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HYSTERESIS WAY OF BIOLOGICAL SYSTEMS EVOLUTION FROM THE PERSPECTIVE OF NON-EQUILIBRIUM THERMODYNAMICS

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Keywords: membrane, protein, cell, hysteresis, non-equilibrium thermodynamics, protein self-organization.

Abstract. The paper investigates from the perspective of non-equilibrium thermodynamics the processes in biological systems such as cell membrane, single-domain and multi-domain globular protein, alga cell *Chara corallina*. State change process of these biological systems follows the same general evolution laws of non-equilibrium thermodynamics systems researched previously for physicochemical processes. It is noted that the lower to higher entropy generation state transitions of these biological systems and reverse transitions are realized through hysteresis. In the course of the transition, the membrane structure (gel or liquid-crystal state), globular protein (denatured or globular state) or alga cell structure, thermophysical and other parameters change dramatically. Transitions occur abruptly with the formation of unsteady structures. The self-organization process of single-domain and multi-domain proteins is described from the perspective of non-equilibrium thermodynamics. Thermodynamic analogues of these processes from “inanimate” nature are given.

ГИСТЕРЕЗИСНЫЙ СПОСОБ ЭВОЛЮЦИИ БИОЛОГИЧЕСКИХ СИСТЕМ С ПОЗИЦИИ НЕРАВНОВЕСНОЙ ТЕРМОДИНАМИКИ

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Ключевые слова: мембрана, белок, клетка, гистерезис, неравновесная термодинамика, самоорганизация белка.

Аннотация. Проанализированы с позиции неравновесной термодинамики процессы, протекающие в таких биологических системах, как мембрана клетки, однодоменный и многодоменный глобулярный белок, клетка водоросли *Chara corallina*. Показано, что процесс изменения состояний рассматриваемых биологических систем подчиняется одним и тем же общим закономерностям эволюции неравновесных термодинамических систем, выявленным для физико-химических процессов. Отмечается, что переходы в рассматриваемых биологических системах из стационарного состояния с меньшим производством энтропии в стационарное состояние с бóльшим производством энтропии и в обратном направлении реализуются через гистерезис. При переходах резко изменяются структура мембраны (гель-состояние или жидкокристаллическое), структура глобулярного белка (развернутое или глобулярное состояние) или клетки водоросли, а также теплофизические и другие параметры. Переходы осуществляются достаточно резко, скачкообразно с образованием нестационарных структур. Описывается процесс самоорганизации однодоменных и многодоменных белков с позиции неравновесной термодинамики. Приводятся термодинамические аналоги этим процессам из «неживой» природы.

Hysteresis has long been observed in "abiological" physical and chemical processes. In biological processes, hysteresis phenomena have been experimentally confirmed only with the advent of modern research methods.

Theoretical and experimental investigations [1–7] have demonstrated that hysteresis phenomena in fluid dynamics, Earth atmosphere, heat and mass transfer, magnetism, catalytic and other physical and chemical processes comply with the same general laws for a non-equilibrium thermodynamic system changing state. Hysteresis involves direct and reverse transitions between two quasi-stationary

states of a non-equilibrium thermodynamic system characterized by different entropy generation. These transitions cause changes in the structures corresponding to the states (for example, current and flow patterns, heat exchange pattern, composition and concentration of reaction products) and other parameters. Moreover, structure and parameters of a thermodynamic system, including entropy generation, change sharply, sometimes even intermittently.

It can be assumed that the revealed general patterns of change in the state of a non-equilibrium thermodynamic system are also applicable to biological systems. Under certain conditions, a multiplicity of stationary states with their own operation modes can be realized in open biological systems, similar to physical systems. A characteristic feature of biological systems is a number of ways for the system transition from one stable stationary state to another, with a different operation mode. In [8] various transition methods were described, the most significant being trigger method and hysteresis, which play an important role in the regulation of biological systems.

Hysteresis phenomena can be experimentally observed in a cell membrane and other membrane systems. Let us consider such a membrane as an example of a thermodynamic system. The packing mode of molecular components in membranes (the structures of a thermodynamic system), their mobility, the process kinetics and other properties can be studied using model bilayer lipid membranes (BLMs) made from natural lipids, for example, bovine brain phospholipids, similar to the biological membranes of the cell in their physical and chemical properties.

Investigation of lipid membranes using various physical methods, including electron microscopy X-ray diffraction, radio spectroscopy (electronic paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR)) and others, demonstrated that the membrane molecules are aligned. Phospholipid molecules are located in a double layer, and their hydrophobic tails and polar hydrophilic heads are orientated in a certain pattern. A physical state characterized by a certain alignment in the mutual orientation and arrangement of molecules, but with liquid aggregate state, is called a liquid crystal (“liquid”) state. Liquid crystals can be formed only in substances consisting of “long molecules” that are shorter in the transverse dimension.

As temperature decreases, transition of the phospholipid membrane from the liquid crystal state to the gel state conventionally referred to as solid crystal takes place. In the gel state, the molecules are more aligned than in the liquid crystal state (Fig. 1). All hydrophobic tails of phospholipid molecules in the gel state are fully stretched out strictly parallel to each other. In the liquid crystal state, due to thermal motion, the molecule tails are bent, their parallel alignment is offset, especially in the middle of the membrane. Since the alignment in the solid-crystal state is more ordered than in the liquid-crystal state, it can be assumed that the former is characterized by lower entropy. As the molecules’ tails can bend in the liquid crystal state, this state has more degrees of freedom in comparison with the solid crystal, and, therefore, higher heat capacity.

