Dynamics and Kinetics of Enzymes
Kinetic Equilibrium of Forces in Biochemistry

Erwin W. Becker*

Institut für Mikrostrukturtechnik der Universität Karlsruhe, Bundesrepublik Deutschland

Z. Naturforsch. **47c,** 628–633 (1992); received May 18, 1992

Enzyme, Mechanism, Free Energy Transfer, ATPase, Calmodulin, Muscle Contraction

To explain the high specificity, high reaction rate, and high thermodynamic efficiency in enzymatic processes, cooperation of the enzyme with a molecular transfer unit is assumed. A "kinetic equilibrium of forces" is suggested, which enables high reaction rates to occur under equilibrium conditions and a thorough examination of the substrate to be made without consumption of free energy. In case of ATPases, ion-binding proteins are the most probable transfer units. By analyzing the elementary effect in muscle contraction it is shown that the new theorem may be of substantial value in elucidating biochemical processes.

Introduction

It is well known that enzymatic processes may proceed with high specificity, high reaction rate, and high thermodynamic efficiency. To explain the specificity, Emil Fischer and Paul Ehrlich introduced their famous lock-and-key hypothesis. According to a suggestion by L. Pauling, the enzyme has to fit the transition state rather than the ground state of the substrate [1]. D. E. Koshland [2] complemented this model by the so-called induced-fit theory, which assumes an adaptation of the enzyme to the substrate. M. Eigen *et al.* [3] have demonstrated that the various steps of enzymatic reactions may proceed at considerable velocity. With the primary objective to explain the high thermodynamic efficiency of enzymatic reactions involving membranes, P. Mitchell introduced the so-called "chemiosmotic equilibrium" [4].

In spite of the vast knowledge existing about the chemistry of enzymatic reactions, the background for the surprising capabilities of the enzymes is still a matter of discussion [5]. In the present paper an attempt is made to substantiate a biomechanical theorem called "kinetic equilibrium of forces" which could be of some help in answering open questions. It is based on an assumed cooperation of the enzyme with a molecular transfer unit. Under the condition that the cooperating partners exhibit opposite relations between their free energy contents and their forces exerted on each other, a reversible exchange of free energy by thermal molecular motion can be expected to take place. The suggested "kinetic equilibrium of forces" enables high reaction rates to occur under equilibrium conditions and a thorough examination of the substrate to be made without consumption of free energy. In case of ATPases, ion-binding proteins are the most probable transfer units. By analyzing the elementary effect in muscle contraction it is shown that the new theorem may be of substantial value in elucidating biochemical processes.

Cooperation of an enzyme with a transfer unit

Considering their importance to the economy of the cell, ATPases are taken as examples in establishing the new theorem. For the reaction

$$ATP + H_2O = ADP + Pi,$$

leading to the products ADP and phosphate ion (Pi), Fig. 1 indicates in a schematic manner the dependence of free energy G on the distance x1 between ATP and H_2O and on the distance x2 between ADP and Pi. The common origin of the distances lies roughly at the center of the transition state. The difference in the free energies of activation, Ga2-Ga1, is the free energy of reaction Gr. Except in close proximity to the transition state, where intramolecular changes take place, free energy is based on repulsive intermolecular forces. During the main part of the interaction, therefore, an increase in free energy is connected with an increase in force.

Under physiological conditions, the thermodynamic equilibrium of the reaction is far to the

* Reprint requests and correspondence to Prof. Dr. E. W. Becker, Strählerweg 18, D-W-7500 Karlsruhe 41.

Verlag der Zeitschrift für Naturforschung,
D-W-7400 Tübingen
0939–5075/92/0700–0628 $ 01.30/0

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

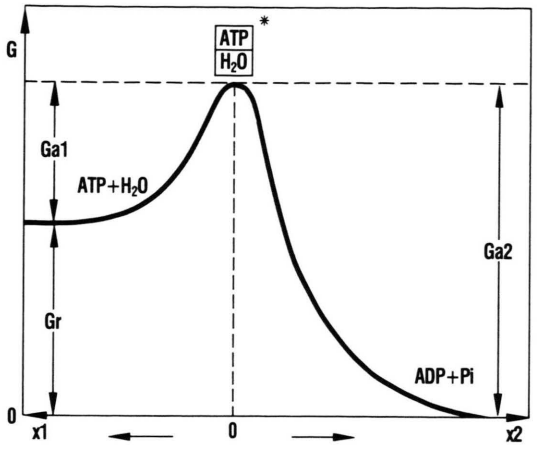

Fig. 1. Schematic representation of the free energy G of ATP hydrolysis as a function of the distance x 1 between ATP and H_2O on the one hand and of the distance x 2 between ADP and phosphate ion (Pi) on the other hand.

