better than the Kramer's equation (16).

3. Dynamic model of protein-ligand complexes formation

According to our model of specific complexes formation the following order of events is assumed (Fig. 2):

1. Ligand (L) collides with the active site (AS), formed usually by two domains, in its open (b) state: the structure of water cluster in AS is being perturbed and water is forced out of AS cavity totally or partially;
2. Transition of AS from the open (b) to the closed (a) state occurs due to strong shift of $[a \leftrightarrow b]$ equilibrium to the left, i.e. to the AS domains large scale dynamics;
3. A process of dynamic adaptation of complex $[L+AS]$ begins, accompanied by the directed ligand diffusion in AS cavity due to its domains small-scale dynamics and deformation of their tertiary structure;
4. If the protein is oligomeric with few AS, then the above events cause changes in the geometry of the central cavity between subunits in the open state leading to the destabilization of the large central water cluster and the shift of the $A \rightleftharpoons B$, corresponding to $R \rightleftharpoons T$ equilibrium of quaternary structure leftward. Water is partially forced out from central cavity.

Due to the feedback mechanism this shift can influence the $[a \leftrightarrow b]$ equilibrium of the remaining free AS and promotes its reaction with the next ligand. Every new ligand stimulates this process, promoting the positive cooperativity. The negative cooperativity also could be resulted from the interaction between central cavity and active sites;

5. The terminal $[protein - ligand]$ complex is formed as a consequence of the relaxation process, representing deformation of domains and subunits tertiary structure. This stage could be much slower than the initial ones [1-3]. As a result of it, the stability of the complex grows up.

Dissociation of specific complex is a set of reverse processes to that described above which starts from the $[a^* \rightarrow b]$ fluctuation of the AS cavity.

In multidomain proteins like antibodies, which consist of 12 domains, and in oligomeric proteins, the cooperative properties of H_2O clusters in the cavities can determine the mechanism of signal transmission from AS to the remote effector regions and allosteric protein properties.

The stability of a librational water effecton as coherent cluster strongly depends on its sizes and geometry. This means that very small deformations of protein cavity, which violate the [cavity-cluster] complementary condition, induce a cooperative shift of $[A \leftrightarrow B]$ equilibrium leftward. The clusterphilic interaction, introduced by us (see section 13.3 of [1] or paper [4]) turns to hydrophobic one due to $[lb/tr]$ conversion.

This process can be developed step by step. For example, the reorientation of variable domains, which form the antibodies active site (AS) after reaction with the antigen determinant or hapten deforms the next cavity between pairs of variable and constant domains forming F_{ab} subunits (Fig.2). The leftward shift of $[A \leftrightarrow B]$ equilibrium of this cavity, in turn, changes the geometry of the big central cavity between F_{ab} and F_c subunits, perturbing the structure of the latter. **Therefore, the signal transmission from the AS to the effector sites of F_c subunits occurs due to the balance shift between clusterphilic and hydrophobic interactions.** This signal is responsible for complement- binding sites activation and triggering the receptors function on the lymphocyte membranes, in accordance to our model.

The leftward shift of $[A \leftrightarrow B]$ equilibrium in a number of cavities in the elongated multidomain proteins can lead to the significant decrease of their linear size and dehydration. The mechanism of muscular contraction is probably based on such phenomena and clusterphilic interactions (see next section).

For such a nonlinear system the energy is necessary for reorientation of the first couple of domains only. The process then goes on spontaneously with decreasing the averaged protein chemical potential.

The chemical potential of the A- conformer is usually lower than that of B- conformer ($\bar{G}_A < \bar{G}_B$) and the relaxation of protein is accompanied by the leftward $A \leftrightarrow B$ equilibrium shift of cavities.

It is predictable, that hydration of proteins will decrease, when clusterphilic [water-cavity] interaction turns to hydrophobic one.

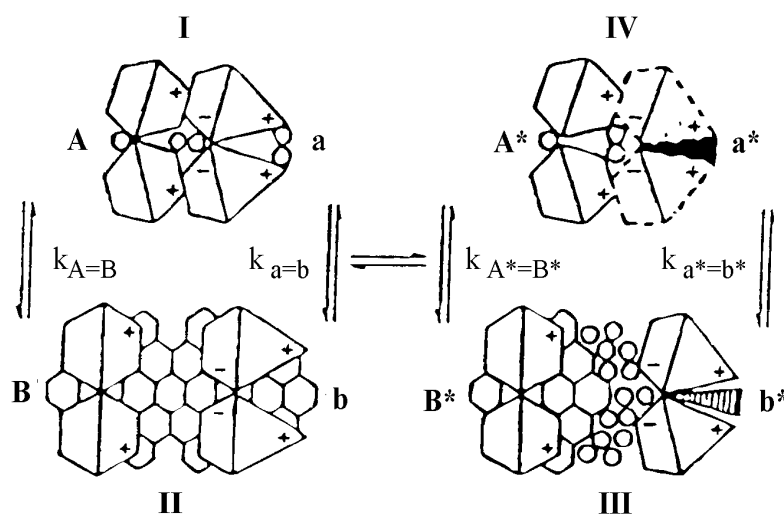


Fig. 2. The schematic picture of the protein association (Fab subunits of antibody with a ligand), which is accompanied by the destabilization of water clusters in cavities,

according to the dynamic model (Käiväräinen, 1985). The dotted line denotes the perturbation of the tertiary structure of the domains forming the active site. Antibodies of IgG type contain usually two such Fab subunit and one Fc subunit, conjugated with 2Fab by flexible hinge, forming the general Y-like structure.

Our dynamic model of protein behavior and signal transmission, described above, is an alternative to solitonic mechanism of non dissipative signal transmission in proteins and in other biosystems proposed by Davidov (1973). Propagation of solitonic wave is a well known nonlinear process in the ordered **homogeneous** mediums. The solitons can originate, when the nonlinear effects are compensated by the wave dispersion effects. Dispersion is reflected in fact that the longer waves spreads in medium with higher velocity than the shorter ones.

However, biosystems of nonregular, fluctuating structure are not the mediums, good for solitons emergency and propagation.