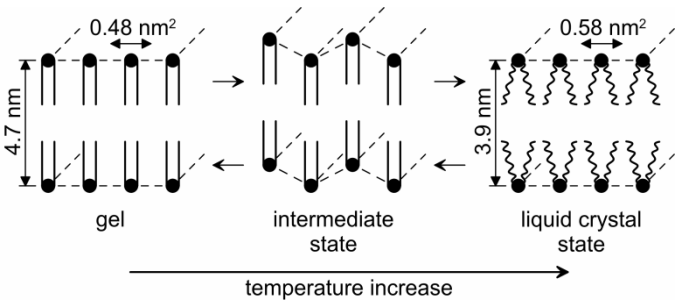

Fig. 1. Schematic of a dipalmitoylphosphatidylcholine membrane structure change (cross-section) during direct and reverse gel to liquid crystal transitions as a function of temperature

Transitions between the states of a model membrane under heating or cooling occur in a narrow temperature range and are characterized by a very sharp change in the system enthalpy. In this case, a shift in the transition temperature range is observed, recorded both during cooling and during subsequent heating of the system. Depending on the membrane system, the temperature hysteresis shift can reach 3–5 K to ~10 K under certain conditions [9]. Using various physical research methods, including differential scanning microcalorimetry, it was found that not only the enthalpy, but also the volume, entropy and other parameters of the thermodynamic system can change quite rapidly during transitions of the membrane structure from the liquid-crystal state to the solid-crystal state and back. For example, during the transition from the gel state to the liquid crystal state, the volume increases due to an increase in the membrane area per one molecule from 0.48 nm^2 to 0.58 nm^2.

For some phospholipid membranes, for example, dipalmitoylphosphatidylcholine (DPPC), intermediate stationary states with their own structure are formed during transition from the liquid crystal state to the gel and back. In this transition, a sharp change in the enthalpy, entropy, and other parameters also occurs in a certain temperature range (Fig. 2). Electron microscopy was used to establish that the bilayer in this state looks like a wavy surface (Fig. 1). The transition from the gel state to the intermediate state occurs at lower temperatures and is referred to as pre-transition. The transition from the intermediate state to the liquid crystal occurs at higher temperatures and is called the main transition. During such transitions, the change in the membrane properties occurs rather sharply in a certain temperature range. We assume that the point of transition from one state to another is the temperature at which the maximum (peak) heat capacity (points T_{C1} and T_{C2} for the corresponding transitions in Fig. 2) is reached during the transition. The heat of the structural transition at the selected point is assumed to be the total heat generated or absorbed during the transition (the shaded area under the curve in Fig. 2 of the corresponding transition). In the isobaric process under consideration, the heat of the structural transition is equal to the change in the enthalpy of the corresponding transition.

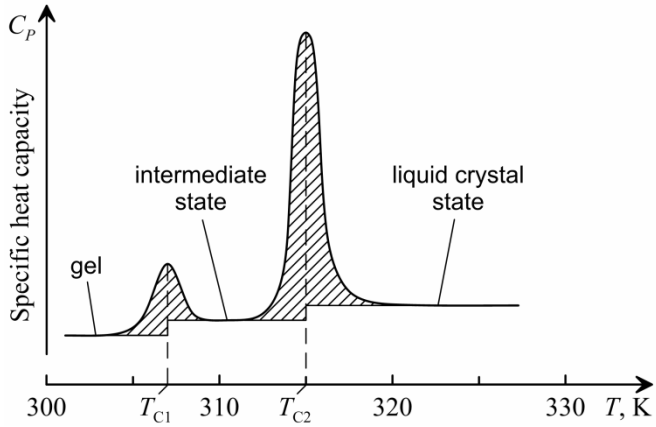

Fig. 2. Specific heat capacity C_P of DPPC dispersion as a function of temperature

Based on these assumptions and thermodynamics perspective we can conditionally view transitions between different membrane states as phase transitions of the first order [10] that are characterized by an abrupt change in a number of thermodynamic parameters. During the phase transition, volume and entropy and, as a consequence, the enthalpy, internal energy, and specific heat change abruptly.

Based on the assumptions made and the isobaric nature of the process, the dependences of the thermodynamic system entropy on temperature T for all three possible stationary states of the membrane are determined by the equations:

$$S_{\mathrm{G}} - S_0 = \int_{T_0}^{T} \frac{C_{\mathrm{G}}}{T}\, dT\,, \tag{1}$$

where S_{G} is the entropy of the system in the gel state; S_0 is the entropy value at the selected reference point T_0; C_{G} is the specific heat capacity at constant pressure of the gel state of the system;

$$S_{\mathrm{I}} - S_0 = \int_{T_0}^{T_{\mathrm{C1}}} \frac{C_{\mathrm{G}}}{T}\, dT + \frac{\Delta H_1}{T_{\mathrm{C1}}} + \int_{T_{\mathrm{C1}}}^{T} \frac{C_{\mathrm{I}}}{T}\, dT\,, \tag{2}$$

where S_{I} is the entropy of a system in the intermediate state; C_{I} is the specific heat capacity at constant pressure of the intermediate state of the system; ΔH_1 is the enthalpy increment during the transition from the gel state to the intermediate state;

$$S_{\mathrm{LC}} - S_0 = \int_{T_0}^{T_{\mathrm{C1}}} \frac{C_{\mathrm{G}}}{T}\, dT + \frac{\Delta H_1}{T_{\mathrm{C1}}} + \int_{T_{\mathrm{C1}}}^{T_{\mathrm{C2}}} \frac{C_{\mathrm{I}}}{T}\, dT + \frac{\Delta H_2}{T_{\mathrm{C2}}} + \int_{T_{\mathrm{C2}}}^{T} \frac{C_{\mathrm{LC}}}{T}\, dT\,, \tag{3}$$

