L.A. Blumenfeld

Physics of Bioenergetic Processes

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With 30 Figures

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Foreword

According to its definition, synergetics is concerned with the cooperation of individual parts of a system that produces macroscopic temporal, spatial or functional structures. A good deal of the volumes published within this series dealt with the formation of truly macroscopic structures which we can see with our eyes. A common scheme could be developed to understand the formation of many patterns through self-organization. In particular, we have to use concepts which go beyond conventional thermodynamics. New ideas became crucial. We have to study kinetic processes, and often few highly excited degrees of freedom play the decisive role in the evolution of structures. Over the past years it has turned out that quite similar lines of approach apply to a world which at first sight would be classified as "microscopic". That world consists of processes in which biomolecules are involved. An important example for the problems occurring there is provided by Manfred Eigen's theory of evolution of life at the molecular level (cf. his contribution to Volume 17 of this series). Another important example has been provided by Blumenfeld's book on problems of biological physics (Vol.7 of this series). There it was proposed to treat biological molecules as machines which, in a certain sense, work through "macroscopic" degrees of freedom. The inadequacy of concepts of equilibrium thermodynamics or irreversible thermodynamics was pointed out and the processes were studied in detail through kinetic models. This line of approach is again taken up in the present book by L.A. Blumenfeld, which deals with various aspects of bioenergetic processes such as muscle contraction, active transport of ions, substrate and membrane phosphorylations. He treats proteins as "molecular machines" and stresses the importance of nonequilibrium states of proteins. The important feature of Blumenfeld's book consists in introducing physical concepts into the bioenergetic processes of molecules whereby he sheds new light on enzymatic catalysis.

In view of the extremely complex processes which go on in living matter some of Blumenfeld's ideas might need further elaboration. But his approach opens new vistas in the treatment of these processes and a study of his book seems to be a must for all those who try to understand this kind of processes. For these reasons I am very happy that we can include Blumenfeld's book in the Springer Series in Synergetics.

Stuttgart, June 1983 *Hermann Haken*

Acknowledgement

Over the recent years the subject matter of this monograph has been repeatedly discussed with my coworkers and colleagues. These discussions were extremely useful and did help me to remove many errors (not all of them, I'm sure) and to formulate more clearly the critical and the positive statements.

I wish to express my deep gratitude to B. Cartling, A. Ehrenberg (Arrhenius Laboratory, Stockholm University), D.Sh. Burbajev, M.G. Goldfeld, R.M. Davydov, A.F. Vanin (Institute of Chemical Physics, USSR Academy of Sciences), L.V. Jakovenko, V.I. Pasechnik, A.N. Tikhonov, V.A. Tverdislov (School of Physics, Moscow University), L.I. Boguslavsky, Yu.A. Chismadzhev (Institute of Electrochemistry, USSR Academy of Sciences), D.S. Chermavsky (Physical Institute, USSR Academy of Sciences).

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Moscow, March 1983 *L.A. Blumenfeld*

Contents

1. Introduction

Achievements of biological chemistry in recent decades are striking. This is true not only for current popular molecular genetics but for all branches of science dealing with chemical compounds of living matter and their transformations. In many laboratories throughout the world highly qualified scientists use first-class physical instruments and sophisticated chemical and biological techniques to isolate, purify, and characterize low- and high-molecular compounds responsible for the functioning of the chemical machinery of cell and tissues, in order to determine the chemical mechanisms and the kinetic and thermodynamic parameters of biochemical processes.

All this also holds true for that branch of biochemistry (or biophysics, biology, physicochemical biology, biophysical chemistry, molecular biology – the number of different names denoting one and the same thing grows exponentially with the number of scientists and scientific publications), which is the subject matter of this book. The volume of material bearing relation to bioenergetic processes is becoming truly immense.

At the same time reading of original articles, reviews, and monographs dealing with the mechanisms of muscle contraction, active transport of ions, or membrane phosphorylation gives one not only an almost aesthetic pleasure (always produced by descriptions of beautiful experiments) but a deep feeling of dissatisfaction as well. This situation was already exhaustively described in 1947 by SZENT-GYORGUI [1.1] who said that as we knew more and more about muscle contraction we understand less and less, and that we should soon know everything and understand nothing. The situation has not improved up to now. This becomes clear, e.g., if we consider the processes of membrane phosphorylation, i.e., the most important processes of transduction and accumulation of light quanta energy in plants and photosynthetic bacteria, and of food oxidation energy in the mitochondria of animals and microbes.