right. In case of muscle contraction, however, a reversible ATP-hydrolysis far from thermodynamic equilibrium is observed [6]. So, cooperation between ATPase and a molecular "transfer unit" must be assumed. During the exergonic phase of the reaction, the transfer unit is expected to store the free energy released so that, during the endergonic phase, it can be fed back to the ATPase. In spite of the fact that the transfer unit may be an integral part of the enzyme it will be treated as a separate entity.

To illustrate a possible way of cooperation, the hinge-cleft model of an enzyme [7] is assumed. According to Fig. 2, the enzyme is to consist of the globular units S 1 a and S 1 b connected by a hinge-like flexible section so that a pair of tongs is formed. The transfer unit T, the specifications of which are to be established, is expected to bridge the ATPase cleft without impairing the material flow.

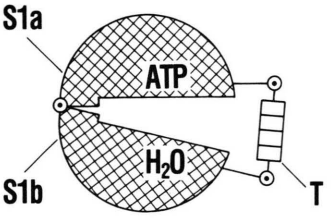

Fig. 2. Hinge-cleft model of the cooperation between a transfer unit T and an ATPase consisting of the globular units S 1 a and S 1 b carrying the reactants ATP and H_2O.

Prerequisites of a reversible transfer of free energy

The prerequisites of a reversible transfer of free energy between an enzyme and a transfer unit, cooperating according to Fig. 2, are illustrated by the model of a mountain railway shown in Fig. 3. The cooperating units are represented by two cars connected by a weightless rope and running on two curved slopes of the mountain. To simulate the conditions in the cell it is assumed that the cars move without mechanical friction, but within a medium, the viscosity of which prevents considerable kinetic energy from occurring. Under these conditions, the free energies of the cars are identical with their potential energies. In order to make the dynamic situation more surveyable, it is further assumed that a frictionless guide forces the rope to run parallel to the slopes. So, the gradients of the slopes at the positions of the cars are measures of the forces exerted upon the rope. The car on the left side, together with the corresponding

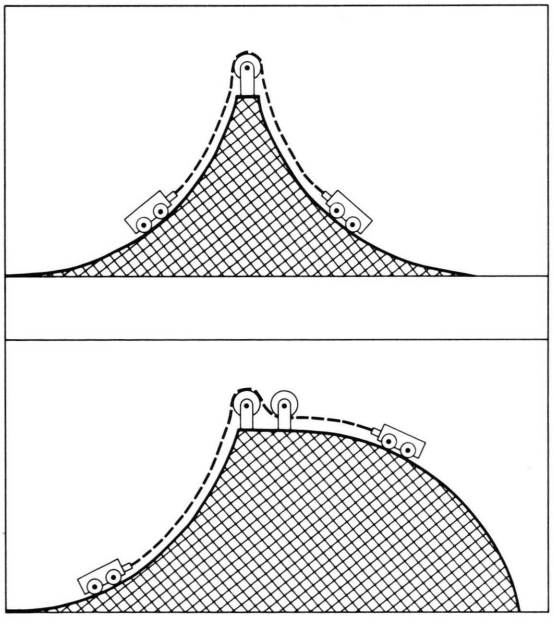

Fig. 3. Model of a mountain railway for illustrating the cooperation between an enzyme (left) and a transfer unit (right). Upper case: Equilibrium of forces in one position of the cars only. Lower case: Equilibrium of forces in any position.

slope, represents the ATPase, whereas the car on the right side represents the transfer unit. In the upper part of the figure, both partners exhibit the relation between free energy and force typical of ATPases (see above). In the lower part, the opposite relation is assumed for the transfer unit.

In the upper case, equilibrium of forces is established in one position of the cars only. With increasing deviation from that point, a corresponding force builds up which pulls the cars back to the equilibrium state. In proportion to the resulting velocity entropy is generated. In the lower case, the equilibrium of forces is maintained in any position of the cars. In such "extended" equilibrium of forces, a small overweight of one car pulls the other car onto the top of the mountain. The rate of the corresponding transfer of free energy is proportional to the unbalance of forces, *i.e.* to the entropy generation. So, in a macroscopic system operating within a viscous medium, there is a rigorous correlation between the "reaction rate" and the entropy generation.