where S_{LC} is the entropy of a system in the liquid crystal state; C_{LC} is the specific heat capacity at constant pressure of the liquid crystal state of the system; ΔH_2 is the enthalpy increment during the transition from the intermediate to the liquid crystal state. The calculations used relative entropy values reckoned from an arbitrary reference point. Since the temperature of phase transitions for membranes from DPPC and a number of other homogeneous lipids exceeds 273 K, we assume T_0 = 273 K for calculation purposes; S_0 = 0. We also assume that at temperatures of the

thermodynamic system that differ only slightly from the temperatures of structural transitions, the specific heat C_G, C_I, and C_{LC} remain constant. Then equations (1) – (3) after integration are written as:

$$S_G = C_G \ln\left(\frac{T}{T_0}\right), \quad (4)$$

$$S_I = C_G \ln\left(\frac{T_{C1}}{T_0}\right) + \frac{\Delta H_1}{T_{C1}} + C_I \ln\left(\frac{T}{T_{C1}}\right), \quad (5)$$

$$S_{LC} = C_G \ln\left(\frac{T_{C1}}{T_0}\right) + \frac{\Delta H_1}{T_{C1}} + C_I \ln\left(\frac{T_{C2}}{T_{C1}}\right) + \frac{\Delta H_2}{T_{C2}} + C_{LC} \ln\left(\frac{T}{T_{C2}}\right). \quad (6)$$

Based on [11] let us assume for the DPPC membrane: ΔH_1 = 9.63 kJ/mol; T_{C1}= 307 K; ΔH_2 = 40.57 kJ/mol; T_{C2} = 315 K.

When calculating the entropy change as a function of temperature for the three possible states of the thermodynamic system under consideration, we assume C_G = 110 J/(K·mol); C_I = 120 J/(K·mol); C_{LC} = 150 J/(K·mol). These values of specific heat capacities for the corresponding membrane states are selected with the account for changes in the specific heat of some polymeric substances used in commercial membranes. Depending on temperature, such polymers can be in a glassy, highly elastic, or liquid states. It should be noted that the adopted values of specific heat capacities do not affect the nature of the change in the entropy and specific generation of entropy (used below) as functions of temperature, and thus do not affect the conclusions below. The change in the nature of these dependences is affected only by the relations between the specific heat capacities of the three possible membrane states.

Fig. 3 shows specific entropy as a function of temperature for the gel (S_G), intermediate (S_I), and liquid crystal (S_{LC}) states of the considered thermodynamic system calculated according to (4) – (6). It can be seen that at the transition points T_{C1} from the gel state to the intermediate state and T_{C2} from the intermediate state to the liquid crystal state, entropy of the thermodynamic system increases sharply.

In previous works, for example [2, 4], it was shown that the magnitude and direction of the change in entropy generation for each of all possible states of the same non-equilibrium thermodynamic system can indicate the directions of evolution of the system and the sequence of its states. Entropy generation $\Pi S = dS/d\tau$ is the amount of entropy that occurs inside a thermodynamic system per time unit. Assuming that during the membrane's heating or cooling up to the transition points, the temperature changes at the same rate ν = 0.01 K/s, the entropy generation will be written as $\Pi S = \frac{dS}{d\tau} = \frac{dS}{dT}\nu$, given that $\delta\tau = \delta T/\nu$. Based on this and equations (4) – (6), we find the specific entropy generation as a function of temperature for the three possible states of the thermodynamic system under consideration:

$$\Pi S_G = \frac{C_G \nu}{T};\ \Pi S_I = \frac{C_I \nu}{T};\ \Pi S_{LC} = \frac{C_{LC} \nu}{T}. \quad (7)$$

Fig. 4. presents the graphs of these dependencies.

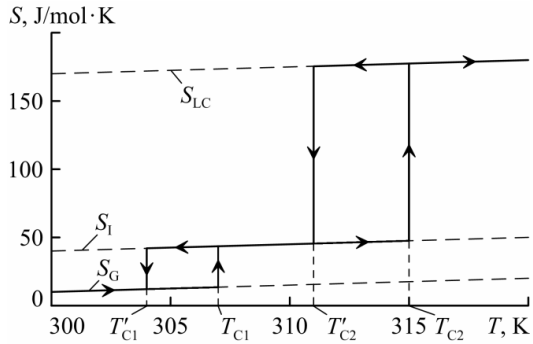

Fig. 3. Specific entropy change as a function of temperature for the possible states of the system

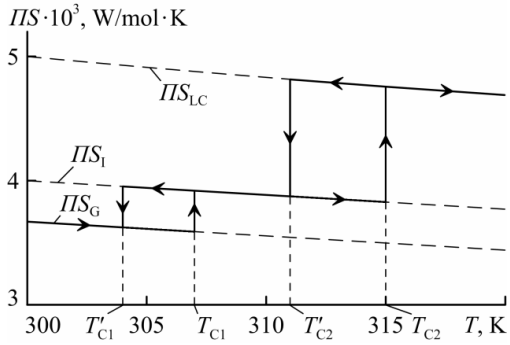

Fig. 4. Specific entropy generation as a function of temperature for the gel (ΠS_G), intermediate (ΠS_I) and liquid crystal (ΠS_{LC}) states of system

In accordance with the general laws of changes in non-equilibrium thermodynamic states revealed in [1, 2], *the transition of a system from a stationary (quasi-stationary) state with a lower entropy generation to a stationary state with a higher entropy generation can occur only if in the process of its evolution, a physical condition is not fulfilled, preventing the existence of the initial state. The transition of a thermodynamic system from a higher to a lower entropy state can be achieved only if in the process of its evolution, the difference in the entropy generation of these two states reaches a certain positive value. Direct and reverse transitions between two stationary states of a non-equilibrium thermodynamic system are characterized by hysteresis. These transitions are accompanied by changes both in the structures characteristic of these states (in this case gel, intermediate or liquid crystal states), as well as thermodynamic and other parameters.*