Unbiased scrutiny of existing theories reveals their intrinsic contradictions and the impossibility to conform them to experimental data. The best illustration of this can probably be found in the presently most popular chemiosmotic concept of membrane phosphorylation mechanism proposed in 1961 by MITCHELL [1.2]. This concept was not readily accepted by scientific public opinion.[1] (Footnote 1 see next page.) At first the chemiosmotic approach seemed to be too revolutionary and unwanted as

compared with the orthodox postulates of chemical concept popular in those days. Experimental data, which could be explained by Mitchell's hypothesis, were, however, gradually accumulated, some predictions of this hypothesis were verified in several laboratories, and in the 1970s the chemiosmotic concept became quite respectable and was universally accepted.

I fully understand that Mitchell's concept was extremely helpful in stimulating a great number of beautiful experiments which essentially enlarged our knowledge of the membrane transfer of protons and other particles, i.e., of the processes whose importance in the regulation of intracellular electron transport and energy transduction is beyond doubt. I have always thought, however, that the physical fundamentals of this concept (i.e., of the postulate that the energy source for membrane phosphorylation is the transmembrane electrochemical potential gradient) are rather doubtful. Detailed examination of the chemiosmotic concept, and its relation to experimental data can, at the same time, help us provide an answer to a more general and probably more important question: what is the reason for theoretical flimsiness of modern biochemistry when its experimental and practical achievements are so remarkable. This reason may be formulated as follows: twentieth century biochemistry uses for theoretical description of biochemical processes nineteenth century physical chemistry, i.e., the concepts developed to describe the behavior of low-molecular compounds in gaseous phase and dilute solutions. In thermodynamics this means the unreserved use of the Van't Hoff equation, and in kinetics of the Arrhenius equation (or, which is the same, of the activated complex theory). I am speaking, of course, not only about the formal use of corresponding mathematical expressions in calculating the thermodynamic and kinetic parameters of chemical reactions, but about the acceptance of postulates underlying these expressions, and the acceptance of the physical models of processes for which these expressions have been derived. One of the aims of the present monograph is to prove the inadequacy of this physical model for the majority of biochemical processes, particularly for the processes of energy transduction in biological systems.

Because of this inadequacy many experimental facts described in scientific publications are not facts in the true sense of the word. For example, the statement "the equilibrium constant of this reaction equals to..." represents for many enzymatic reaction not an experimental fact but its interpretation. The experimental facts in this case are the measured values of reagent concentrations constant with time in the reaction mixture. The statement concerning the equilibrium constant is in this case an interpretation based on the assumption that the mass action law holds true for this reaction. This assumption for a process involving ordered macro-

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1 All scientists that have worked long enough know that in science public opinion probably plays a more important role than in other human undertakings. Respectability of research directions and points of view often determine for many years the progress of a branch of science and the status of a scientist.

molecular structures cannot be considered a priori as true, and requires special discussion.

This monograph is to a certain extent the continuation of the book *Problems of Biological Physics* [1.3] the first edition of which was published in the USSR in 1974 [1.4]. The theoretical basis in the present book, as it was in [1.3], is the concept that the physical mechanisms of processes involving high-ordered macromolecules require, as a rule, not a statistical, but a mechanical approach. This is primarily true for bioenergetic processes briefly discussed in the last chapter of [1.3]. These processes are actualized by means of elementary chemico-mechanical transducers – the machines of molecular dimensions.

In several laboratories of different countries experimental and theoretical studies directly or indirectly based on these ideas have been carried out in recent years. My co-workers in the Laboratory of the Physical Chemistry of Biopolymers (Institute of Chemical Physics, USSR Academy of Sciences) and in the Biophysical Department (School of Physics, Moscow University) have obtained new results, and bioenergetic processes can now be discussed much more comprehensively than they were in [1.3]. All the aforesaid can be considered to justify this book.

