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Enzyme kinetics and the maximum entropy production principle

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ABSTRACT

A general proof is derived that entropy production can be maximized with respect to rate constants in any enzymatic transition. This result is used to test the assumption that biological evolution of enzyme is accompanied with an increase of entropy production in its internal transitions and that such increase can serve to quantify the progress of enzyme evolution. The state of maximum entropy production would correspond to fully evolved enzyme. As an example the internal transition $ES \leftrightarrow EP$ in a generalized reversible Michaelis–Menten three state scheme is analyzed. A good agreement is found among experimentally determined values of the forward rate constant in internal transitions $ES \rightarrow EP$ for three types of β-Lactamase enzymes and their optimal values predicted by the maximum entropy production principle, which agrees with earlier observations that β-Lactamase enzymes are nearly fully evolved. The optimization of rate constants as the consequence of basic physical principle, which is the subject of this paper, is a completely different concept from a) net metabolic flux maximization or b) entropy production minimization (in the static head state), both also proposed to be tightly connected to biological evolution.

1. Introduction

Accommodation of the species to changes of the environment is, according to the Darwinian theory of evolution, an inherent property of living beings. Ability of living beings to survive environmental changes depends on complex interconnections of biochemical fluxes. Speed of biochemical reactions is mostly determined by structure and function of enzymes.

Any attempt to understand why kinetic parameters of enzymes are such as experimentally measured must take into account the fact that biological systems are, in contrast to chemical systems of an inanimate nature, the outcome of biological evolution [1,2].

The fact that enzymes evolve under selection pressure raises the question if it is possible to find some physical quantity that can measure the degree of enzyme evolution. The maximum metabolic flux has been frequently proposed as such a measure and even as the goal of the evolution [3–7].

Metabolic fluxes are increasing functions of forward and decreasing functions of backward rate constants, respectively. In the case of a three state enzyme reaction depicted in the Fig. 1 overall flux is proportional to the product of forward rate constants. If there were no constraints the maximum flux value would diverge. However, various constraints impede the divergence of fluxes. Firstly, enzymes are quantum mechanical objects and relaxation times of excited states limit fluxes. Secondly, there is a diffusion limit with characteristic relaxation times for binding substrates to an enzyme. In the case of three state enzyme kinetic scheme (Fig. 1) the enzyme can be either in free state E, enzyme-substrate state ES or enzyme-product state EP [3]. The reactions $E+S \rightarrow ES$ and $EP \rightarrow E+P$ depend not only on the transition state between free states, $E+S$ and $E+P$, and intermediate states, ES and EP, but on the diffusion, too. Diffusion is an additional source of the flux limitation. Microscopic description of these constraints is a formidable task. In order to overcome these difficulties one usually imposes constraints on some of rate constants [3,6] in enzymatic reaction and searches for a maximum value of flux.

The standard constraint in enzymatic reaction is overall equilibrium constant since it depends on the values of equilibrium concentrations of ligands (i.e. substrates and products). Usually one assumes additional constraints. Very often it is assumed that second-order rate constants are not the subject to evolutionary variation but are fixed due to diffusion constraints [3,4].

For example, in the case of three state enzymatic reaction depicted in the Fig. 1 Albery and Knowles [3] have assumed $k_1^{+\,*} = k_3^{-\,*} = const.$ The stationary overall flux is then the function of three rate constants, instead of six ones that determine the reaction. They have proposed that mutations leading to changes in the Gibbs free energies can be understood within three different types of variation of binding energies. These are named uniform binding, differential binding and

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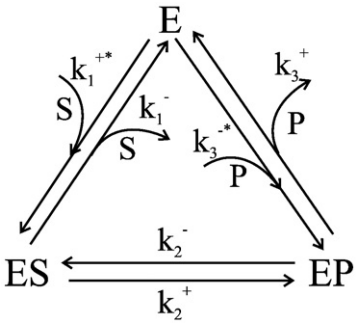

Fig. 1. Triangle kinetic scheme for the three state enzyme reaction.

catalysis of an elementary step. In the uniform binding model, binding energies of intermediate states and their transition state change equally leaving rate constants of internal transition k_2^+ and k_2^- unchanged. In the case of differential binding the relative free energy of the complex intermediate states is changed. Finally in the case of catalysis of an elementary step, free energy of transition state flanked by intermediates states is changed.