In the case under consideration, a physically justified condition that determines the transition at the increased temperature from the state with a lower generation of entropy (gel state) to the intermediate state with a higher generation of entropy is the property of membranes of some lipids, including DPPC, to change the location of hydrocarbon chains at the temperature T_{C1}. This leads to the structure of the membrane becoming distorted and the flat layers becoming wave-shaped (Fig. 1). With a further increase in temperature up to T_{C2}, the membrane structure changes again and the intermediate state transforms into a liquid crystalline state, which has a higher entropy generation compared to the intermediate state. These transitions are indicated in Fig. 4 by vertical lines with an upward arrow.

According to the discovered laws and the axiom of the perfection of the natural processes [12], in transition from a lower entropy generation state to a higher entropy generation state, if several stationary states are possible, a thermodynamic system first assumes the state with the lowest value of entropy generation of all possible transition states, provided that physical conditions permit this. In the case under consideration, a transition from the gel state to the intermediate one occurs. If the physical conditions do not allow this, then the

system will go into the next state, which has a minimum entropy generation of all the remaining states that a non-equilibrium thermodynamic system can assume. For example, for some lipids other than DPPC, the membrane molecules due to certain physical conditions cannot have the wave-shaped surface corresponding to the structure of the intermediate state of the thermodynamic system (Fig. 1). In this case, with increasing temperature, the gel state at T_{C2} becomes liquid crystal state, because the intermediate state cannot be realized due to certain reasons.

With decreasing temperature of the liquid-crystal thermodynamic system, the difference in the entropy generation ($\Pi S_{LC} - \Pi S_I$) increases and at temperature T'_{C2} reaches a certain positive value of liquid crystal to intermediate state transfer. This transition is represented in Fig. 4 by a vertical line with a downward arrow. If the temperature continues to increase after that, the reverse transition will occur at $T_{C2} > T'_{C2}$. Direct and reverse transitions exhibit hysteresis as noted for example in [9]. Similarly, in accordance with the general laws of non-equilibrium thermodynamic system evolution, at temperature T_{C1} transition from the gel state to the intermediate state should occur and the reverse transition from the intermediate state to gel should occur at $T'_{C1} < T_{C1}$. The realization of this transition is experimentally confirmed in [13]. Fig. 5 shows the experimental data of the spin-spin relaxation time as a function of temperature upon heating and cooling of the system obtained with the proton magnetic resonance method. It can be seen that the heating and cooling curves form hysteresis in the region of transitions from the gel state to the intermediate and vice versa. These results were well reproduced using the EPR and NMR spectroscopy.

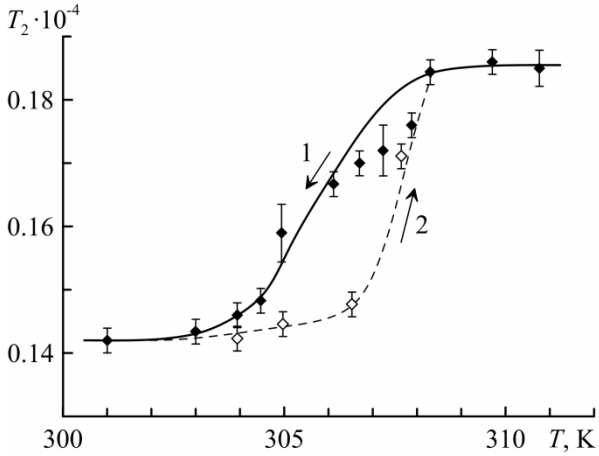

Fig. 5. Spin-spin relaxation time as a function of temperature (1 – cooling; 2 – heating) of DPPC water dispersions

In the process of transition of a non-equilibrium thermodynamic system from one stationary state to another, unsteady (unstable) structures are formed, as was shown in previous works [1–6].

Thus, in Rayleigh-Benard convection, a thermodynamic system transiting from a stationary state with a honeycomb fluid motion structure to a roll flow

stationary state forms some non-stationary structures. A detailed description of these nonstationary flow structures is given, for example, in [14].

The transition of the laminar gas flow in the pipe to a stable turbulent flow also occurs through non-standard flow structures in a certain narrow range of Reynolds numbers, and there is a continuous fluctuation both in time and in space of the turbulent and laminar gas flow regions [1].

When the nucleate boiling of free volume water, which corresponds to a lower entropy generation, evolves into film boiling, which corresponds to a higher entropy generation, various combinations of film and nucleate boiling modes form and randomly replace each other in the heat transfer surface [2].

The unsteady nature of transition structures formed in periodic chemical reactions has been experimentally proved (for example [6]). Such transitions also occur in other physical chemical processes. Moreover, the non-stationary structures formed during the transition can represent randomly varying in time and space combinations of stationary structures characteristic of the beginning and end of the process. However, non-stationary structures can also be formed that differ from stationary structures.

It follows from all of the above that in the temperature ranges of the direct and reverse transitions between gel-intermediate and intermediate-liquid crystal states, unsteady structures should be formed, which may differ from stationary ones. According to [11], both bilayer regions corresponding to the liquid crystal structure and solid crystal regions are formed within this temperature range. Flawed packing of hydrocarbon chains at the boundaries of these sections determines the phenomena occurring in the transition zone. In addition, through the transition from the liquid crystal to the gel state in the lipid bilayer, channels (pores) with a radius of 1–3 nm are formed [15]. As a result, the ionic conductivity of the membrane changes sharply at transition temperatures.