2. Phenomenology of Bioenergetic Processes

In this chapter the basic data concerning the most important bioenergetic processes will be given. These data are necessary to grasp the essence of solved and unsolved problems arising when we consider the relevant physical mechanisms. During recent decades scientists of different specialities in many laboratories throughout the world have obtained numerous facts concerning the structures of intracellular energy-transducing systems, and the biochemical mechanisms of corresponding reactions. It is impossible to touch even briefly upon all these highly interesting questions in such a short book. Therefore in the first chapter I shall describe only the strictly established and generally accepted facts without the knowledge of which the reader will not be able to understand the solutions let alone the formulations of corresponding physical problems. More detailed descriptions of experimental data can be found in the list of references.

2.1 Muscle Contraction

The approach taken to bioenergetic problems is based on a postulate according to which all the bioenergetic processes are actualized by means of elementary chemico-mechanical transducers, that is the molecular machines capable of exciting specific degrees of freedom and realizing the coherent states of mechanical motion by utilizing the local statistical acts of chemical transformations [2.1-3]. We shall see that mechanisms of this type are able to provide for various bioenergetic processes: overcoming an activation barrier in enzymatic catalysis, transferring ions across membranes against their electrochemical potential gradients, synthesizing thermodynamically unfavorable compounds by means of certain energy-donating reactions. It is natural to begin with the processes for which the excitation of specific degrees of freedom and mechanical motion directly represent the purpose of the functioning of elementary chemico-mechanical transducers, i.e., with the processes of biological motility.

Biological motility is a traditional field of biophysical research. All living things move (although not everything that moves is living). Macroscopic (mechanical) motion is the first phenomenon confronting a scientist studying biological objects at different levels of organization. Some examples are the continuous motion of pro-

toplasm, mechanical motion of cell components in the course of cell life cycle, the motion of spermatozoa, various kinds of muscle activity, the relative motion of ribosomes and messenger RNA molecules during protein biosynthesis. The structure and phenomenology of the functioning of striated muscle are known quite well. Since the physico-chemical principles of the contraction of all biological systems are rather alike, it is therefore appropriate, to describe here the contraction process of vertebrate striated muscle.

An elementary part of a muscle contractile fiber is a polynuclear cell with thickness of 20-100 μm and with a length of up to a few cm. The cell is filled with sarcoplasmatic reticulum and subcellular particles (nuclei, mitochondria) but a substantial part of its volume is occupied by myofibriles – elongated contractile structures with a diameter of 1-2 μm (one fiber contains $\sim 10^3$ myofibrils). Myofibrils contain two types of filaments, thick and thin ones, packed in comparatively small repeating units, the so-called sarcomeres, with a length (for a resting muscle) of ~2.2 μm. Sarcomeres are separated by protein Z-discs, with which thin filaments are connected. Thin filaments are mainly composed of actin, but also include Ca^{2+}-sensitive regulatory complexes of the proteins tropomyosin and troponin. An actin filament is a fibrillar polymer of monomeric actin globules (G-actin, molecular weight ~46000 D, diameter ~5.5 nm) packed in a double helix with an axial period of ~71 nm. Thick filaments are composed of myosin molecules (about 350 protein molecules per one thick filament). The elongated myosin molecule, molecular weight (MW):~470000 D, has a length of ~150 nm and a diameter (in the core region) of ~2 nm. The thickened end of a myosin molecule (the so-called head) has a length of ~20 nm and a diameter of ~4 nm. Each sarcomere contains $\sim 10^6$ thick and thin filaments. The structure of resting muscle at different organization levels is shown in Fig.2.1. In thick filaments the heads of myosin molecules form periodically localized projections, the miosin cross-bridges. The axial distance between identically positioned bridges equals ~43 nm.

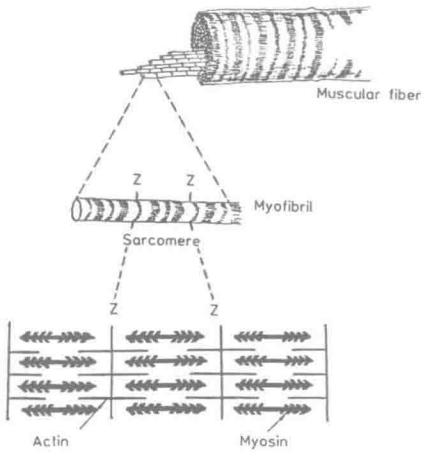
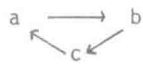


Fig.2.1. The structure of a resting muscle at different organization levels

In a resting muscle the myosin and actin filaments do not interact. After muscle activation the myosin bridges bind to the actin monomers of thin filaments. The actomyosin complex plays a role of a chemicomechanical energy transducer. During muscle contraction the length of thin and thick filaments does not change: they slide relative to one another. This theory of muscle contraction, "the sliding filament model" [2.4], is now generally accepted. Filament structures do not change during contraction. Only the relative positions of bridges are changed, since bridges represent the sole movable components of the whole structure. Not discussed here are Ca^{2+}-dependent displacements of the troponin – tropomyosin complex relative to the actin filament, which have a regulatory function making actin centers available to myosin bridges [2.5].