Further progress has been done by Heinrich et al. [6,8]. They have replaced Albery and Knowles equality $k_1^{+*} = k_3^{-*} = k_d = const$ with inequality $k_1^{+*}, k_3^{-*} < k_d$ and have introduced additional inequalities for the rest of rate constants k_1^-, $k_3^+ < k_r$, k_2^-, $k_2^+ < k_m$ (see [6,8]). In this way analysis has become more detailed but more complex at same time. Using the stationary description of the biochemical reaction, and taking into account imposed constraints, one gets overall flux as function of free rate constants. The requirement that flux assumes its maximum value determines the values of the free rate constants [3–6,8,9]. Once the constraints are defined and fluxes are determined it is possible to offer an answer to the question how far enzymes are from fully evolved states. Burbaum et al. [4] proposed the ratio between actual and maximum possible metabolic flux, named the catalytic efficiency, as the measure (the degree) of evolution of enzyme.

The assumption that maximum metabolic flux is the goal of enzyme evolution does not exclude possibility that some other physical quantity can be a measure of enzyme evolution [8]. Here we invoke observations that the emergence of complex dissipative structures [10,11] and highly evolved living beings [12,13] is as a rule associated with greater entropy production. It seems to us quite natural to make the correlation between increases of entropy production due to evolution of species with evolution of enzymes. In other words we assume that biological evolution of enzyme is accompanied with an increase of the entropy production in enzymatic reaction. An additional assumption in this paper is that transitions described with first order rate constants are under strongest evolutionary pressure. We call them internal transitions. If internal transitions are in the state of maximum entropy production (MEP) we can associate such optimal state with fully evolved enzyme. While both of above mentioned optimization principles: maximal metabolic flux [6,8] and optimal catalytic efficiency [4,9] are based on maximization of metabolic flux, the MEP principle involves optimization of product between metabolic flux and corresponding thermodynamic force-affinity of enzymatic reaction.

As we have mentioned the complexity of enzyme reaction cycle forces one to introduce constraints. Since these necessarily introduce uncertainty into a theory one tends to reduce the number of constraints. The MEP principle optimizes fluxes and thermodynamic forces with the outcome that one less constraint is needed for each internal transition in comparison to the principle of maximum metabolic flux. For example let us assume, for the sake of simplicity, that k_2^+ is the only undetermined rate constant in three state enzyme cyclic reaction (Fig. 1). Then the method of maximum metabolic flux predicts infinite value of this constant and one is forced to introduce constraints in order to avoid this divergence. The method of maximum metabolic flux encounters additional substantial problems in case of branching of fluxes. The following question arises: which flux must be maximized? Then it is not surprising that only single cycle or linear chain mechanisms of coupled enzyme reactions (reactions where product of one reaction is a substrate for only one subsequent reaction) have been considered using this method [3,4,7,9]. These problems are easily solved by the application of MEP principle — predicted maximal metabolic flux is not infinite and artificial constraints are not needed to limit it, while optimal distribution of branching fluxes can also be found (that latter point is left out from this paper which uses only the examples of single cycle kinetic schemes).

We stress that the MEP principle is not offered here as an alternative to biological selection and evolution. Rather, it is the unique physical selection principle [13] which can be regarded as a good candidate for acting in concert (not in opposition) with biological selection and evolution. The MEP principle has already been used as the selection principle helping to illuminate the evolution of biota [14], the Earth system [15] and environmental and ecological systems [16].

In this paper we first consider a single internal transition of an arbitrary complex kinetic scheme for enzyme catalyzed reaction. It will be shown that entropy production achieves its maximum for a certain optimal value of the forward rate constant in a chosen transition. As an example the three state reaction of the Michaelis–Menten type for β-Lactamase enzymes are considered too. In the latter case we have found that the theoretically predicted optimal values of the forward rate constant k_2^+, which are calculated for the internal enzyme transition of three different types of β-Lactamase enzymes (β-Lactamase I, PC1 β-Lactamase and RTEM β-Lactamase) correspond well (within the order of magnitude) with measured values from the reference [5].

The MEP principle is not the only principle proposed for nonequilibrium processes. The well known one is the Prigogine's principle of minimum entropy production. It looks like the antipode of the MEP principle. However, as it was stressed by Martyushev and Seleznev [11], these two principles should not be considered as contradictory or opposed to each other, since they include different constraints and different variable parameters. We argue in Section 5 that the Prigogine's principle is less suitable for a description of energy transduction in biological systems.