The non-stationary nature of the structures formed within the transition temperature range is indicated by the appearance of jump-like current fluctuations during transitions. The experimental current phase diagrams of flat BLM from DPPC presented in [16] show that at temperatures corresponding to the steady liquid crystal state and temperatures below the pre-transition corresponding to the gel state, current fluctuations are practically absent, and its value is close to zero. The region of transition from one state to another is accompanied by random current surges of various sizes and durations. All this indicates that in the process of transition from one stationary structure to another in the thermodynamic system, unsteady, chaotically changing structures are formed.

Biological membranes possess an even greater variety of non-stationary structures with the unique properties. This is due to the fact that the lipid layer of biological membranes is formed by various lipid mixtures (phospholipids, glycolipids, etc.). Individual phospholipids of biological origin are also a mixture of compounds that have the same polar “head” and different hydrocarbon chains, so state transitions in membranes always occur in a heterogeneous system in a wide temperature range. For example, in some natural lipids, the transition temperature

range reaches 40 K [11]. The composition of biological membranes also includes other chemical compounds (proteins, water, cholesterol, etc.). With a prolonged decrease in ambient temperature, a change in the chemical composition of the biological membrane is observed, which also provides an extension of the transition temperature range. The transition temperature decreases for example due to an increase in the number of unsaturated bonds in the tails of lipid molecules

In “in vitro” experiments, reverse structural rearrangements are also observed in proteins when exposed to temperature or denatured alcohol (for example, urea or an aqueous solution with a different pH value). The decomposition of the initial (“solid”) protein structure and its self-re-organization occurs also in a living cell (“in vivo”), for example, during the transport of proteins through membranes.

Before analyzing structural transitions in a protein from the perspective of non-equilibrium thermodynamics, we will briefly consider the composition and possible structures of a protein [17]. A protein is based on a polymer consisting of amino acid residues of the polypeptide chain. The number of links of the protein chain can vary from several dozens to many thousands. In a “functioning” protein, its chain is folded in a strictly defined way. Of all the protein classes, we will further consider water-soluble globular proteins as the most researched proteins to date. Proteins are distinguished from ordinary molecules primarily by their size. The smallest proteins have a molar mass of the order of tens of thousands, but for some it reaches a million. Proteins can comprise tens or even hundreds of thousands of different atoms, so they are often called macromolecules. The prefix "macro" emphasizes that they possess the properties of large, macroscopic systems, and therefore must comply with the general laws of thermodynamics. Protein molecules have an ordered structure. Each atom in this macroscopic system occupies a certain position in space, similar to a crystal. However, in proteins, unlike crystals, there is no regularity in the arrangement of atoms, no established symmetry.

Small proteins are formed by their polypeptide chain laid in a compact globule 25–40 Å in diameter. Larger proteins are made up of several such sub-globules called the domain. The protein chain is relatively compactly packed into the globule. A protein with such a structure is called a native protein. It has been experimentally shown that denaturation of small proteins (thermal or denaturant-induced) leads to the transition of its structure to a fully developed (denatured) state (Fig. 6). This transition is accompanied by a sharp change in many characteristics of the protein (density, viscosity, fluorescence, heat capacity, and a number of others) and is conditionally referred to as “melting”. For example, the density of a protein decreases significantly when it transits to a denatured state.

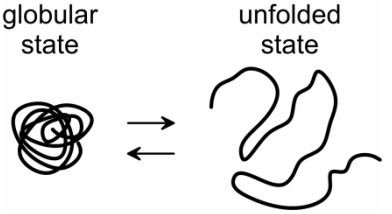

Fig. 6. Schematic of a single-domain protein folding and unfolding

Fig. 7 shows microcalorimetry of specific heat at constant pressure C_P of a single-domain (small) protein as a function of temperature [18]. The peak in the curve is determined by the maximum heat absorption during the macromolecule transition from the native state to the denatured state. T_C is the calculated transition temperature ("melting point") considered as for a membrane; ΔT is the transition width; the shaded area under the curve is the heat absorbed during the transition in terms of a mole of protein. The dependence of C_P from T in Fig. 7 is similar to the dependence shown in Fig. 2 for the main transition of the membrane from an intermediate state to a liquid crystalline state. Therefore, the dependences of the entropy change and specific entropy generation upon the transition of a single domain protein from the native state to the denatured state will be similar to the dependences shown in Fig. 3 and Fig. 4 for the transition of a membrane from an intermediate state to a liquid crystalline state, since the same equations are used to construct these dependences.

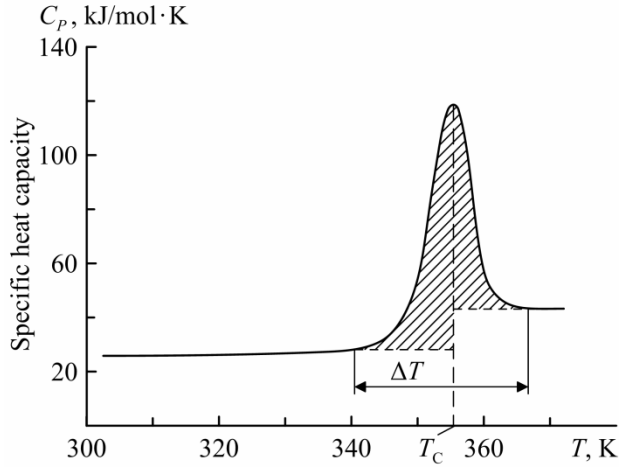

Fig. 7. Specific heat capacity of a single-domain protein – myoglobin in aqueous solution with pH = 10

Based on the previous analysis of non-equilibrium thermodynamic systems [1–6], the transition of a protein from the native state to the denatured state should occur through unstable structures that randomly change in time and space. And since the transition process occurs in a narrow temperature range and in a short period of time, it seems that there are only two stationary states and at a certain moment either one or the other is realized. Therefore, such a transition was termed as "all-or-nothing".