Our dynamic model takes into account the real multidomain and multiglobular structure of a proteins and properties of their hydration shell fractions. In contrast to Davidov's solitonic model, the dissipation processes like reversible "melting" of water clusters, accompanied by large-scale dynamics of proteins, are the necessary stages of our [hydrophobic \rightleftharpoons clusterphilic] mechanism of signal transmission in biosystems.

The evolution of the ideas of the protein-ligand complex formation proceeded in the following sequence:

- 1. "Key-lock" or the rigid conformity between the geometry of an active site and that of a ligand (Fisher, 1894);**
- 2. "Hand-glove" or the so-called principle of induced conformity (Koshland, 1962);**
- 3. At the current stage of complex-formation process understanding, the crucial role of protein dynamics gets clearer. Our model allows us to put forward the "Principle of Stabilized Conformity (PSC)" instead that of "induced conformity" in protein-ligand specific reaction.**

Principle of Stabilized Conformity (PSC) means that the geometry of the active site (AS), optimal from energetic and stereochemical conditions, is already existing BEFORE reaction with ligand. The optimal geometry of AS is to be the only one selected among the number of others and stabilized by ligand, but not induced "de nova".

For example, the $[a \leftrightarrow b]$ large-scale pulsations of the active sites due to domain fluctuations and stabilization of the closed (a) state by ligand are necessary for the initial stages of reaction. Such active site pulsations decreases the total activation energy necessary for the terminal complex formation as multistage process.

4. The life-time of quasiparticles and frequencies of their excitation

The set of formula, describing the dynamic properties of quasiparticles, introduced in mesoscopic theory was presented at Chapter 4 of book [1] and paper [2]:

The frequency of c- Macrotransitons or Macroconvertions excitation, representing [dissociation/association] of primary librational effectons - "flickering clusters" as a result of interconversions between primary [lb] and [tr] effectons is:

$$F_{cM} = \frac{1}{\tau_{Mc}} \cdot P_{Mc}/Z \quad 17$$

where: $P_{Mc} = P_{ac} \cdot P_{bc}$ is a probability of macroconvertion excitation;
 Z is a total partition function (see eq.4.2 of [1, 2]);
the life-time of macroconvertion is:

$$\tau_{Mc} = (\tau_{ac} \cdot \tau_{bc})^{1/2} \quad 18$$

The cycle-period of (ac) and (bc) convertions are determined by the sum of life-times of intermediate states of primary translational and librational effectons:

$$\begin{aligned} \tau_{ac} &= (\tau_a)_{tr} + (\tau_a)_{lb}; \\ \tau_{bc} &= (\tau_b)_{tr} + (\tau_b)_{lb}; \end{aligned} \quad 19$$

The life-times of primary and secondary effectons (lb and tr) in a - and b -states are the reciprocal values of corresponding state frequencies:

$$[\tau_a = 1/\nu_a; \tau_{\bar{a}} = 1/\nu_{\bar{a}}]_{tr,lb}; \quad [\tau_b = 1/\nu_b; \tau_{\bar{b}} = 1/\nu_{\bar{b}}]_{tr,lb} \quad 20$$

$[(\nu_a) \text{ and } (\nu_b)]_{tr,lb}$ correspond to eqs. 4.8 and 4.9 of [1, 2];

$[(\nu_{\bar{a}}) \text{ and } (\nu_{\bar{b}})]_{tr,lb}$ could be calculated using eqs.4.16; 4.17 [1, 2].

The frequency of (ac) and (bc) convertions excitation [lb/tr]:

$$F_{ac} = \frac{1}{\tau_{ac}} \cdot P_{ac}/Z \quad 21$$

$$F_{bc} = \frac{1}{\tau_{bc}} \cdot P_{bc}/Z \quad 22$$

where: P_{ac} and P_{bc} are probabilities of corresponding convertions excitations (see eq.4.29a of [1, 2]).

The frequency of Supereffectons and Superdeformons (biggest fluctuations) excitation is:

$$F_{SD} = \frac{1}{(\tau_{A^*} + \tau_{B^*} + \tau_{D^*})} \cdot P_S^{D^*}/Z \quad 23$$

It is dependent on cycle-period of Supereffectons: $\tau_{SD} = \tau_{A^} + \tau_{B^*} + \tau_{D^*}$*

and probability of Superdeformon activation ($P_S^{D^}$), like the limiting stage of this cycle.*

The averaged life-times of Supereffectons in A^* and B^* state are dependent on similar states of translational and librational macroeffectons :

$$\tau_{A^*} = [(\tau_A)_{tr} \cdot (\tau_A)_{lb}] = [(\tau_a \tau_{\bar{a}})_{tr} \cdot (\tau_a \tau_{\bar{a}})_{lb}]^{1/2} \quad 24$$

and that in B state:

$$\tau_{B^*} = [(\tau_B)_{tr} \cdot (\tau_B)_{lb}] = [(\tau_b \tau_{\bar{b}})_{tr} \cdot (\tau_b \tau_{\bar{b}})_{lb}]^{1/2} \quad 25$$

The life-time of Superdeformons excitation is determined by frequency of beats between A^ and B^* states of Supereffectons as:*

$$\tau_{D^*} = 1/|(1/\tau_{A^*}) - (1/\tau_{B^*})| \quad 26$$

The frequency of translational and librational macroeffectons $A \rightleftharpoons B$ cycle excitations could be defined in a similar way:

$$\left[F_M = \frac{1}{(\tau_A + \tau_B + \tau_D)} \cdot P_M^D/Z \right]_{tr,lb} \quad 27$$

where:

$$(\tau_A)_{tr,lb} = [(\tau_a \cdot \tau_{\bar{a}})_{tr,lb}]^{1/2} \quad 28$$

and

$$(\tau_B)_{tr,lb} = [(\tau_b \cdot \tau_{\bar{b}})_{tr,lb}]^{1/2} \quad 29$$

$$(\tau_D)_{tr,lb} = 1/|(1/\tau_A) - (1/\tau_B)|_{tr,lb} \quad 30$$

The frequency of primary translational effectons ($a \rightleftharpoons b$)_{tr} transitions could be expressed like:

$$F_{tr} = \frac{1/Z}{(\tau_a + \tau_b + \tau_t)_{tr}} \cdot (P_d)_{tr} \quad 31$$

where: $(P_d)_{tr}$ is a probability of primary translational deformons excitation (eq. 4.25 of [1, 2]); $[\tau_a; \tau_b]_{tr}$ are the life-times of (a) and (b) states of primary translational effectons (eq. 20).