2. The trade-off between affinity and flux in the single transition of an enzyme reaction

In accordance with Terrel Hill's approach [17] we assume that each transition between any enzyme discrete states is associated with calculable entropy production when both forward k_{ij} and backward k_{ji} rate constants are known. This assumption connects enzyme kinetics and thermodynamics and makes it possible to ask more specific question connecting physics, chemistry and biological evolution: is it possible to find the optimal forward rate constant k_{ij} associated with maximal entropy production in any $i \leftrightarrow j$ transition. The answer to that question is positive and easy to prove. Let us consider two states i and j, with a nonzero transition flux connecting these states, so that the corresponding equilibrium constant is not equal to one. The stationary probability of the i-th macromolecular state is

$$p_i = \Sigma_i / \Sigma \tag{1}$$

where Σ_i and Σ are respectively the sum of the directional diagrams toward state i and the sum of the directional diagrams of all the states [17].

The directional diagrams for state i can be subdivided into diagrams $a_{ji}k_{ji}$, including the rate constant k_{ji}, and diagrams b_i,

excluding the rate constant k_{ji}. Similarly, the directional diagrams for state j can be subdivided into diagrams $a_{ij}k_{ij}$, including the rate constant k_{ij}, and diagrams b_j, excluding the rate constant k_{ij}. In other words, we can write

$$\Sigma_i = a_{ji}k_{ji} + b_i,\ \Sigma_j = a_{ij}k_{ij} + b_j. \tag{2}$$

To see that

$$a_{ij} = a_{ji} \tag{3}$$

we can introduce directional diagrams for the transition $i \leftrightarrow j$ in complete analogy to the definition of the state directional diagrams [17]. Such transitional diagrams flow into the transition $i \leftrightarrow j$ and cannot be different for these two states. They do not connect all states, like state directional diagrams, because they lack the arrow corresponding to the rate constant k_{ij} or k_{ji}. We have already labeled these diagrams for the $i \leftrightarrow j$ transition as a_{ij} or a_{ji}. The state and transition directional diagrams for a simple three state enzyme reaction are depicted in Figs. 2 and 3, respectively.

After this short introduction of Hill's diagrammatic technique, with the addenda of transition directional diagrams, we turn to the thermodynamic properties of transitions. The affinity of the $i \rightarrow j$ transition is

$$X_{ij} = RT\ln\frac{k_{ij}p_i}{k_{ji}p_j}. \tag{4}$$

Using Eqs. (1) and (2) we get

$$X_{ij} = RT\ln\frac{K_{ij}b_i + k_{ij}a_{ij}}{b_j + k_{ij}a_{ij}}, \tag{5}$$

where $K_{ij} = k_{ij}/k_{ji}$ is the equilibrium constant for this transition.

The flux associated with the $i \rightarrow j$ transition is

$$J_{ij} = n\left(k_{ij}p_i - k_{ji}p_j\right), \tag{6}$$

where n is the number of moles of enzyme molecules. Using Eqs. (1) and (2) we get

$$J_{ij} = n\frac{b_i - b_j/K_{ij}}{\Sigma/k_{ij}}. \tag{7}$$

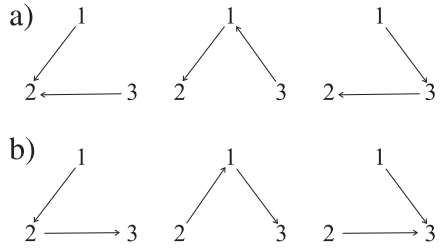

Fig. 2. Directional diagrams for the state 2 a) and state 3 b) in the three state enzyme reaction.

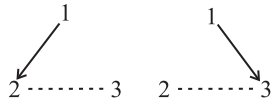

Fig. 3. Directional diagrams for the transition 2 ↔ 3 in the three state enzyme reaction.

The entropy production σ_{ij} associated with the $i \rightarrow j$ transition is defined in the standard manner as the product of the flux J_{ij} and the corresponding affinity X_{ij},

$$\sigma_{ij}T = X_{ij}J_{ij}. \tag{8}$$

It comes from Eqs. (5) and (7) that affinity and flux are the decreasing and increasing functions of the forward rate constant k_{ij} respectively if the condition

$$K_{ij}b_i > b_j \tag{9}$$

is satisfied. This trade-off between affinity and flux is a necessary condition for the maximum of entropy production.

In the opposite case,

$$K_{ij}b_i < b_j, \tag{10}$$

affinity and flux are negative but entropy production is positive. The negative values mean that reaction is going in the reverse direction. In this case affinity and flux are the increasing and decreasing functions of the forward rate constant k_{ij}, respectively.