The sequence of processes occurring during muscle contraction can be visualized as follows. The binding of Ca^{2+} ions by troponin-tropomyosin complex leads to conformational changes in protein thin filaments. These conformational changes result in the "opening" of monomeric actin "beads" and, consequently, in the attachment of myosin bridges to corresponding (i.e., those situated at this moment of time opposite them) centers on thin filaments. In its attached state every bridge exerts a pushing force which pushes the corresponding thin filament to the sarcomere center. This leads to the sliding of thin filaments relative to thick ones and, consequently, to the diminishing of sarcomere length. The pushing force value depends on the bridge displacement (i.e., on the bridge deformation relative to its equilibrium position in a free state), and if a displacement is large enough the pushing force changes its sign, i.e., becomes a hindering force. The sliding of filaments leads not only to a change in the pushing force but also to a change in the binding constant of a myosin bridge with actin centers, so the probability of dissociation increases. The bridge passes from the hindering to the free state, relaxes to equilibrium conformation relative to the myosin framework and is now capable of binding again the next free actin center. In the region of thin and thick filaments, overlapping myosin bridges function independently – the forces exerted by them are additive.

An elementary mechanical cycle for one bridge can be formally represented by the following scheme [2.6]:



(2.1)

Here a, b, c are states of free bridge, attached pushing bridge, and attached hindering bridge, respectively. The rate of $b \rightarrow c$ transitions depends on the filament-sliding velocity. Each myosin bridge is thus largely an independent mechanical arrangement able to perform mechanical work. This molecular machine functions cyclically. The work is performed at the $b \rightarrow c$ stage of (2.1). The energy source of the cyclic process (2.1) is enzymatic ATP hydrolysis. The discovery of actomyosin ATPase activity by ENGELHARDT and LYUBIMOVA in 1939 [2.7] was the first step in the analysis

of chemicomechanical energy transducers of living matter. Today, the biochemistry of myosin and actomyosin ATP hydrolysis by isolated proteins in solution is known rather well.

Let us first consider the data concerning the catalytic activity of myosin. As a matter of fact all research has been carried out under physiological conditions (pH, ionic strength) at which myosin is insoluble. Therefore, as a rule, the biochemical activity of heavy meromyosin or of subfragment -1 (S-1) was studied (heavy meromyosin is soluble fragment of myosin containing catalytic center; S-1 is catalytically active subunits of heavy meromyosin whose molecular weight is about 1/3 of that of the latter). It can be assumed that enzymatic properties of myosin, heavy meromyosin and S-1 are alike.

It has been established that during ATP hydrolysis protein conformation undergoes marked changes, and the protein intermediate state differs from the free myosin state and the equilibrium complex state of myosin with reaction products (ADP and P_i) as well [2.8-13]. Heat liberation during ATP hydrolysis by myosin in solution is realized not at the time moment of ATP ester-phosphate bond splitting but after 15-20 s [2.14]. One can conclude that after hydrolysis the energy is stored for some time in the conformationally nonequilibrium state of the protein.

Detailed investigations carried out in different laboratories [2.15-20] have resulted in a currently generally accepted scheme of ATP hydrolysis by myosin. This scheme includes ordinary chemical reactions and the stages of protein conformational changes:

$$M + ATP \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} M \cdot ATP \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} M^* \cdot ATP \underset{k_{-3}}{\overset{k_{+3}}{\rightleftarrows}} M^{**} \cdot ATP \underset{k_{-4}}{\overset{k_{+4}}{\rightleftarrows}} M^* \cdot ADP \cdot P_i$$

$$\underset{k_{-5}}{\overset{k_{+5}}{\rightleftarrows}} M^* \cdot ADP + P_i \underset{k_{-6}}{\overset{k_{+6}}{\rightleftarrows}} M \cdot ADP + P_i \underset{k-7}{\overset{k_{+7}}{\rightleftarrows}} M + ADP + P_i \quad . \qquad (2.2)$$