The frequency of primary librational effectons as ($a \rightleftharpoons b$)_{lb} cycles excitations is:

$$F_{lb} = \frac{1/Z}{(\tau_a + \tau_b + \tau_t)_{lb}} \cdot (P_d)_{lb} \quad 32$$

where: $(P_d)_{lb}$ is a probability of primary librational deformons excitation; τ_a and τ_b are the life-times of (a) and (b) states of primary librational effectons defined as (20).

The life-time of primary transistons (tr and lb) as a result of quantum beats between (a) and (b) states of primary effectons could be introduced as:

$$[\tau_t = |1/\tau_a - 1/\tau_b|^{-1}]_{tr,lb} \quad 33$$

For the case of $(a \rightleftharpoons b)^{1,2,3}$ transitions of primary and secondary effectons (*tr and lb*), their life-times in (a) and (b) states are the reciprocal value of corresponding frequencies: $[\tau_a = 1/\nu_a]$ and $[\tau_b = 1/\nu_b]_{tr,lb}^{1,2,3}$. These parameters and the resulting ones could be calculated from eqs.(2.27; 2.28 of [1]) for primary effectons and (2.54; 2.55 of [1]) for secondary ones.

The results of calculations, using eqs. (31, 32) for frequency of excitations of primary *tr and lb* effectons are plotted on Fig. 3a,b.

The frequencies of Macroconverters and Superdeformons were calculated using eqs.(17 and 23).

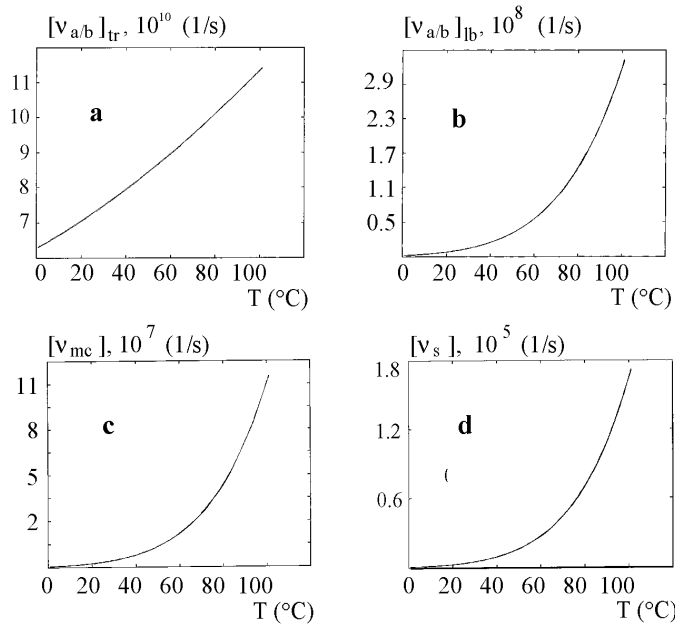


Fig. 3. (a) - Frequency of primary [tr] effectons excitations, calculated from eq.(31); (b) - Frequency of primary [lb] effectons excitations, calculated from eq.(32); (c) - Frequency of [lb/tr] Macroconverters (flickering clusters) excitations, calculated from eq.(17); (d) - Frequency of Superdeformons excitations, calculated from eq.(28).

At the temperature interval $(0-100)^{\circ}C$ the frequencies of translational and librational

macrodeformons (tr and lb) are in the interval of $(1.3-2.8) \cdot 10^9 s^{-1}$ and $(0.2-13) \cdot 10^6 s^{-1}$ correspondingly. The frequencies of (ac) and (bc) convertons could be defined also using our software and formulae, presented at the end of Chapter 4 of [1, 2].

The frequency of primary translational effectons $[a \leftrightarrow b]_{tr}$ excitations at 20^0C , calculated from eq.(31) is $\nu \sim 7 \cdot 10^{10} (1/s)$. It corresponds to electromagnetic wave length in water with refraction index ($n = 1.33$) of:

$$\lambda = (cn)/\nu \sim 6mm \quad 34$$

For the other hand, there are a lot of evidence, that irradiation of very different biological systems with such coherent electromagnetic field exert great influences on their properties (Grundler and Keilman, 1983).

Between the dynamics/function of proteins, membranes, etc. and dynamics of their aqueous environment the strong interrelation is existing.

The frequency of macroconvertons, representing big density fluctuation in the volume of primary librational effecton at $37C$ is about $10^7 (1/s)$ (Fig 3c).

The frequency of librational macrodeformons at the same temperature is about $10^6 s^{-1}$, i.e. coincides with frequency of large-scale protein cavities pulsations between open and closed to water states (see Fig.2). This confirm our hypothesis that the clusterphilic interaction is responsible for stabilization of the proteins cavities open state and that transition from the open state to the closed one is induced by coherent water cluster dissociation.

The frequency of Superdeformons excitation (Fig.3d) is much lower:

$$\nu_s \sim (10^4 - 10^5) s^{-1} \quad 35$$

Superdeformons are responsible for cavitational fluctuations in liquids and origination of defects in solids. Dissociation of oligomeric proteins, like hemoglobin or deassembly (peptization)of actin and microtubules could be also related with such big fluctuations.

5. Mesoscopic mechanism of enzyme catalysis

The mechanism of enzyme catalysis is one of the most intriguing and unresolved yet problems of molecular biology. It becomes clear, that it is interrelated not only with a spatial, but as well with hierarchical complicated dynamic properties of proteins (see book: "The Fluctuating Enzyme" , Ed. by G.R.Welch, 1986).

The [proteins + solvent] system should be considered as a cooperative one with feedback links (Kaivarainen, 1985, 1992). Somogyi and Damjanovich (1986) proposed a similar idea that collective excitations of protein structure are interrelated with surrounded water molecules oscillations.

The enzymatic reaction can be represented in accordance with our dynamic model as a consequence of the following stages (Käiväräinen, 1989; [1]).

The first stage:



- the collision of the substrate (S) with the open (b) state of the active site [AS] cavity of enzyme (E).