The optimal values for the forward rate constant are associated with the extreme value of entropy production. The zero of the first derivation of entropy production leads to the equation

$$\ln\frac{K_{ij}b_i + k_{ij}a_{ij}}{b_j + k_{ij}a_{ij}} = \frac{a_{ij}\left(K_{ij}b_i - b_j\right)k_{ij}\left(k_{ij}\Sigma_a + \Sigma_b\right)}{\Sigma_b\left(a_{ij}k_{ij} + K_{ij}b_i\right)\left(a_{ij}k_{ij} + b_j\right)}, \tag{11}$$

where $k_{ij}\Sigma_a$ and Σ_b are the sums of all state directional diagrams with or without line describing the transition between states i,j, respectively. In other words, $\Sigma = k_{ij}\Sigma_a + \Sigma_b$. The foregoing Eq. (11) has the solution k_{ij}^0 for both of the above conditions. The extreme value of the entropy production for the optimal value of the forward rate constant k_{ij}^0, is then given by the expression

$$\sigma\left(k_{ij}^0\right) = \frac{nR}{K_{ij}}\frac{a_{ij}\left(k_{ij}^0\right)^2\left(K_{ij}b_i - b_j\right)^2}{\Sigma_b\left(a_{ij}k_{ij}^0 + K_{ij}b_i\right)\left(a_{ij}k_{ij}^0 + b_j\right)}. \tag{12}$$

The second derivation of the entropy production function,

$$\frac{d^2\sigma\left(k_{ij}^0\right)}{dk_{ij}^2} = -\frac{nR}{K_{ij}}a_{ij}\frac{\left(K_{ij}b_i - b_j\right)^2\left(a_{ij}b_jk_{ij}^0 + a_{ij}K_{ij}b_ik_{ij}^0 + 2K_{ij}b_ib_j\right)}{\left(a_{ij}k_{ij}^0 + K_{ij}b_i\right)^2 + \left(a_{ij}k_{ij}^0 + bj\right)^2\left(k_{ij}^0\Sigma_a + \Sigma_b\right)}, \tag{13}$$

is always negative for $k_{ij} = k_{ij}^0$. Thus, for k_{ij}^0 entropy production has the maximum value given by the expression (12).

3. Entropy production in the enzyme internal transition

In order to demonstrate how the MEP principle works, let us consider a passive device with resistance R attached to the source of electromotive force E with an internal resistance R_i. Assuming that whole network is at fixed temperature we get, after simple algebra, that the optimal value of a resistance R, determined by maximum entropy production in the passive device, should be equal to the internal one, that is $R = R_i$. The elements of electric circuit are fixed. In order to optimize circuit in the sense of maximum entropy production in passive device one has to adjust the resistance of the passive devices in such a way to make it equal to the internal resistance of the source of electromotive force.

The search for the maximum entropy production in one transition of enzymatic reaction as a function of transition rate constant is

analogous to adjustment of the resistance of the passive device described above. However, parameters of enzymatic reaction are not fixed since they are exposed to the evolution pressure. In the three state model of the enzymatic reaction the internal transition is the only transition that is directly connected neither with a substrate nor with a product concentration. This makes it the most sensitive one to the evolution pressure. We can ask ourselves if there is some physical quantity that accompanies biological selection. We argue here that biological evolution, within fixed concentrations of substrate and product molecules, is accompanied with an increase of the entropy production in the internal transition. If an enzyme in its evolution process has become fully evolved, then this should correspond to the maximum entropy production in the internal transition. We stress that MEP principle is not an alternative to the biological selection. As it was pointed out in Introduction the MEP principle just serves as a test whether enzyme has approached a fully evolved state or not.

In this section we apply the MEP principle to the three state enzyme reaction shown in Fig. 1. The standard notation of the kinetic parameters and enzyme states in this section is slightly different from the one we used in the previous section. In order to bring this text closer to the reader we adopt here the standard notation shown in Fig. 1. Table 1 links the standard notation with the one used in the previous section.

