THE RELEVANCE OF MAXIMUM ENTROPY PRODUCTION PRINCIPLE AND MAXIMUM INFORMATION ENTROPY PRINCIPLE IN BIOLOGY

P. Županović, D. Juretić

*University of Split, Faculty of Science, Teslina 12, 21000 Split, Croatia

ABSTRACT

Prigogine and Wiame noticed correctly that biological processes are irreversible and as such should be described within irreversible thermodynamics. They took the entropy production as the basic quantity for the description of biological processes. In contrast to Prigogine. who suggests the principle of minimum entropy production as a relevant principle for the biology, we take maximum entropy production principle as a relevant one. We argue here that maximum entropy production principle is more suitable than minimum entropy production principle for a description of energy transduction in biological systems.Our basic assumption is that the biological evolution through enzyme catalysis, is accompanied by an entropy production increase in internal transitions and with the Shannon's information entropy increase of all enzyme states. In such a way the increase of entropy production is understood as being tightly coupled to biological evolution. Fully evolved enzymes are characterized by maximum possible entropy production in internal transitions.

INTRODUCTION

In this paper we examine whether the distribution of optimal rate constant values for transitions among functionally important states in enzymes can be predicted by maximum entropy production (MaxEP) principle, by maximum of Shannon information entropy (MaxEnt) or as the combination of these two principles.

The MaxEP and MaxEnt principles applied to Michaelis-Menten kinetics of β-Lactamase enzymes [1,2] give a good agreement of optimal rate constant values for internal transition ES → EP with experimentally determined values. We also found that the functional design of rotary enzyme ATP synthase is consistent with the MaxEP and MaxEnt principle applied in combination [3] to the extent that predicted optimal angular position for the ATP-binding transition agrees within experimental error with the experimental value.

In the Discussion section we maintain that the MaxEP principle is much more relevant than Prigogine's principle [4,5]. Furthermore, the successful application of the MaxEP and MaxEnt principle, reviewed in this paperr, argues for the point of view that physical and biological evolution cannot be considered separately one from another.

ENTROPY PRODUCTION FOR MICHAELIS-MENTEN KINETICS

In often used three state model for enzyme kinetics (Michaelis-Menten kinetics, Fig. 1), the internal transition ES↔EP is the only one not directly connected with a substrate or product concentration. Our conjecture here is that biological evolution, within fixed concentrations of substrate and product molecules, is accompanied with an increase of the entropy production in the internal transition. For fully evolved enzyme (the "perfect enzyme" concept of Albery and Knowles [6]) maximum entropy production is expected to be associated with that transition. We also propose that biological evolution is accompanied with an increase in Shannon information entropy of the entire cyclic reaction scheme. The perfect, fully evolved enzymes, are rare in nature, if they exist at all, and we do not expect to find more than approximate correspondence among predicted optimal rate constants by using the MaxEP or MaxEnt principle and measured rate constants for enzymes considered to be highly evolved. The MaxEP principle can be used as a test whether the enzyme has approached a fully evolved state or not, but cannot be considered as an alternative to the biological selection and evolution.

Enzyme reactions involve metabolic fluxes (J) and thermodynamic forces (X) that govern these fluxes. The associated entropy production rate is defined as the product of the metabolic flux and the corresponding thermodynamic force, divided by absolute temperature

$$\sigma = \frac{JX}{T}. \quad (1)$$

For the three-state model (Fig. 1), J is the net flux of any given transition, because there is only one cycle and only one flux (which must be the same for all transitions in accordance with Kirchhoff's junction rule). For given substrate and product concentrations, the total thermodynamic force of the overall reaction is a constant. The sum of the affinities (i.e. thermodynamic forces) associated with chosen transitions equals the total thermodynamic force X (in accordance with Kirchhoff's loop rule). One of our basic results from previous research [1] shows that there is a unique maximum for the entropy production of any given transition with respect to variation in its forward rate constant. This is because the

associated transition flux and affinity are, respectively, monotonically increasing and decreasing functions of the forward rate constant. In other words, there is a simple trade-off between thermodynamic flux and force.