A microscopic system cooperating with a transfer unit in an extended equilibrium of forces, however, may avoid the detrimental correlation just mentioned by profiting from thermal molecular motion. Of course, the resulting statistical transfer of free energy is not directed. On the other hand, under an extended equilibrium of forces, every point on the reaction coordinate pertinent to the next step of the reaction is reached in due time. It is concluded, therefore, that a "kinetic equilibrium of forces" is the basis of the surprising combination of high specificity, high reaction rate, and high thermodynamic efficiency in enzymatic processes. Here "kinetic" means subject to thermal motion.

A prerequisite of the supposed "kinetic" equilibrium of forces is the "extended" equilibrium of forces which is demonstrated for macroscopic systems in the lower part of Fig. 3.

Conceivable realization of a kinetic equilibrium of forces

Probable partners of enzymes in establishing a kinetic equilibrium of forces are ion-binding proteins. In the presence of a target protein, binding of calcium ions to calmodulin, *e.g.*, transforms the calmodulin chain into a more compact configuration [8]. Under the influence of the target protein, binding of ions proceeds in a pronounced cooperative manner [9]. So, an increasing traction force should result. As the force is based mainly on ionic attraction, the free energy content of the ion-binding protein is expected to decrease with increasing traction force. In the cooperation, according to Fig. 2, of an ATPase with a calmodulin-like protein, therefore, the prerequisites of a kinetic equilibrium of forces should be fulfilled. Under such cooperation, ATP hydrolysis will result in a reversible stretching of the protein chain which is accompanied by a release of ions. By reabsorption of ions, ATP can be resynthesized.

Like calmodulin, many other proteins combine preferably with calcium ions [10]. Other ions, *e.g.* magnesium ions, on the other hand, are far more abundant in the cell. So, a considerable number of possibilities exist for achieving a kinetic equilibrium of forces in enzymatic reactions. As already mentioned, the transfer unit may be a separate entity as well as an integral part of the enzyme.

Relevance of the kinetic equilibrium of forces to the specificity of enzymes

In Fig. 4 it is shown how the high specificity of enzymes can be explained on the basis of the kinetic equilibrium of forces. To make the essential arguments more transparent, a monomolecular reaction without a net free energy of reaction is assumed. The tasks of the enzyme, therefore, are restricted to selecting the proper substrate S and to starting the reaction making use of the highly efficient storage of free energy by the transfer unit T.

In Fig. 4a, the cleft of the enzyme is empty. It is kept open by the mutual repulsion of the internal negative charges of the ion-binding protein chain which acts as a transfer unit. Under that condition, the affinity of the positive ions to the protein chain is not sufficient to start the cooperative ion-binding process. The situation is different when a substrate enters the cleft. By means of its non-specific attractive forces, the substrate tries to narrow the cleft. The corresponding shortening of the protein chain starts the cooperative binding of positive ions which results in squeezing of the substrate. In a first approximation, the squeezed substrate will react as an elastic body. As such, it exhibits a relation between the free energy and the force opposite

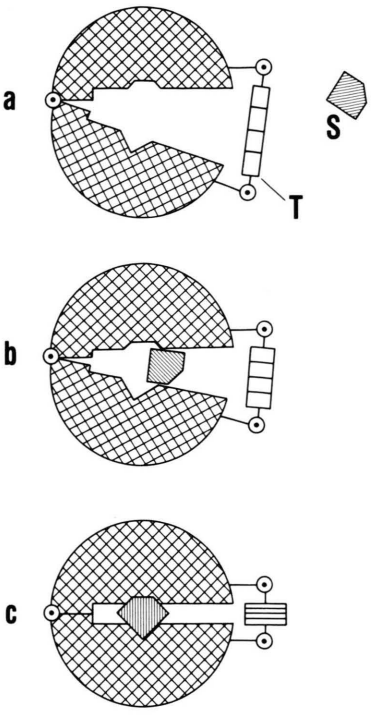

Fig. 4. Different phases of the examination of the substrate S by the combination of an enzyme and a transfer unit T.

to that of the transfer unit T. So, in a kinetic equilibrium of forces a thorough examination of the substrate by the enzyme is feasible. If, according to Fig. 4c, the transition state of the substrate fits the enzyme, the protein chain T becomes shorter. The corresponding improvement of ion-binding causes the free energy of activation to be supplied in a kinetic equilibrium of forces, and the reaction is started. The repulsive forces of the reaction products force the two globular units of the enzyme apart. In this way, the ions bound to the protein chain are liberated, and the free energy of activation is restored. After the reaction products have been released the system again becomes functional.