We are looking for the maximum entropy production in $ES \rightarrow EP$ transition in order to find the optimal value of the forward rate constant k_2^+. The general result derived in the previous section guarantees that a maximum of entropy production does exists in this transition. Using Figs. 2 and 3 we find:

$$\Sigma_a = k_3^+ + k_1^{+*}[S] + k_3^{-*}[P], \tag{14}$$

$$\Sigma_b = k_1^- k_2^- + k_1^- k_3^+ + \left(k_2^- + k_3^+\right)k_1^{+*}[S] + (k_1^- + k_2^-)k_3^{-*}[P], \tag{15}$$

$$a_{23} = k_1^{+*}[S] + k_3^{-*}[P], \tag{16}$$

$$b_2 = k_1^{+*}k_3^+[S], \tag{17}$$

$$b_3 = k_1^- k_3^{-*}[P]. \tag{18}$$

The conditions (9) and (10) now read

$$K > 1 \tag{19}$$

and

$$K<1, \tag{20}$$

respectively, where $K = K^* \frac{[S]}{[P]}$. K^* is the overall equilibrium constant of the three state enzyme reaction. It is defined as a product of the equilibrium constants K_i of each transition of the kinetic scheme and it reads

$$K^* = K_1^* K_2 K_3^* = \frac{k_1^{+*}k_2^+k_3^+}{k_1^- k_2^- k_3^{-*}} = \frac{[P]_e}{[S]_e}. \tag{21}$$

Here $[P]_e$ and $[S]_e$ are concentrations of the product and substrate in equilibrium. An asterisk in superscript is added in the case of second order rate and equilibrium constants. By means of Eq. (21) conditions (19) and (20) become, respectively,

$$\frac{[S]}{[P]} > \frac{[S]_e}{[P]_e}, \tag{22}$$

$$\frac{[S]}{[P]} < \frac{[S]_e}{[P]_e}. \tag{23}$$

In the former or latter case the relative concentration of substrate molecules is higher or lower than the corresponding equilibrium concentration and the reaction goes in a forward $S \rightarrow P$ or reverse $S \leftarrow P$ direction, respectively. In this way we get a clear physical interpretation of conditions (9) and (10).

The right-hand-side of Eq. (21) shows that the value of the overall equilibrium constant is expressed by the quotient between product and substrate equilibrium concentrations. This means that any configuration of the rate constants has to meet the same equilibrium condition and the overall equilibrium constant is not subject to evolution [8]. In addition, it is widely accepted that second order rate constants (k_1^{+*} and k_3^{-*}) are also not subjected to evolution, because they depend on the kinetic properties of substrate and product [3,18]. In addition to this assumption, and considering the discussion of Heinrich et al. [8] on free energy changes in a pure internal enzyme transition, we take that the rate constants k_3^+ and k_1^- are constant, hence the equilibrium constants K_1^* and K_3^* are fixed parameters. Then, due to the relation between transition equilibrium constants given by Eq. (21), the equilibrium constant of the pure internal transition K_2 is a fixed parameter, too. Now the reaction has only one degree of freedom since five constraints are imposed on six rate constants. We choose the k_2^+ forward rate constant as the variable.

Substituting expressions for flux (6) and affinity (5) in Eq. (8) the entropy production in the transition $ES \leftrightarrow EP$, written in standard notation, becomes

$$\sigma\left(k_2^+\right) = nR\frac{b_2 - b_3/K_2}{\Sigma_a + \Sigma_b/k_2^+} ln\frac{K_2 b_2 + k_2^+ a_{23}}{b_3 + k_2^+ a_{23}}. \tag{24}$$

Eq. (11)

$$ln\frac{K_2 b_2 + k_2^+ a_{23}}{b_3 + k_2^+ a_{23}} = \frac{a_{23}(K_2 b_2 - b_3)k_2^+(k_2^+\Sigma_a + \Sigma_b)}{\Sigma_b(K_2 b_2 + k_2^+ a_{23})(k_2^+ a_{23} + b_3)}, \tag{25}$$

implicitly gives the optimal value of the forward rate constant k_2^+. In next section we check the validity of the MEP principle when applied to enzyme reaction for several enzymes.

4. Comparison with experimental results

In order to evaluate the validity of model predictions we turn to the β-Lactamase enzymes. These enzymes are interesting for two reasons. Firstly, it was concluded on the basis of kinetic studies that β-Lactamase enzymes are nearly fully evolved enzyme [5] and secondly, these enzymes play an important role in the resistance of pathogens to β-lactam antibiotics [5]. The kinetics of these enzymes has been well explored and we refer to data published in reference [5]. In reference [5] the reactions of β-Lactamase enzymes are taken as irreversible reactions. It can be described by the three state kinetic scheme given in Fig. 1, neglecting the k_2^- and k_3^{-*} backward rate constants.