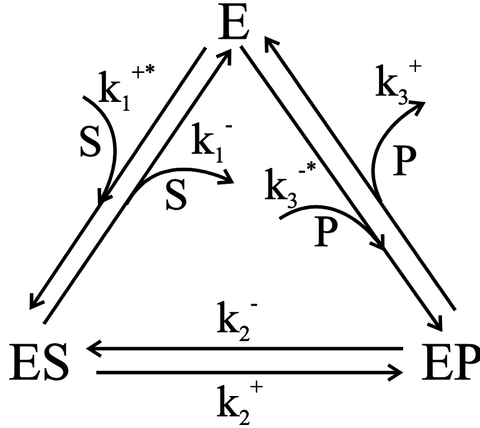

Figure 1. Michaelis-Menten reversible kinetic scheme.

Here we calculate the entropy production of the internal enzyme transition ES↔EP, and the Shannon entropy of the entire reaction, as functions of the forward rate constant k_2^+. The net metabolic flux for ES↔EP is

$$J \equiv \frac{d[P]}{dt} = k_2^+[ES] - k_2^-[EP] \,, \qquad (2)$$

where [P],[ES] and [EP] are the concentrations of product and complex states ES and EP, respecively. Thermodynamic force (or affinity) is the difference in chemical potentials between states ES and EP,

$$A_{23} = RT \ln\left(K_2 \frac{[ES]}{[EP]} \right), \qquad (3)$$

where R is the gas constant and K_2 is the equilibrium constant for ES↔EP. The affinity (3) is a function of the complex concentrations [ES] and [EP].

After lenghtly, bur otherwise straightforward calculation, we get entropy production as a function of forward kinetic constant in the internal transition,

$$\sigma(k_2^+) = R[E]_t \frac{k_2^+(DK_2 - F)}{K_2(Gk_2^+ + H)} \ln\left(K_2 \frac{Ck_2^+ + D}{Ek_2^+ + F} \right), \qquad (4)$$

Here $A=k_3^+ + k_1^-/K_2$, $B=k_3^+k_1^-$, $C=(k_1^{+*}[S]+k_3^{-*}[P])/K_2$, $D=k_3^+k_1^{+*}[S]$, $E=CK_2$, $F=k_1^-k_3^{-*}[P]$, $G=A+C+E$, $H=B+D+F$.

SHANNON INFORMATION ENTROPY FOR MICHAELIS-MENTEN KINETICS

The Shannon information entropy of the enzyme model in Fig. 1 is defined as

$$H = -\sum_{i=1}^{3} p_i \ln(p_i) \,, \qquad (5)$$

where p_i (i=1...3) are probabilities that the enzyme is found in one of its functional states (E , ES or EP, respectively). These probabilities are given by:

$$p_i = \frac{[X_i]}{[E]_t} \,, \qquad (6)$$

where $[E]_t$ is the total enzyme concentration and $[X_i]$ are the concentrations of the enzyme species E, ES or EP (for i=1,2,3, respectively).

Similiarly to entropy production we get the Shannon information entropy as a function of k_2^+

$$H(k_2^+) = -\left(\frac{1}{k_2^+G + H}\right) \left[(k_2^+A + B)\ln\left(\frac{k_2^+A + B}{k_2^+G + H}\right) + (k_2^+C + D)\ln\left(\frac{k_2^+C + D}{k_2^+G + H}\right) + (k_2^+E + F)\ln\left(\frac{k_2^+E + F}{k_2^+G + H}\right) \right] \qquad (7)$$

COMPARISON OF PREDICTED OPTIMAL VALUES OF FORWARD KINETIC CONSTANT WITH EXPERIMENTAL RESULTS

Optimal values of the forward rate constant k_2^+ predicted by MaxEP and MaxEnt are then obtained from the conditions

$$\frac{d\sigma}{dk_2^+} = 0 \,, \qquad (8)$$

$$\frac{dH}{dk_2^+} = 0 \,. \qquad (9)$$

The predicted and observed value of forward kinetic constants for three types of β-Lactamase enzymes are shown in Table. 1.

Table 1. The comparison of experimental and predicted values of the forward rate constants k_2^+ for three types of β-Lactamase enzymes.

Enzyme	k_2^+ [s^{-1}] (MaxEP)	k_2^+ [s^{-1}] (MaxEnt)	k_2^+ [s^{-1}] (Observed)
PC1 – β Lactamase	281	94.5	173
RTEM β Lactamase	4034	1091	2800
β Lactamase I	6669	3548	4090

The values of k_2^+ predicted by MaxEP and MaxEnt are of the same order of magnitude as the observed values.