If the protein polypeptide chain unfolds and loses its ordered configuration during denaturation, the protein can fold back to its original configuration when favorable conditions are restored. In this case, the unfolding and folding processes are realized through non-stationary states and are accompanied by hysteresis. This follows from the thermodynamic analysis of various physical chemical processes, including those in the membrane described above. Moreover, the protein refolded into the initial three-dimensional configuration (globule) completely restores its functions. From the perspective of thermodynamics, it signifies a non-equilibrium

transition from a higher entropy state to a lower entropy state in accordance with the axiom about the perfection of natural processes [11]:

Of the all possible stationary states of a non-equilibrium thermodynamic system complying with the laws of nature, irreversible thermodynamics, boundary and other physical conditions, the state with the minimally possible entropy generation is the most likely.

This axiom has now been tested for many physical chemical processes [1–7].

A kind of a thermodynamic "abiotic" analogue of the protein folding process is the process of capture of a certain volume of air with a fixed mass by a sea wave ([1], Problem 2). When a non-equilibrium process of transferring into a stationary state occurs in such a thermodynamic system, of all the various shapes that the captured air volume of air can take (for example, toroidal, egg-shaped, disk-shaped, and many other configurations), only the spherical configuration corresponds to the minimum entropy generation, which is observed in reality as air bubbles in the water.

Hysteresis effects have been experimentally confirmed in other biological macromolecules, such as DNA and RNA, which have a biopolymer three-dimensional protein-like structure. It was experimentally established in [19] that the renaturation rate of DNA macromolecules is maximum at the temperature about 25 K below its "melting" temperature T_C, when denaturation rate is at maximum. Hysteresis effect is especially noticeable when measuring the optical properties of a solution. The experimental results presented in [19] show that the degree of renaturation strongly depends on the cooling rate. If the temperature of the solution is sharply reduced from a value lying well above the "melting point" to a value lying well below T_C, the probability of complete renaturation is very small. In this case, the intra-chain secondary structure formed during cooling will be very stable and the restoration of the native structure will be completely blocked. It should be noted that some steels behave in a similar way, changing their structure upon heating to a certain temperature, and then rapid cooling. Moreover, in some metal alloys, thermal hysteresis also appears during heat treatment (for example, [20]).

The results of atomic force microscopy experiments in stretching a DNA molecule are presented in [21]. In this case, a hysteresis of the applied force was observed during the stretching of the molecule and its subsequent relaxation. Also in [21] for the P5abc domain of the *Tetrahymena thermophila* ribozyme, experimental dependences of its unfolding strength under tension are presented, with the subsequent transition to the domain folding upon relaxation. It was shown that the processes of the domain unfolding and folding are also realized through hysteresis.

Only small proteins can comprise one domain. Most proteins contain more than one domain, while the largest proteins can comprise dozens. Each domain in a protein behaves more or less independently of the rest of the macromolecule when its structure changes. Therefore, the native structure of a large protein decomposes in discrete steps, absorbing discrete portions of energy of the order of the domain fusion heat. Each step corresponds to the destruction of a domain in a certain region of the macromolecule, while all such regions are destroyed or restored similarly to a single domain protein.

Fig. 8 shows the specific heat capacity C_P of myosin as a function of temperature obtained by scanning microcalorimetry [22]. The numbers indicate the sequence of "smelting" domains in this protein. The schematic arrangement of domains in the macromolecule is shown in the upper part of Fig. 8. A similar study of other multi-domain proteins confirmed that with increasing temperature, their native structures also decompose in discrete steps. Fig. 8 demonstrates that the heat capacity of the denatured domains ($C_{P,1} - C_{P,6}$) are greater than the heat capacities of the native protein $C_{P,N}$ increasing stepwise each time another domain is unfolded.

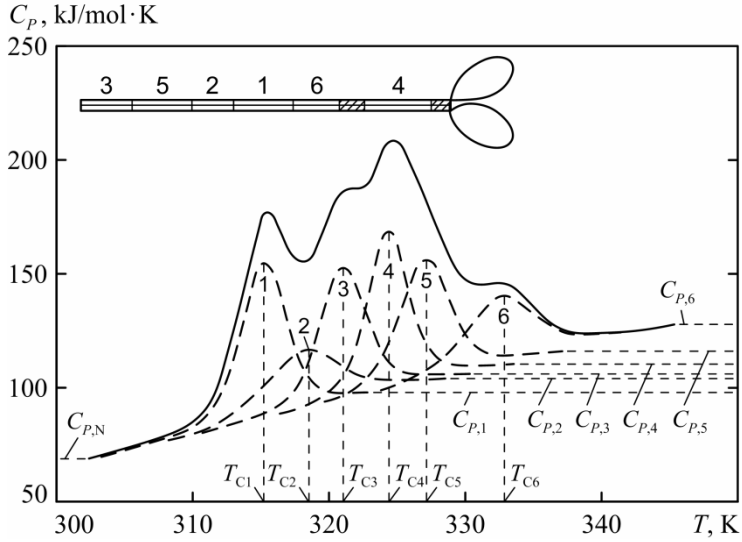

Fig. 8. Specific heat capacity of myosin as a function of temperature and a schematic of the macromolecular structure

To simplify the calculation, we will further assume that at the initial moment there was a single-domain macromolecule for which the dependence $C_P = f(T)$ corresponded to curve No.1 in Fig. 8. Using the above formulas obtained in the analysis of membrane states, we find the specific entropy generation for native ΠS_N and denatured ΠS_1 states of a single domain protein (Fig. 9). In this case, the transition of the macromolecule to the unfolded (denatured) state occurs at the domain melting temperature T_{C1} and is shown in Fig. 9 as a solid line with an upward arrow.