For our calculations we use the reversible three state kinetic scheme from Fig. 1. The values of rate constants k_1^{+*}, k_1^-, k_2^+ and k_3^+ for the kinetic mechanism are taken from reference [5], for three different types of β-Lactamases: β-Lactamase I, PC1 β-Lactamase and RTEM β-Lactamase. The missing values of the backward rate constants k_2^- and k_3^{-*} are estimated in a reasonable manner. The irreversibility of

Table 1
Equivalency between the notation of rate constants and enzyme states used in Section 2 and the standard notation used in this section for the three state enzyme reaction.

1	2	3	k_{12}	k_{21}	k_{23}	k_{32}	k_{31}	k_{13}
E	ES	EP	$k_1^{+*}[S]$	k_1^-	k_2^+	k_2^-	k_3^+	$k_3^{-*}[P]$

Table 2
Rate constants used in three state enzyme reaction.

Enzyme	k_1^{+*} $[s^{-1}\mu M^{-1}]$	k_1^- $[s^{-1}]$	k_2^+ $[s^{-1}]$	k_2^- $[s^{-1}]$	k_3^+ $[s^{-1}]$	k_3^{-*} $[s^{-1}\mu M^{-1}]$	K_1^* $[\mu M^{-1}]$	K_2	K_3^* $[\mu M]$
PC1 β-Lactamase	22	196	173	1.3	96	22	0.11	133	4.4
RTEM β-Lactamase	123	11800	2800	47	1500	123	0.01	59	12
β-Lactamase I	41	2320	4090	141	3610	41	0.018	29	88

the kinetic scheme for β-Lactamases proposed in reference [5] implies that backward rate constants k_2^- and k_3^{-*} are much smaller than the corresponding forward rate constants, but they are not equal to zero. The value of k_3^{-*} is determined by using the assumption that second order rate constants are not subjected to evolution but fixed by diffusion constraints, so that the equality $k_1^{+*}=k_3^{-*}$ holds [8]. Values of the backward rate constants k_2^- are calculated by using the expression of the Michaelis-Menten constant K_M for reversible three state enzyme reaction

$$k_2^- = \frac{k_1^{+*}k_2^+K_M + k_1^{+*}k_1^-K_M - k_2^+k_3^+ - k_1^-k_3^{-*}}{k_1^- - k_1^{+*}K_M}, \tag{26}$$

where the value of k_3^{-*} is estimated as described above and the values of all other parameters are taken from reference [5]. Values of all rate constants used in our calculations are presented in Table 2.

In addition, the following data for substrate concentration and the total concentration of enzymes taken from reference [5] are also used in model calculations: $[S]=1500\mu M$ and $[E]_t=1\mu M$, respectively. The in vivo concentration of product, $[P]$, for β-Lactamase enzymes is not well defined. Therefore it is argued in reference [5] for these enzymes that the back reaction may be neglected because the concentration of product is negligible. Since we need a value of $[P]$ in our calculation we calculate it on the basis of experimentally determined values of the ratio $F=[EP]/[E]_t$. Considering the kinetic equations for substrate, product and enzyme states at stationary conditions, as well as the conservation equation for total enzyme concentration, we find

$$[P] = K_3^* \frac{F}{1-F\left(\frac{1}{K_2}+1\right)}. \tag{27}$$

The values of parameter F and the corresponding ratios of product and substrate concentrations are shown in Table 3. Moreover, a comparison of data in fourth and fifth column in Table 3 shows that all reactions of β-Lactamase enzymes are far from equilibrium.

Now we turn to our main goal and ask whether the value of the forward rate constant k_2^+ is optimal in the sense that entropy production is maximal possible in the internal transition. The comparison of our model predictions with the experimental results of reference [5] are given in Table 4.

Affinity in Eq. (4) is a logarithmic function of the ratio of products of reactant concentrations and corresponding rate constants. This is a free gas approach to the chemical potentials, which neglects interactions between molecules. The quantitative agreement between measured and predicted values of k_2^+ can be improved if one takes into account that enzymatic reaction takes part in water. Assuming that the contribution of the interactions between water and reacting molecules does not depend on the type of enzyme, we take the values of the predicted and experimentally measured forward rate constants, respectively, for PC1 β-Lactamase as a unit. The results presented in the fourth and fifth column in Table 4 shows a surprisingly good agreement between the theoretically determined and experimentally found values of the normalized forward rate constant k_2^+. Error is less than 15%.

5. Prigogine's principle and biological systems

The MEP principle is not the only one proposed for nonequilibrium processes. The Prigogine's principle of minimum entropy production [19] is better known and much more often discussed in scientific papers dealing with thermodynamics and even with possible biological applications [20].