The frequency of collisions between the enzyme and the substrate, whose concentrations are

$[C_E]$ and $[C_S]$, respectively, is expressed with the known formula (Cantor and Schimmel, 1980):

$$v_{col} = 4\pi r_0(D_E + D_S) \cdot N_0[C_E] \cdot [C_S] \quad 37$$

where: $r_0 = a_E + a_S$ is the sum of the enzyme's and substrate's molecular radii; N_0 is the Avogadro number;

$$D_E = \frac{kT}{6\pi\eta a_E} \quad \text{and} \quad D_S = \frac{kT}{6\pi\eta a_S} \quad 38$$

- are the diffusion coefficients of the enzyme and substrate; k is the Boltzmann constant; T is absolute temperature; η is a solvent viscosity.

The probability of collision of (b) state of the active site with substrate is proportional to the ratio of the b -state outer cross section area to the whole enzyme surface area:

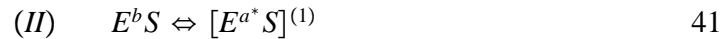
$$P_b = \frac{\theta^b}{\theta_E} \cdot F_b \quad 39$$

where: $F_b = \frac{f_b}{f_b + f_a}$ is a fraction of time, the active site [AS] spend in the open (b) - state.

So, the frequency of collision between the substrate and (b) state of the active site (AS) with account for (40), meaning the first stage of reaction is:

$$v_{col}^b = v_{col} \cdot P_b = k_I \quad 40$$

The second stage of enzymatic reaction is a formation of the primary enzyme-substrate complex:



It corresponds to transition of the active site cavity from the open (b) state to the closed (a) one and stabilization the latter state by a ligand.

The rate constant of the [$b \rightarrow a$] transitions is derived with the Stokes-Einstein and Eyring-Polanyi generalized equation (3):

$$k_{II}^{b \rightarrow a^*} = \frac{kT}{\eta V} \exp\left(-\frac{G_{st}^{b \rightarrow a}}{RT}\right) \quad 42$$

where: η is the solvent viscosity; V is the effective volume of the enzyme domain, whose diffusional reorientation accompanies the ($b \rightarrow a$) transition of the active site [AS].

The leftward shift of the [$a \Leftrightarrow b$] equilibrium between two states of the active site is concomitant with this stage of the reaction. It reflects the *principle of stabilized conformity*, related to AS domains movements that we have put forward in the previous section.

The third stage:



represents the formation of the secondary specific complex. This process is related to directed ligand diffusion into the active site cavity and the dynamic adaptation of its geometry to the geometry of the active site.

Here the Principle of Stabilized microscopic Conformity is realized, when the AS change its geometry from (a) to (a^*) without domains reorientation. The rate constant of this stage is determined by the rate constant of substrate diffusion in the closed (a) state of the active site cavity. It is also expressed by **generalized kinetic equation** (42), but with other values of variables:

$$k_S^{1 \rightarrow 2^*} = \frac{1}{\tau_s^*} \exp\left(-\frac{G_s^a}{RT}\right) = k_{III} \quad 44$$

where:

$$\tau_s = (v_s/k)\eta^{in}/T \quad 45$$

is the correlation time of substrate of volume (v_s) in the (a) state of the active site; η^{in} is the internal effective viscosity; G_s^a is the activation energy of thermal fluctuations of groups, representing small-scale dynamics (SS), which determine the directed diffusion of a substrate in the active site [AS] closed cavity.

The directed character of ligand diffusion in AS can be determined by the relaxation of a

protein structure, due to perturbation of AS domains by ligand. The relaxation changes were observed in many reactions of specific protein-ligand complexes formation (Käiväräinen, 1985, 1989).

The complex formation [pair of domains forming the AS + substrate], followed by these domain immobilization can be considered as an emergency of a new enlarged protein primary effecton from two smaller ones, corresponding to less independent AS domains or their compact "nodes".

We assume that at this important stage, the waves B of the attacking catalytic atoms (λ_B^c) and the attacked substrate atoms (λ_B^s) start to overlap and interfere in such a way that conditions for quantum-mechanical beats between them become possible.

Let us consider these conditions in more detail.

According to classical statistics, every degree of freedom gets the energy, which is equal to $kT/2$. This condition corresponds to harmonic approximation when the mean potential and kinetic energies of particles are the same:

$$V = T_k = mv^2/2 \approx kT/2 \quad 46$$

The corresponding to such ideal case the de Broglie wave (wave B) length is equal to:

$$\lambda_B = \frac{h}{mv} = \frac{h}{(mkT)^{1/2}} \quad 47$$

For such condition the wave B length of proton at room temperature is nearly 2.5\AA , for a carbon atom it is about three times smaller and for oxygen - four times as small.

In the latter two cases, the wave B lengths are comparable and even less than the sizes of the atoms itself. So, their waves can not overlap and, beats between them are not possible.

However, in real condensed systems with quantum properties, including the active sites of enzymes, the harmonic approximation is not valid because $(T_k/V) \ll 1$ (see Fig. 5 of [1]). Consequently, the kinetic energy of atoms of AS:

$$T_k \ll (1/2)kT \quad \text{and} \quad \lambda_B \gg h/(mkT)^{1/2} \quad 47a$$

It must be taken into account that librations, in a general case are presented by rotational-translational anharmonic oscillations of atoms and molecules, but not by their rotational motions only (Coffey et al., 1984).

The length of waves B of atoms caused by a small translational component of most probable impulse, related to librations is bigger than that related to pure translations:

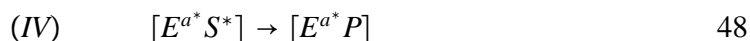
$$[\lambda_{lb} = (h/P_{lb})] > [\lambda_{tr} = (h/P_{tr})] \quad 47b$$

Even in pure water the linear sizes of primary librational effectons are several times bigger than that of translational effectons and the size of one H_2O molecule (see Fig. 7 of [1] or Fig.4 of [2]).

In composition of the active site rigid core the librational waves B of atoms can significantly exceed the sizes of the atoms themselves. In this case their superposition, leading to quantum beats of waves B in the [active site - substrate] complex, accelerating the enzyme reaction is quite possible.