In accordance with the assumption in [18], we suppose that during evolution, the initial protein combined with another independent single-domain protein corresponding to curve No. 2 in Fig. 8. As a result, a two-domain protein was formed, which has a heat capacity $C_{P,2}$ in a fully denatured state and which is shown in Fig. 9 as the calculated specific entropy generation ΠS_2. In this two-domain protein, the transition from the native state to the fully denatured state will take place in two stages in accordance with the above basic principles of the evolution of non-equilibrium thermodynamic systems. The state of a thermodynamic system with a lower entropy generation ΠS_N (globule structure) can transit into a state with a higher entropy generation ΠS_1 or ΠS_2 only if such conditions are realized that prevent a state with a minimum entropy generation. Such a condition is the heating

of the protein to the "melting point" of the first domain in the T_{C1} globule. In this case, in accordance with the above provisions of non-equilibrium thermodynamics, in the presence of several such possible states, the transition occurs to the lowest entropy generation state of all states possible, i.e. to a state with ΠS_1. As the temperature continues to increase to T_{C2}, the system changes from a state with ΠS_1 entropy generation to a state with higher entropy generation ΠS_2 corresponding to the fully unfolded state of domain No. 2 (two-domain protein). This transition is plotted as a solid line with an upward arrow in Fig. 9. After that, a third domain joins the two-domain macromolecule. This reasoning is used to build the entire chain of the transition of the multi-domain protein under consideration from the native state (globule) to the fully denatured (unfolded) state. Fig. 9 shows the entire transition chain in terms of the specific entropy generation – temperature. The process of a globule unfolding is plotted as solid lines with an upward arrow.

Protein folding occurs in the reverse order. Moreover, according to the axiom of the perfection of natural processes, a series of transitions happen from a higher entropy generation state to the lowest possible entropy generation state. Moreover, complying with all of the above, direct and reverse transitions occur through hysteresis. Reverse transitions are plotted in Fig. 9 as dashed lines with a downward arrow. This illustrates self-organization of protein during folding from the perspective of non-equilibrium thermodynamics.

The dependences in Fig. 9 are constructed under the assumption that the estimated rate of the protein temperature change ν allows the non-equilibrium thermodynamic system under consideration to transit into a stationary (quasi-stationary) state. This means that at the estimated velocity ν and lower, the specific heat capacities as a function of temperature will remain unchanged.

A thermodynamic "abiotic" analogue for the case under consideration is the Rayleigh-Benard convection in the horizontal layer of a liquid when heated from below. Depending on the heating temperature, and more precisely on the Rayleigh number, various stationary stable states of fluid motion occur in the layer in the form of: honeycomb Benard cells; two-dimensional cells "rolling" in opposite directions; three-dimensional cells and other patterns (for example, [14]). With heating of the liquid layer increases, similar to a multi-domain protein, a transition is made from a state with a lower entropy generation to a state with a higher entropy generation, and the structure of the fluid flow changes. The transition from one stationary (stable) flow structure to another occurs through non-stationary (unstable) flow structures. With a decrease of the heat flux into the thermodynamic system under consideration, and hence the Rayleigh number, its reverse transition from a high entropy generation stationary state to a lower entropy generation stationary state occurs at lower Rayleigh numbers than does direct transition. Thus, transitions between two stationary states of a non-equilibrium thermodynamic system demonstrate hysteresis. Moreover, of the all possible types of two and three-dimensional structures of the fluid flow only those structures can be realized that correspond to the stability conditions, and from these stable states only those that comply with the laws of irreversible thermodynamics.

To compare the differences in specific heat capacity change as a function of temperature for multi-domain proteins (Fig. 8) and single-domain proteins, Fig. 10 shows the melting curves of barnase in solutions with different pH, with the values indicated above each curve [23]. It is evident from Fig. 10 that despite a significant increase in the melting temperature of the protein with increasing pH, its heat capacity in the denatured state remains practically unchanged. This confirms that the protein under consideration consists of one domain and in accordance with the calculations using the above formulas has two values of entropy generation, the smaller corresponding to the native state and the greater value to the denatured state.

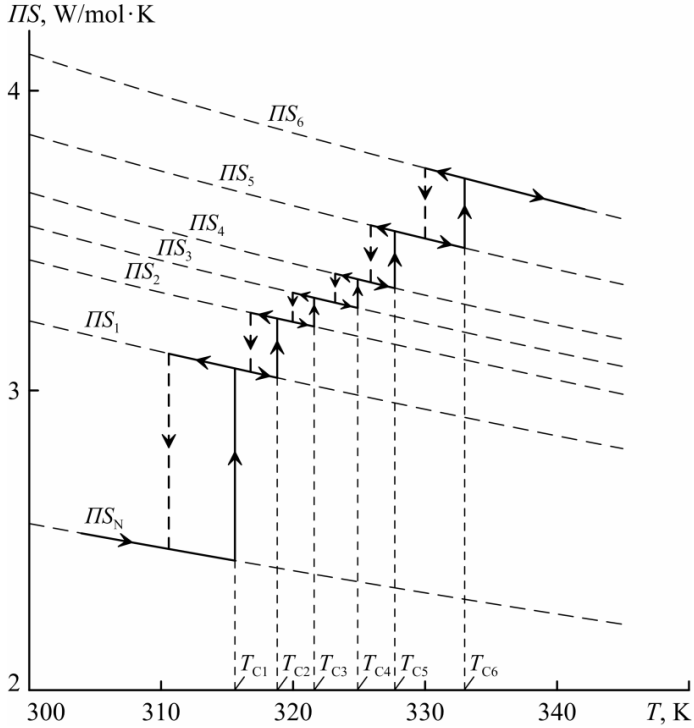

Fig. 9. Change in specific entropy generation during protein unfolding (solid lines) and folding (dashed lines)