Although both principles seek the extremum of the same function, the entropy production, they do not oppose each other since they are derived under entirely different constraints and have vastly different validity regions [11].

In its simplest form the Prigogine's theorem [21] is derived from linear relationships among two fluxes, j_1, j_2 and conjugated forces X_1, X_2 close to equilibrium, when Onsager's reciprocity relations are valid [22,23]. The Prigogine's approach is characterized by the fact that the experiment is designed in such a way that the driven flux j_2 spontaneously reaches a zero value in the stationary state. The chosen variable is secondary thermodynamic force X_2 (the output force). By elementary algebra one finds that the induced maximal value of the secondary (driven) force in the stationary state, named the static head state, is associated with minimum entropy production. Due to constraints used during this derivation, the static head state is necessarily close to the equilibrium stationary state, and can be regarded as the closest relative to the state of thermodynamic equilibrium. Indeed, the free energy transduction efficiency of the static head state, $\eta=-X_2j_2/X_1j_1$, is equal to zero. Biological processes that ensure living beings to remain alive have a requirement for nonzero free energy transduction and do not support blockages in the hierarchy of free-energy transducers. Prigogine's result predicts zero transduction of free energy, blocked free-energy transduction and zero efficiency in creating power needed to drive uphill thermodynamic synthesis of macromolecules or power needed to drive

Table 3
Estimated ratios of products and substrates concentrations and corresponding equilibrium values. Much smaller ratios between concentrations of products and substrates (fourth column) than corresponding ratio estimated for equilibrium (fifth column) indicate that reactions are far from equilibrium.

Enzyme	F	$[P]$[μM]	$[P]/[S]$	$[P]_e/[S]_e=K_1^*K_2K_3^*$
PC1 β-Lactamase	0.64	7.9	0.053	64
RTEM β-Lactamase	0.65	23	0.015	7.1
β-Lactamase I	0.51	95	0.063	43

Table 4
Optimal values of the rate constant k_2^+ calculated by MEP principle for enzymes PC1 β-Lactamase, RTEM β-Lactamase and β-Lactamase I, and comparison of predicted values with experimental data of reference [5].

Enzyme	$k_2^+[s^{-1}]$ (predicted)	$k_2^+[s^{-1}]$ (measured)	$k_2^+/281$ (theory)	$k_2^+/173$ (experiment)
PC1 β-Lactamase	281	173	1	1
RTEM β-Lactamase	4034	2800	14.3	16.2
β-Lactamase I	6669	4090	23.7	23.6

numerous biological nanomotors. Evidently, it cannot be applied to the most important processes in bioenergetics, biochemistry and to enzymatic processes that couple downhill and uphill free energy changes (for instance the operation of symport and antiport integral membrane proteins involved in ATP synthesis, potassium accumulation, sodium ejection and so on).

Applications of Prigogine's principle in biological systems often lead to impasse situations. An example can be seen in [24,25] where mitochondrial oxidative phosphorylation has been considered by using the formalism of linear nonequilibrium thermodynamics. Starting model had been a simple scheme of two loop network where phosphorylation is driven by respiration. For mitochondria incubated in the presence of oxygen and oxidizable substrates, but without ADP, the system naturally evolves to static head identified by author as the state 4 with vanishing net ATP synthesis and vanishing free-energy transduction efficiency. With a goal to breach this impasse and to show that optimal efficiency of the reaction is in accordance with Prigogine's principle the author has first introduced an additional nonspecified ATP-utilization flux and then assumed that the corresponding driving force is proportional only to phosphate potential [25]. In this way the author defined an optimal steady state of oxidative phosphorylation, still labeled as the static-head state and described as the natural state of optimal efficiency, although the basic premises of Prigogine's theorem, the vanishing of driven fluxes and open-circuited static-head situation were no longer fulfilled.

Another example of misapplication of Prigogine's principle in bioenergetics is more recent and more drastic. The claim that photosynthesis is in agreement with minimum entropy production [20] is based on simultaneous minimization of all of present forces [26], the mathematical procedure which can only ensure that thermodynamic equilibrium is established with zero entropy production.

In our test case of one loop enzymatic reaction Prigogine's theorem cannot be applied at all, since one loop enzymatic reaction is driven by one thermodynamic force (chemical affinity) and produces one flux (of substrate being transformed into product), while Prigogine's static head state with minimal entropy production can be established only with the minimum of one driving and one driven force.