In accordance to our model, periodic energy exchange resulting from such beats occurs between waves B of the substrate and active site atoms.

The reaction $[S^* \rightarrow P]$, accelerating by these quantum beats, is the next 4th stage of enzymatic process - the chemical transformation of a substrate into product:



The angular wave B frequency of the *attacked atoms of substrate* with mass m_S and the amplitude A_S can be expressed by eq.(2.20):

$$\omega^S = \hbar/2m_S A_S^2 \quad 49$$

The wave B frequency of the attacking catalytic atom (or a group of atoms) in the active site is equal to:

$$\omega^{\text{cat}} = \frac{\hbar}{2m_c A_c} \quad 50$$

The frequency of quantum beats which appear between waves B of catalytic and substrate atoms is:

$$\omega^* = \omega^{\text{cat}} - \omega^S = \hbar \left(\frac{1}{2m_c A_c} - \frac{1}{2m_s A_s} \right) \quad 51$$

The corresponding energy of beats:

$$E^* = E^{\text{cat}} - E^S = \hbar \omega^* \quad 52$$

It is seen from these formulae that the smaller the wave B mass of the catalytic atom (m_c) and its amplitude (A_c), the more frequently these beats occur at constant parameters of substrate (m_s and A_s). **The energy of beats is transmitted to the wave B of the attacked substrate atom from the catalytic atom, accelerating the reaction.**

According to our model, the perturbations in the region of the active site are accompanied by the appearance of phonons and acoustic deformons in a form of small-scale dynamics of protein structure. They provide the signal transmission in oligomeric proteins to the central cavity and other active sites leading to allosteric effects.

It is known from the theory of oscillations (Grawford, 1973) that the effect of beats is maximal, if the amplitudes of the interacting oscillators are equal:

$$A_c^2 \approx A_s^2 \quad 53$$

The [substrate \rightarrow product] transformation can be considered as a result of the substrate wave B transition from the main [S] state to excited [P] state. The rate constant of such a reaction in the absence of the catalyst ($k^{S \rightarrow P}$) can be presented by the modified Eyring-Polany formulae, leading from eq.(2.27 of [1]) at condition: $\exp(hv_p/kT) \gg 1$

$$v_A^S = k^{S \rightarrow P} = \frac{E^P}{h} \exp\left(-\frac{E^P - E^S}{kT}\right) = v_B^P \exp\left[-\frac{h(v_B^P - v_B^S)}{kT}\right] \quad 54$$

where: $E^S = hv^S$ and $E^P = hv^P$ are the main and excited - transitional to product energetic states of substrate, correspondingly; v^S and v^P are the substrate wave B frequencies in the main and excited states, respectively.

If catalyst is present, which acts by the above described mechanism, then the energy of the substrate E^S is increased by the magnitude E^{cat} with the quantum beats frequency ω^* (51) and gets equal to:

$$E^{Sc} = E^S + E^{\text{cat}} \quad 55$$

Substituting $E^P = hv_B^P$ and $E^{Sc} = h(v_B^S + v_B^{\text{cat}})$ in (54), we derive the rate constants for the catalytic reaction in the moment of beats ($k^{Sc \rightarrow P}$). This corresponds to the 4th stage of enzymatic reaction:

$$(IV): k^{Sc \rightarrow P} = v^P \exp\left[-\frac{h(v^P - v^S - v^{\text{cat}})}{kT}\right] = k_{IV} \quad 56$$

where: v^P , v^S and v^c are the most probable B wave frequencies of the transition [S \rightarrow P] state, of the substrate and of the catalyst atoms, correspondingly.

Hence, in the presence of the catalyst the coefficient of acceleration (q) is equal to:

$$q_{\text{cat}} = \frac{k^{Sc \rightarrow P}}{k^{S \rightarrow P}} = \exp\left(\frac{hv^{\text{cat}}}{kT}\right) \quad 57$$

For example, at

$$h\nu^c/kT \approx 10; \quad q_{\text{cat}} = 2.2 \cdot 10^4 \quad 58$$

At room temperatures this condition corresponds to

$$E^{\text{cat}} = h\nu^{\text{cat}} \approx 6 \text{ kcal/mole.}$$

The beating acts, followed by transitions of a substrate molecule to activated by catalyst excited state ($S \rightarrow Sc$), can be accompanied by the absorption of phonons or photons with the frequency ω^* (51). In this case, the insolation of a [substrate - catalyst] system with ultrasound or electromagnetic field of the frequency ω^* should strongly accelerate the reaction when the resonance conditions are satisfied.

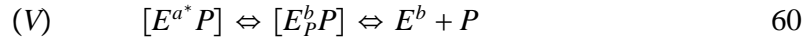
It is possible that the resonance effects of this type can account for the experimentally revealed response of various biological systems to electromagnetic field radiation with the frequency about $6 \cdot 10^{10} \text{ Hz}$ (Deviatkov et al., 1973). We have to point out that just such frequency is close to the frequency of $(a \rightleftharpoons b)_{ir}$ transitions excitation of primary translational water effectons. **The strict correlation between the dynamics of water and that of biosystems should exist on each hierarchic level of time and space.**

Changes in the volume, geometry and electronic properties of the substrate molecule, resulting from its transition to the product, change its interaction energy with the active site by the magnitude:

$$\Delta E_{SP}^{a^*} = (E_S^{a^*} - E_P^{a^*}) \quad 59$$

It must destabilize the closed state of the active site and increase the probability of its reverse $[a^* \rightarrow b^*]$ transition.