Here superscripts * and ** designate different conformationally changed protein states. At room temperatures (21-24°C), pH 8.0 and ionic strength 0.1, the following values of equilibrium constants (K_i) and rate constants (k_i) for individual stages of (2.2) have been obtained:

$$K_1 = 4.5 \times 10^3 M^{-1}; \quad K_2 > 2\ 10^4; \quad k_{+2} = 400\ s^{-1}; \quad k_{-2} < 0.02\ s^{-1};$$

$$K_3 = 9 \times k_{+3} = 160\ s^{-1}; \quad k_{-3} = 18\ s^{-1}; \quad K_4 < 2 \times 10^7; \quad k_{+4} = 0.06\ s^{-1};$$

$$k_{-4} > 3 \times 10^{-9}\ s^{-1}; \quad K_5 > 1.5 \times 10^{-3}\ M; \quad K_6 = 3.5 \times 10^{-3}; \quad K_7 = 2.7 \times 10^{-4}\ M \quad .$$

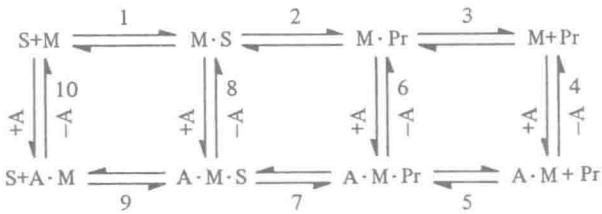
Equilibrium constant for the overall reaction ($ATP \rightleftarrows ADP + P_i$) under these conditions is $K = K_1 K_2 \ldots K_7 = 1.7 \times 10^7$ M [2.21].

These values show that in the course of ATP hydrolysis the free energy of the system decreases mainly at stages 2 and 4 connected with conformational changes of macromolecular enzyme–substrate and enzyme–product complexes. The slowest stage is not the act of ATP hydrolysis (stage 3) but the conformational change of enzyme–product complex following stage 3 and preceding the liberation of products. Some constants (K_2, k_{-2}, K_4, k_{-4}, K_5) have not been determined precisely. The "true" values (i.e., the values satisfying (2.2) and the overall reaction equilibrium constant K) cannot, however, differ greatly from the upper or lowest limiting values given above. Some of these constants were measured for ATP analogs and the "true" values almost coinciding with the above limiting values were obtained. For instance, k_{-2} value for ATP (β, γ-NH) (S'-adenylimidodiphosphate) is exactly 0.02 s^{-1} [2.19]. We shall return later to the discussion of the reality of scheme (2.2) and the values of thermodynamic and kinetic constants of its individual stages.

ATP hydrolysis by myosin is a rather slow process. In muscle fibers enzymatic hydrolysis and energy liberation proceed by 2-3 orders of magnitude faster, which is causally related to the periodic formation and dissociation of actomyosin complexes, i.e., to the attachment and dissociation of myosin bridges.

Deshchervsky's model of an elementary mechanical cycle of striated muscle contraction described above does not refer mechanical stages to biochemical ones. This can be done, however, if we assume that biochemical mechanism of enzymatic ATP hydrolysis by ordered actomyosin systems in a muscle resembles that of ATP hydrolysis by soluble actomyosin complexes studied experimentally.

The currently generally accepted cyclic mechanism of actomyosin ATPase was independently proposed by LYMN and TAYLOR [2.22], and by BUKATINA and DESHCHEREVSKY [2.23] (see also [2.3,24]). The most important and apparently surprising experimental fact is the observation that although actin accelerates ATP hydrolysis by myosin about 200 times [2.25], the ATP addition to actomyosin leads to a rise in the actomyosin effective dissociation constant. It means that during ATP hydrolysis actomyosin disappears, and the concentration of a less effective catalyst, myosin, increases. Analysis carried out in [2.22,23] has, however, shown that actin accelerates the stages of reaction product dissociation, and not the stage of ATP hydrolysis. A rather simplified scheme of ATP hydrolysis by actomyosin according to the authors cited above can be presented as follows:



(2.3)