In the case of processes close to equilibrium Onsager has proposed the principle of the least dissipation of energy [22,23]. Since linear nonequilibrium thermodynamics can be derived from the MEP principle [27,28] there is an equivalency between Onsager principle of the least dissipation of energy and the MEP principle [29,30]. One can conclude that Prigogine's theorem is a simple consequence of the MEP principle in the case of linear relationships between forces and fluxes close to equilibrium when the external constraint is imposed on the system. Ross and Vlad [31] also offer well grounded critique of attempts to present the principle of minimum entropy production as a fundamental law of nature relevant for biochemistry and molecular biology. They explicitly state that Prigogine's principle (not the theorem) is an artifact based on a mathematical error.

For nonlinear relationships between forces and fluxes, that are commonly found for enzyme transitions among functional states and enzyme catalyzed reactions, the MEP principle can still be applied [32] but Prigogine's theorem is no longer relevant [33]. For such a far-from-equilibrium case the increase in efficiency tends to be accompanied by an increase in free-energy dissipation as well [33]. We cannot expect metabolism to be at the same time maximally efficient and minimally wasteful of free energy. For instance, futile proton circuits produced by mitochondria in brown adipose tissue serve mainly to increase entropy production and maintain nonshivering thermogenesis — a very useful capability for small and young mammals, and may be even crucial for the evolutionary success of mammals [34], but impossible to explain from the point of view that the fitness arrow of biological evolution points toward minimal entropy production [35].

6. Conclusions

Strictly speaking, the MEP principle is valid in linear nonequilibrium processes [30]. It has been successfully applied to the problem of current distribution in linear electric networks [36,37] and flux distribution in the linear network of chemical reactions [38]. In spite of the fact that the MEP principle has not been theoretically rigorously proved [11,39–41] it has been successfully applied in numerous problems in physics, chemistry and biology (see reference [11] and references therein). We single out recent work done by Bordel and Nielsen [32]. Although the MEP principle is rigorously valid only in a network of linear biochemical reactions they have found that this principle works very well even in the case of a non-linear network of biochemical reactions. Among other applications of the MEP principle we single out ones used to predict rate constants in relevant transitions of bacteriorhodopsin light-cycle and bacterial photosynthesis [42] and an optimal angular position for the ATP-binding transition in the ATPase nanomotor [43]. The successful applications of the MEP principle to these intramolecular transitions (predictions in qualitative agreement or close to experimental values) has encouraged us to test this principle as physical selection principle acting in concert with enzyme biological evolution.

In this paper we have tested the entropy production that is tightly connected with biological evolution of β-Lactamase enzymes. Firstly, we have worked out a general proof, which shows that, when a single intra-enzymatic transition of an arbitrary enzyme reaction is taken into consideration, there always exists a unique maximum in entropy production with respect to the forward (k_{ij}) or backward (k_{ji}) rate constant of this transition. Secondly, we have applied the MEP principle to the internal transition $ES \leftrightarrow EP$ of three β-Lactamase enzymes, reactions which are described by the three state kinetic scheme. Our results show a surprisingly good agreement of the theoretically predicted and experimentally determined values of rate constant k_2^+ with small deviations of 15%. These results are in favor of our assumption that entropy production in internal transition is associated with biological evolution. The measured values of forward rate constants in β-Lactamase enzymes are very close to those obtained by using the MEP principle. This fact indicates that β-Lactamases are nearly fully evolved enzymes.

Burbaum et al. [4] have used Albery and Knowles approach to the problem of enzyme evolution briefly exposed in the Introduction. This method is based on maximum of metabolic flux and, as it is pointed in the Introduction, additional constraints or hypothesis must be added in order to avoid the divergence of forward rate constant. An additional hypothesis here is the model of catalysis in elementary step model described briefly in the Introduction. Within this model the assumption is $k_2^+ = k_2^-$. They have found that equality $k_2^+ = k_3^+$ should hold for fully evolved enzyme, if enzyme cycles far from equilibrium. Although the measured values of these forward rate constants are comparable for each enzyme we note that assumption $k_2^+ = k_2^-$ is not fulfilled (see Table 2).

General enzyme cycles can be described with kinetic schemes much more complex than the three state scheme. Our method can be applied to these schemes, too. Qian aptly mentioned [44] that without free energy dissipation many cellular networks cannot function. The aim of our further work will be to check the validity of the MEP principle as the physical connection with biological evolution of enzymes and cellular networks with more complex kinetic schemes for free-energy transduction.

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