MAXENT AND MAXEP RELEVANCE FOR THE FUNCTIONAL DESIGN OF THE ROTARY ENZYME ATP

ATP synthase is an important biomolecular nanomotor. From an evolutionary viewpoint it is a very ancient secondary proton pump, which exploits the proton motive force created by respiration or photosynthesis to drive the synthesis of adenosine triphosphate (ATP), the most commonly used "energy currency" in living cells. ATP synthase is embedded in the inner membrane of mitochondria or in the thylakoid membrane of chloroplasts. ATP is formed from adenosine diphosphate (ADP) and inorganic phospate (P), assuming that activation energy is available. This activation energy is stored and released as elastic energy in the stalk-like axle of the ATP synthase nanomotor. The rotary mechanism is well understood [7]. The stator is an ensemble of three structural subunits. Translocation of protons through this protein, driven by the transmembrane proton gradient, is accompanied by a stepped rotation of the stalk-like axle. Each 120° clockwise (or counter-clockwise) rotation is accompanied by the synthesis (or hydrolysis) of ATP. Here we will consider only the ATP synthase of chloroplast thylakoid membranes.

The number of protons translocated through the thylakoid membrane that is necessary for the synthesis of one ATP molecule is called the gearing ratio, $g \equiv H^{+} / ATP$. The gearing ratio g is related to the free energy input E per revolution,

$$E = 3g\Delta\mu_{H^{+}} \qquad (10)$$

where

$$\Delta\mu_{H^{+}} = 2.3RT\Delta pH - F\Delta\Psi, \qquad (11)$$

is the transthylakoid proton motive force. F is the Faraday constant, while ΔpH and $\Delta\Psi$ are the transmembrane differences in pH and electric potentials, respectively. The 120° stalk rotation has a short ($\approx 2ms$) pause, called the catalytic dwell, at a certain relative angular position of stalk, denoted by κ (with $0 \le \kappa \le 1$). The catalytic dwell is so-called because it is associated with the internal transition (synthesis or hydrolysis of ATP) of ATP synthase. In accordance with our starting assumption this internal transition is most sensitive to evolution. Therefore we take κ as the variable that is optimized during evolution. The free energy (E) partly depends on external conditions (the difference between pH factors outside and inside of the membrane), and we take this to be an adjustable parameter as explained below.

We describe the synthesis and hydrolysis of ATP using the five-state kinetic model shown in Fig.4. The problem can be solved analytically, either by solving the steady-state rate equations directly [8] or by using Hill's diagram method [9]. Using experimental data obtained by Pänke and Rumberg [8, 10], we calculated the state probabilities $p_E(i|\kappa)$, the forward fluxes,

$$J_{E+}(\kappa) = k_{Esyn}(\kappa)\, p(O*P*ADP|\kappa)_E \qquad (12)$$

and the backward fluxes

$$J_{E-}(\kappa) = k_{E\,hyd}(\kappa)\, p(O*ATP|\kappa)_E. \qquad (13)$$

Rate coefficients $k_{E\,syn}(\kappa)$ and $k_{E\,hyd}(\kappa)$ are calculated within the transition state theory [11] and are given by

$$k_{E\,syn}(\kappa) = k^{0}_{syn} \exp(\kappa E / 3RT), \qquad (14)$$

$$k_{E\,hyd}(\kappa) = k^{0}_{hyd} \exp(-(1-\kappa)E / 3RT). \qquad (15)$$

The values of specific binding change constants $k^{0}_{syn}(\kappa) = 1.15\cdot 10^{-3} s^{-1}$ $k^{0}_{hyd}(\kappa) = 4.5\cdot 10^{5} s^{-1}$ are taken from [8, 10]. Under controlled experimental conditions, the enzyme was illuminated in the presence of 1mM ADP, 1 mM P and 10μM ATP at T=300K [8, 10]. Fixed kinetic rate constants are given in the legend of Fig. 4.

The number of ATP molecules produced per enzyme p per second is then

$$J_E(\kappa) = J_{E+}(\kappa) - J_{E-}(\kappa). \qquad (16)$$

The Shannon information entropy of state probabilities, and entropy production of the internal enzymatic transitions, are

$$H_E(\kappa) = -\sum_{1}^{5} p_E(i|\kappa) \log p_E(i|\kappa), \qquad (17)$$

$$\sigma_E(\kappa) = RJ_E(\kappa) \log \frac{J_{E+}(\kappa)}{J_{E-}(\kappa)}, \qquad (18)$$

respectively.