The reaction rate, *i.e.* the probability of the reaction cycle occurring, may be regulated by the concentration of the ions pertinent to the transfer unit. Another possibility for regulation seems to be the supply or the withdrawal of the free energy of activation stored within the transfer unit. This could be done, *e.g.* by phosphorylation or dephosphorylation of a participant in the reaction.

Elementary effect in muscle contraction

It is well known that muscle contraction is based on the relative sliding of myosin and actin filaments, the necessary free energy being supplied by the hydrolysis of ATP [11–13]. Although muscle contraction is one of the principal themes of biological research, the exact mechanism whereby the chemical free energy of ATP hydrolysis is converted into mechanical work is still a matter of discussion [14]. Above all, the high thermodynamic efficiency of the process [15], and the precise adaptation of ATP hydrolysis to the mechanical requirements are difficult to explain on the basis of present theories.

Recently, the author proposed a model of the elementary effect in muscle contraction based on the assumption of free energy transfer by the ion-binding myosin light chains [16]. Taking into consideration the experimental results described in the literature, it was concluded that the detour of free energy *via* the light chains, under a so-called chemimechanic equilibrium, accounts for the high thermodynamic efficiency. In the present paper, the term "chemimechanic equilibrium" has been substituted by the more comprehensive "kinetic equilibrium of forces". The emphasis of the fundamental role of thermal molecular motion allows a more precise interpretation of kinetic details. This will be demonstrated along the line of Fig. 5.

The left hand column of Fig. 5, in a schematic manner, indicates the different phases of muscular contraction [16]. In the nomenclature of Fig. 1, the right-hand column denotes the distribution of free energy among the components involved. In Fig. 5A, the free energy Ga2 is concentrated on the transition state of the ATP/H_2O system. The light chains LC and the elastic element E do not contain free energy. In Fig. 5B, the reaction ATP + H_2O → ADP + Pi has taken place and Ga2 is taken over by the light chains. In Fig. 5C, loss of the Pi has caused the so-called weak binding state to pass into the strong binding state [17]. Under the new condition, the light chains LC pass the free energy of reaction, Gr, to the elastic element E, while retaining the free energy of activation, Ga1. Simultaneously, ADP is exchanged for ATP + H_2O.

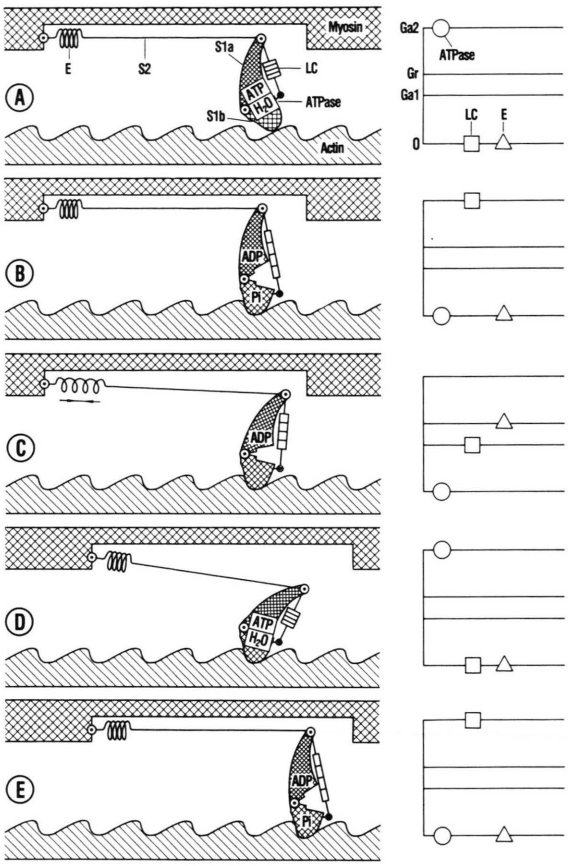

Fig. 5. Mechanical diagrams representing the different phases of muscle contraction according to the proposed model.