Such transition promotes the last 5th stage of the catalytic cycle - the dissociation of the enzyme-product complex:



The resulting rate constant of this stage, like stage (II), is described by the generalized Stokes-Einstein and Eyring-Polany equation (42), but with different activation energy $G_{st}^{a^* \rightarrow b}$ valid for the $[a^* \rightarrow b]$ transition:

$$k_P^{a^* \rightarrow b} = \frac{kT}{\eta V} \exp\left(-\frac{G_{st}^{a^* \rightarrow b}}{kT}\right) = 1/\tau_P^{a^* \rightarrow b} \quad 61$$

If the lifetime of the (a^*) state is sufficiently long, then the desorption of the product can occur irrespective of $[a^* \rightarrow b]$ transition, but with a longer characteristic time as a consequence of its diffusion out of the active site's "closed state". The rate constant of this process (k_P^*) is determined by small-scale dynamics. It practically does not depend on solvent viscosity, but can grows up with rising temperature, like at the 3d stage, described by (44):

$$k_P^* = \frac{kT}{\eta_{a^*}^{\text{in}} \cdot v_P} \exp\left(-\frac{G_P^{a^*}}{kT}\right) = 1/\tau_P^* \quad 62$$

where $\eta_{a^*}^{\text{in}}$ is active site interior viscosity in the closed (a^*) state; v_P is the effective volume of product molecules; $P_{a^*} = \exp(-G_P^{a^*}/RT)$ is the probability of small-scale, *functionally important motions* necessary for the desorption of the product from the (a^*) state of the active site; $G_P^{a^*}$ is the free energy of activation of such motions.

Because the processes, described by the eq.(61) and (62), are independent, the resulting product desorption rate constant is equal to:

$$k_V = k_P^{a^* \rightarrow b} + k_P^* = 1/\tau_P^{a^* \rightarrow b} + 1/\tau_P^* \quad 63$$

The characteristic time of this final stage of enzymatic reaction is:

$$\tau_V = 1/k_V = \frac{\tau_P^{a^* \rightarrow b} \cdot \tau_P^*}{\tau_P^{a^* \rightarrow b} + \tau_P^*} \quad 64$$

This stage is accompanied by the relaxation of the perturbed AS domain and remnant protein

structure to the initial state.

After the whole reaction cycle is completed the enzyme gets ready for the next cycle. The number of cycles (catalytic acts) in the majority of enzymes is within the limits of $(10^2 - 10^4) s^{-1}$. It means that the $[a \rightleftharpoons b]$ pulsations of the active site cavities must occur with higher frequency as far it is only one of the five stages of enzymatic reaction cycle.

In experiments, where various sucrose concentrations were used at constant temperature, the dependence of enzymatic catalysis rate on solvent viscosity (T/η) was demonstrated (Gavish and Weber, 1979). The amendment for changing the dielectric penetrability of the solvent by sucrose was taken into account. There are reasons to consider stages (II) and/or (V) in the model described above as the limiting ones of enzyme catalysis. According to eqs.(43) and (61), these stages depend on (T/η) , indeed.

The resulting rate constant of the enzyme reaction could be expressed as the reciprocal sum of life times of all its separate stages (I-V):

$$k_{res} = 1/\tau_{res} = 1/(\tau_I + \tau_{II} + \tau_{III} + \tau_{IV} + \tau_V) \quad 65$$

where

$$\tau_I = 1/k_I = \frac{1}{v_{col} \cdot P_b}; \quad \tau_{II} = 1/k_{II} = \frac{\eta V}{kT} \exp\left(\frac{G_{st}^{b \rightarrow a}}{RT}\right); \quad 65a$$

$$\tau_{III} = 1/k_{III} = \frac{\eta^{in} v_S}{kT} \exp\left(\frac{G_{SS}^a}{RT}\right); \quad 65b$$

$$\tau_{IV} = 1/k_{IV} = \frac{1}{(v^p)} \exp\left[\frac{h(v^s - v^p - v^c)}{kT}\right];$$

τ_V corresponds to eq.(64).

The slowest stages of the reaction seem to be stages (II), (V), and stage (III). The latter is dependent only on the small- scale dynamics in the region of the active site.

Sometimes *product desorption* goes on much more slowly than other stages of the enzymatic process, i.e.

$$k_V \ll k_{III} \ll k_{II}$$

Then the resulting rate of the process (k_{res}) is represented by its limiting stage (eq. 63):

$$k_{res} \approx \frac{kT}{\eta V} \exp\left(-\frac{G_{st}^{a \rightarrow b}}{RT}\right) + k_p^* = 1/\tau_{res} \quad 66$$

The corresponding period of enzyme turnover: $\tau_{res} \approx \tau_V$ (eq 64). The internal medium viscosity (η^{in}) in the protein regions, which are far from the periphery, is 2-3 orders higher than the viscosity of a water-saline solvent (0.001 P) under standard conditions:

$$(\eta^{in}/\eta) \geq 10^3$$

Therefore, the changes of sucrose concentration in the limits of 0-40% at constant temperature can not influence markedly internal small- scale dynamics in proteins, its activation energy (G^{a*}) and internal microviscosity (Käiväräinen, 1989b). This fact was revealed using the spin-label method. It is in accordance with viscosity dependencies of tryptophan fluorescence quenching in proteins and model systems related to acrylamide diffusion in protein matrix (Eftink and Hagaman, 1986). In the examples of parvalbumin and ribonuclease T_1 it has been shown that the dynamics of internal residues is practically insensitive to changing solvent viscosity by glycerol over the range of 0.01 to 1 P.

It follows from the above data that the moderate changes in solvent viscosity (η) at constant temperature do not influence markedly the k_p^* value in eq.(66).

Therefore, the isothermal dependencies of k_{res} on $(T/\eta)_T$ with changing sucrose or glycerol

concentration must represent straight lines with the slope:

$$tg\alpha = \frac{\Delta k_{res}}{\Delta(T/\eta)_T} = \frac{k}{V} \exp\left(-\frac{G_{st}^{a^* \rightarrow b}}{RT}\right)_T \quad 67$$

The interception of isotherms at extrapolation to $(T/\eta \rightarrow 0)$ yields (62):

$$(k_{res})_{(T/\eta) \rightarrow 0} = k_P^* = \frac{kT}{\eta_{a^*}^{in} V_P} \exp\left(-\frac{G^{a^*}}{RT}\right) \quad 68$$

The volume of the Brownian particle (V) in eq.(67) corresponds to the effective volume of one of the domains, which reorientation is responsible for ($a \rightleftharpoons b$) transitions of the enzyme active site.

Under conditions when the activation energy $G_{st}^{a^* \rightarrow b}$ weakly depends on temperature, it is possible to investigate the temperature dependence of the effective volume V, using eq.(67), analyzing a slopes of set of isotherms (67).