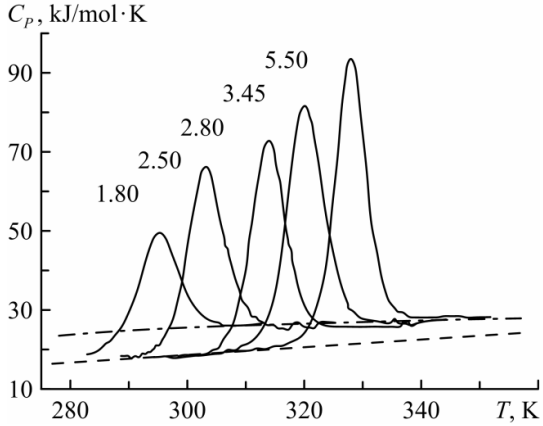

Fig. 10. Specific heat capacity of barnase as a function of temperature in solutions with different pH

Hysteresis phenomena are also observed in plant cells. It was shown in [24] that under illumination of a cell of the *Chara corallina* algae, zones with different pH and transmembrane potentials may appear along its surface. In this case, the difference ΔpH in the pH values of fully formed zones can reach 2.5–3 units. It was experimentally observed that in a certain range of illumination there can exist two stable types of pH distribution along the cell surface: homogeneous pH and fully formed longitudinal profile pH. Each of them can be realized at the same light intensity value, depending on whether its intensity had been increased or decreased in the experiment before. This indicates a hysteresis effect inherent to the ongoing process in the non-equilibrium thermodynamic system under consideration.

Fig. 11 shows the normalized difference in ΔpH values as a function of light intensity when illuminating the algae cell. In the illumination range up to ~1 W/m^2, hysteresis is observed in the ΔpH distribution near the cell membrane. With a stepwise increase in the light intensity from zero (light circles), a homogeneous longitudinal pH profile with $\Delta pH \approx 0$ is maintained. When the light intensity approaches $\sim 10^{-1}$ W/m^2, a sharp transition to the second stable pH profile with $\Delta pH \approx 1.0$ occurs. With a further increase in light intensity, this profile does not change. With a decrease in light intensity (dark squares), the second pH profile is maintained up to a light intensity of $\sim 2 \cdot 10^{-3}$ W/m^2, followed by a sharp ("jump-like") transition to the first homogeneous pH distribution profile.

It follows from Fig. 11 that with an increase in light intensity up to ~1 W/m^2, the algae cell sharply starts photosynthesis process. In this case, the entropy generation in the non-equilibrium thermodynamic system under consideration increases sharply, reaches its maximum value and then remains practically unchanged. This state corresponds to a stationary (quasi-stationary), stable second profile of the longitudinal pH distribution, which corresponds to $\Delta pH \approx 1.0$ in Fig. 11. With a further decrease in the light intensity reaching $\sim 10^{-3}$ W/m^2, the cell "falls asleep", which leads to a jump-like realization of a homogeneous longitudinal pH distribution, which corresponds to $\Delta pH \approx 0$. At the same time, entropy generation decreases sharply reaching minimum values at a new steady state. Based on the above, it follows that the processes occurring in an algae cell comply with the evolutionary principles of non-equilibrium thermodynamic systems analyzed above.

Natural processes are mainly non-equilibrium. Unlike physical chemical processes, equilibrium in a biological system is tantamount to its death. All living systems are non-equilibrium. Classical thermodynamics describes equilibrium processes and is essentially thermostatics. The main provisions of non-equilibrium thermodynamics for processes that only slightly deviate from the thermodynamic equilibrium state were formulated in the second half of the twentieth century and are presented in linear non-equilibrium thermodynamics, a natural development of equilibrium thermodynamics. However, its provisions are not applicable to processes far removed from the equilibrium state.

Experimental and theoretical research allowed identifying the general change patterns in the non-equilibrium thermodynamic systems where physical chemical processes occur. These patterns were applied to a number of biological systems.

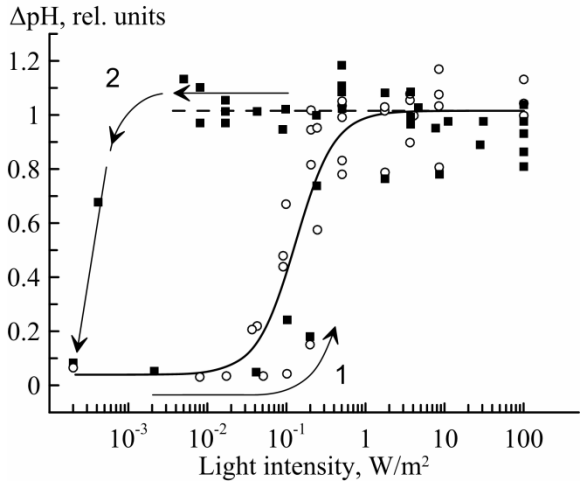

Fig. 11. Hysteresis in pH distribution near the *Chara coralline* algae cell membrane (⇑ – increased intensity; ⇓ – decreased intensity)

In conclusion it should be noted that from the perspective of non-equilibrium thermodynamics, the processes in biology and abiology are subject to the same experimentally confirmed general laws (rules) of changes in the states of non-equilibrium thermodynamic systems given in [1, 2, 12].

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