When, according to Fig. 5D, the free energy content Gr of the elastic element E is taken over by the muscle filaments, the ATPase cleft is further reduced, and the free energy of activation, Ga 1, is transferred from LC to the ATPase. In this way, the transition state of the fresh ATP/H_2O load is reached. The phosphate ion Pi formed by ATP-hydrolysis transfers the strong binding state back to the weak binding state. Thus, the myosin head can follow the shift of the myosin strand and leap over an actin unit (Fig. 5E). The system thus becomes functional again.

The oscillation between states A and B under a kinetic equilibrium of forces allows relative sliding of the filaments to take place with reduced ATP hydrolysis observed in a fast, unloaded contraction [18]. As free energy of activation is not delivered to the ATP/H_2O system before the muscle strands have taken over the free energy stored in the elastic element E (transition C–D in Fig. 5), the muscle can maintain isometric tension with relatively low energy consumption ("Fenn effect"). Within the proposed mechanism, the theory of calcium regulation remains essentially unaffected [16].

The analysis indicates that the kinetic equilibrium of forces is essential not only for the efficiency of free energy transfer between the muscle components but also for the precise adaptation of ATP hydrolysis to the mechanical requirements.

Summary

In the paper, the astonishing capabilities of enzymes are explained by the assumption of a specific thermodynamic state of living matter, called "kinetic equilibrium of forces". It is characterized by the reversible transport of free energy under the action of thermal molecular motion. The ability to avoid entropy generation in spite of high reaction rates seems to be a peculiarity of biological macromolecules. If the suggested theorem of kinetic equilibrium of forces turns out to be of general significance to living matter, it should be an important guide in elucidating biochemical processes.

Acknowledgements

The author is grateful to Professors W. Hasselbach, B. Hess, K. C. Holmes, L. Jaenicke, U. Schindewolf, D. M. Taylor, F. Wieland, and Th. Wieland for illuminating discussions and critically reading the manuscript.

[1] L. Pauling, Chem. Eng. News **24,** 1375 (1946).
[2] D. E. Koshland, Jr., Sci. Am. **229/4,** 52–64 (1973).
[3] M. Eigen and G. G. Hammes, Adv. Enzym. **25,** 1–38 (1963).
[4] P. Mitchell, Nature **191,** 144–148 (1961); J. Biochem. **97,** 1–18 (1985).
[5] L. Kovac, Curr. Topics in Bioenergetics **15,** 331–372 (1987).
[6] C. R. Bagshaw and D. R. Trentham, Biochem. J. **133,** 323–328 (1973).
[7] B. Mao, M. R. Pear, J. A. McCammon, and F. A. Quiocho, J. Biol. Chem. **257,** 1131–1133 (1982).
[8] M. Kataoka, J. F. Head, B. A. Seaton, and D. M. Engelman, Proc. Natl. Acad. Sci. U.S.A. **86,** 6944–6948 (1989).
[9] J. A. Cox, M. Comte, A. Mamar-Bachi, M. Milos, and J.-J. Schaer, Cation Binding to Calmodulin and Relation to Function, in: Calcium and Calcium Binding Proteins (Ch. Gerday, R. Gilles, and L. Bolis, eds.), pp. 141–162, Springer, Heidelberg 1988.
[10] C. W. Heizmann and W. Hunziker, TIBS **16/3,** 98–103 (1991).
[11] H. E. Huxley, Science **164,** 1356–1366 (1969); H. E. Huxley and M. Kress, J. Musc. Res. Cell Motil. **6,** 153–161 (1985).
[12] A. F. Huxley, J. Physiol. **243,** 1–43 (1974); A. F. Huxley, Ann. Rev. Physiol. **50,** 1–16 (1988).
[13] R. S. Goody and K. C. Holmes, Biochim. Biophys. Acta **726,** 13–39 (1983).
[14] M. A. Geeves, Biochem. J. **274,** 1–14 (1991).
[15] R. C. Woledge, Nature **334,** 655 (1988).
[16] E. W. Becker, Naturwissenschaften **78,** 445–449 (1991).
[17] B. Brenner, Muscle Mechanics and Biochemical Kinetics, in: Molecular Mechanisms in Muscular Contraction (J. M. Squire, ed.), pp. 77–149, Macmillan Press, London 1990.
[18] T. Yanagida, T. Arata, and F. Oosawa, Nature **316,** 366–369 (1985).