Our model predicts the increasing of V with temperature rising. This reflects the dumping of the large-scale dynamics of proteins due to water clusters melting and enhancement the Van der Waals interactions between protein domains and subunits (Käiväräinen, 1985; 1989b, Käiväräinen et al., 1993). The contribution of the small-scale dynamics (k^*) to k_{res} must grow due to its thermoactivation and the decrease in η^{in} and G^{a^*} (eq.32).

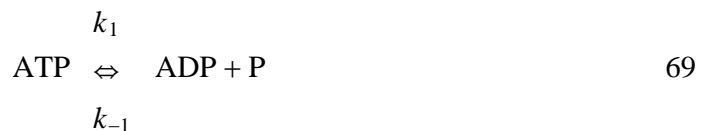
The diffusion trajectory of ligands, substrates and products of enzyme reactions in "closed" (a) states of active sites is probably determined by the spatial gradient of minimum wave B length (maximum impulses) values of atoms, forming the active site cavity.

We suppose, that *functionally important motions (FIM)*, introduced by H. Frauenfelder et al., (1985, 1988), are determined by specific geometry of the impulse space characterizing the distribution of small-scale dynamics of domains in the region of protein's active site.

The analysis of the impulse distribution in the active site area and energy of quantum beats between de Broglie waves of the atoms of substrate and active sites, modulated by solvent-dependent large-scale dynamics, should lead to complete understanding of the physical background of enzyme catalysis.

6. The mechanism of ATP hydrolysis energy utilization in muscle contraction and protein polymerization

A great number of biochemical reactions are endothermic, i.e. they need additional thermal energy in contrast to exothermic ones. The most universal and common source of this additional energy is a reaction of adenosinetriphosphate (ATP) hydrolysis:



The reaction products are adenosinediphosphate (ADP) and inorganic phosphate (P).

The equilibrium constant of the reaction depends on the concentration of the substrate [ATP] and products [ADP] and [P] like:

$$K = \frac{k_1}{k_{-1}} = \frac{[\text{ADP}] \cdot [\text{P}]}{[\text{ATP}]} \quad 70$$

The equilibrium constant and temperature determine the reaction free energy change:

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \quad 71$$

where: ΔH and ΔS are changes in enthalpy and entropy, respectively.

Under the real conditions in cell the reaction of ATP hydrolysis is highly favorable energetically as is accompanied by strong free energy decrease: $\Delta G = -(11 \div 13)$ kcal/M. It follows from (71) that $\Delta G < 0$, when

$$T\Delta S > \Delta H \quad 72$$

and the entropy and enthalpy changes are positive ($\Delta S > 0$ and $\Delta H > 0$). However, the specific molecular mechanism of these changes in different biochemical reactions, including muscle contraction, remains unclear.

Acceleration of actin polymerization and tubulin self-assembly to the microtubules as a result of the ATP and nucleotide GTP splitting, respectively, is still obscure as well.

Using our model of water-macromolecule interaction [6], we can explain these processes by the "melting" of the water clusters - librational effectons in cavities between neighboring domains and subunits of proteins. This melting is induced by absorption of energy of ATP or GTP hydrolysis and represents [lb/tr] conversion of primary librational effectons to translational ones. It leads to the partial dehydration and rapprochement of domains and subunits. The concomitant transition of interdomain/subunit cavities from the "open" B-state to the "closed" A-state should be accompanied by decreasing of linear dimensions of a macromolecule. This process is usually reversible and responsible for the large-scale dynamics.

In the case when disjoining clusterphilic interactions that shift the $[A \leftrightarrow B]$ equilibrium to the right are stronger than Van der Waals interactions stabilizing A-state, the expansion of the macromolecule can induce a mechanical "pushing" force.

In accordance to our model, this "swelling driving force" is responsible for shifting of myosin "heads" as respect to the actin filaments and muscle contraction.

Such **FIRST relaxation "swelling working step"** is accompanied by dissociation of products of ATP hydrolysis from the active sites of myosin heads (heavy meromyosin).

The SECOND stage of reaction, the dissociation of the complex: [myosin "head" + actin], is related to the absorption of ATP at the myosin active site. At this stage the $A \leftrightarrow B$ equilibrium between the heavy meromyosin conformers is strongly shifted to the right, i.e. to an expanded form of the protein.

The THIRD stage is represented by the ATP hydrolysis, ($ATP \rightarrow ADP + P$) and expelling of (P) from the active site. The concomitant local enthalpy and entropy jump leads to the melting of the water clusters in the cavities, $B \rightarrow A$ transitions and the contraction of free meromyosin heads.

The energy of the clusterphilic interaction at this stage is accumulated in myosin like in a squeezed spring.

After this 3d stage is over the complex [myosin head + actin] forms again.

We assume here that the interaction between myosin head and actin induces the releasing of the product (ADP) from myosin active site. It is important to stress that the driving force of "swelling working stage" : $[A \rightarrow B]$ transition of myosin cavities - is represented by our clusterphilic interactions (see Section 13.3 of [1] and paper [5]).

A repetition of such a cycle results in the relative shift of myosin filaments with respect to actin ones and finally in muscle contraction.

The mechanism proposed does not need the hypothesis of Davydov's soliton propagation (Davydov, 1984) along a myosin macromolecule. It seems that this nondissipative process scarcely takes place in strongly fluctuating biological systems. Soliton model does not take into account the real mesoscopic structure of macromolecules and their interaction with water as well.

Polymerization of actin, tubulin and other globular proteins composing cytoplasmic and extracellular filaments due to hydrophobic interaction can be accelerated as a result of their selected dehydration due to local temperature jumps in mesoscopic volumes where the ATP and GTP hydrolysis takes place.

The [assembly \leftrightarrow deassembly] equilibrium is shifted as a result of such mesophase transition to the left in the case when [protein – protein] interface Van-der-Waals interactions are stronger than a clusterphilic one. The latter is mediated by librational water effecton stabilization in interdomain or intersubunit cavities.

It looks that the clusterphilic interactions play an extremely important role on mesoscale in the self-organization and dynamics of biological systems.

7. Water activity as a regulative factor in the intra- and inter-cell processes

Three most important factors can be responsible for the spatial processes in living cells:

1. Self-organization of supramolecular systems in the form of membranes, oligomeric proteins and filaments. Such processes can be mediated by water-dependent hydrophilic, hydrophobic and introduced by us clusterphilic interactions [1, 5];

2. Compartmentalization of cell volume by semipermeable lipid- bilayer membranes and due to different cell's organelles formation;

3. Changes in the volumes of different cell compartments by osmotic process correlated in space and time. These changes are dependent on water activity regulated, in turn, mainly by [assembly \rightleftharpoons deassembly] equilibrium shift of microtubules and actin's filaments.

Deassembly of filaments leads to water activity decreasing due to increasing the fraction of vicinal water, representing a developed system of enlarged primary librational effectons near the surface of proteins (see section 13.5 of [1] and [5]). The thickness of vicinal water layer is about 50 Å, depending on temperature and mobility of intra-cell components.

The vicinal water with more ordered and cooperative structure than that of bulk water represents the dominant fraction of intra-cell water. A lot of experimental evidences, pointing to important role of vicinal water in cell physiology were presented in reviews of Drost-Hansen and Singleton (1992) and Clegg and Drost-Hansen (1991).

The dynamic equilibrium: $\{ I \leftrightarrow II \leftrightarrow III \}$ between three stages of macromolecular self-organization, discussed in Section 13.5 of [1] (Table 2) and in paper [5], has to play an important role in biosystems: blood, lymph as well as inter- and intra-cell media. This equilibrium is dependent on the water activity (inorganic ions, pH), temperature, concentration and surface properties of macromolecules.

Large-scale protein dynamics, decreasing the fraction of vicinal water (Käiväräinen, 1986, Käiväräinen et al., 1990) is dependent on the protein's active site ligand state. These factors may play a regulative role in [coagulation \leftrightarrow peptization] and [gel \leftrightarrow sol] transitions in the cytoplasm of mobile cells, necessary for their migration.

A lot of spatial cellular processes such as the increase or decrease in the length of microtubules or actin filaments are dependent also on their *self-assembly* from corresponding subunits (α , β tubulins and actin).

The self-assembly of such superpolymers is dependent on the [association (A) \leftrightarrow dissociation (B)] equilibrium constant ($K_{A \leftrightarrow B} = K_{B \leftrightarrow A}^{-1}$). In turn, this constant is dependent on water activity (a_{H_2O}), as was shown earlier (eq.13.11a and 13.12 of [1] and [5]).

The double helix of actin filaments, responsible for the spatial organization and cell's shape dynamics, is composed of the monomers of globular protein - actin (MM 42.000). The rate of actin filament polymerization or depolymerization, responsible for cells shape adaptation to environment,

is very high and strongly depends on ionic strength (concentration of $NaCl$, Ca^{++} , Mg^{++}). For example, the increasing of $NaCl$ concentration and corresponding decreasing of a_{H_2O} stimulate the actin polymerization. The same is true of α and β tubulin polymerization in the form of microtubules.

The activity of water in cells and cell compartments can be regulated by $[Na^+ - K^+]$ ATP –dependent pumps. Even the equal concentrations of Na^+ and K^+ decrease water activity a_{H_2O} differently due to their different interaction with bulk and, especially with ordered vicinal water (Wiggins 1971, 1973).

Regulation of pH by proton pumps, incorporated in membranes, also can be of great importance for intra-cell a_{H_2O} changing.

Cell division is strongly correlated with dynamic equilibrium: [assembly \Leftrightarrow deassembly] of microtubules of centrioles.. Inhibition of tubulin subunits dissociation (deassembly) by *addition of D_2O* , or *stimulation* this process by *decreasing temperature or increasing hydrostatic pressure* stops cell mitosis - division (Alberts et al., 1983).

The above mentioned factors enable to affect the $A \Leftrightarrow B$ equilibrium of cavity between α and β tubulins, composing microtubules. These factors action confirm our hypothesis, that microtubules assembly are mediated by clusterphilic interaction (see section 17.5 of [1] and paper [5]).

The decrease in temperature and increase in intra-microtubules pressure lead to the increased dimensions of librational water effectons, clustrons and finally this induce deassembly of microtubules.

Microtubules are responsible for the coordination of intra-cell space organization and movements, including chromosome movement at the mitotic cycle, coordinated by centrioles.

The communication between different cells by means of channels can regulate the ionic concentration and correspondent a_{H_2O} gradients in the embryo.

In accordance with our hypothesis, the gradient of water activity, regulated by change of vicinal water fraction in different compartments of cell can play a role of so-called morphogenic factor necessary for differentiation of embryo cells.

8. Water and cancer

We put forward a hypothesis that unlimited cancer cell division is related to partial deassembly of cytoskeleton's actin-like filaments due to some genetically controlled mistakes in biosynthesis and increasing the osmotic diffusion of water into transformed cell.

Decreasing of the intra-cell concentration of any types of ions (Na^+ , K^+ , H^+ , Mg^{2+} etc.), as the result of corresponding ionic pump destruction, incorporated in biomembranes, also may lead to deassembly of filaments.

The shift of equilibrium: [assembly \Leftrightarrow deassembly] of microtubules (MTs) and actin filaments to the right increases the amount of intra-cell water, involved in hydration shells of protein and decreases water activity. As a consequence of concomitant osmotic process, cells tend to swell and acquire a ball-like shape. The number of direct contacts between transformed cells decrease and the water activity in the intercell space increases also.

We suppose that certain decline in the external inter-cell water activity could be a triggering signal for the inhibition of normal cell division. The shape of normal cells under control of cell's filament is a specific one, providing good dense intercell contacts with limited amount of water, in contrast to transformed cells.

If this idea is true, the absence of contact inhibition in the case of cancer cells is a result of insufficient decreasing of intercell water activity due to loose [cell-cell] contacts.

If our model of cancer emergency is correct, then the problem of tumor inhibition is related to the problem of inter - and intra-cell water activity regulation by means of

chemical and physical factors.

Another approach for cancer healing we can propose here is the IR laser treatment of transformed cells with IR photons frequencies, stimulating superdeformons excitation and collective deassembly of MTs in composition of centrioles. This will prevent cells division and should have a good therapeutic effect. This approach is based on assumption that stability of MTs in transformed cells is weaker and/or resonant frequency of their superdeformons excitation differs from that of normal cells.

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