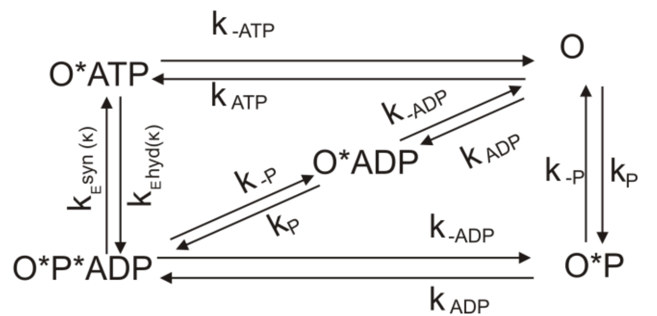

Figure 4. Kinetic model of ATP synthase cycle for transitions between enzyme open (O) states. O*P, O*ADP, O*ATP and O*P*ADP are states which bind P, ADP, ATP, and ADP respectively. Rate constants are expressed in same units when second-order rate constants are multiplied by substrate concentrations: k_{ATP}=20.8s^{-1}, k_{-ATP}=270s^{-1}, k_{ADP}=8900s^{-1}, k_{-ADP}=490s^{-1}, k_P =810s^{-1} and k_{-P}=2030s^{-1}.

Our hypothesis is that the information entropy $H_E(\kappa)$ and entropy production $\sigma(\kappa)$ of a fully evolved enzyme are maximized at a common value of κ, the relative angular position of the catalytic dwell. That is,

$$\frac{\partial H_E(\kappa)}{\partial \kappa} = 0, \quad (19)$$

$$\frac{\partial \sigma_E(\kappa)}{\partial \kappa} = 0. \quad (20)$$

In order to obtain a solution, we adjust the free energy input E until there is a common value of κ that satisfies both equations (19) and (20). In other words, we are simultaneously optimizing κ and E.

The numerical calculations are shown in Fig. 5. The solution yields optimal values $\kappa_{opt} = 0.598$ and $E_{opt} = 161.4\,\text{kJ/mol}$. The former value is very close to the empirical estimate $\kappa_{opt} = 0.6$ [8]. From equation (10), the optimal proton motive force $\Delta\mu_H = 13.4\,\text{kJ/mol}$ and calculated free energy $E_{opt} = 161.4\,\text{kJ/mol}$ corresponds to the gearing ratio g=4 observed in chloroplasts [12].

In summary, our calculations show that ATP synthase is a fully evolved enzyme in the sense of MaxEnt and MaxEP. It is also interesting that the optimal solution of MaxEnt and MaxEP coincides with an inflection point of the curve of ATP synthesis rate (J_E) versus proton motive force (Fig. 5); this feature allows fast metabolic control with respect to short-term changes in proton motive force, as well as a high optimal output/input free energy ratio of 69% [9].

CONLUSIONS

Energy transduction is the central concept in physics, from the energy conservation principle to thermodynamics. One of the better known results from non-equilibrium thermodynamics is Prigogine's theorem of minimal entropy production [6]. It is valid close to thermodynamic equilibrium. The theorem defines a non-equilibrium stationary state, caled the static head state. Non-equilibrium stationary states are the main interest to us here. Free-energy transduction and efficiency are zero in the static head stationary state, which can be considered as the closest non-equilibrium relative of the equilibrium state. Coupling downhill and uphill free energy changes is essential for all life, but this is impossible in the static head state. Life must look to other non-equilibrium steady states with a non-vanishing efficiency of free-energy transduction.

In contrast to static head state, which is the steady state with zero efficiency of free-energy transduction, MaxEP principle applied to β-lactamase enzymes and ATP synthase has predicted good order of magnitude relevant kinetic constants and reasonable efficiency of energy conversion.

Beside MaxEP we use MaxEnt principle and apply it to predict the probabilities of biomolecular states. It is a powerful inference algorithm for solving problems with incomplete available information. In physics, the whole of equilibrium statistical physics can be derived from this principle [13, 14]. At first sight, it might seem that biological evolution, by building ever more structurally complex macromolecules (i.e. of low configurational entropy), has proceeded in the direction of entropy decrease rather than entropy increase. But when we look at the kinetics of β-lactamase enzymes and functional design of ATP synthase, as we have done here, we find that biological evolution is consistent with MaxEnt. There is no contradiction with the second law. The evolutionary optimization of β-lactamase enzymes and ATP synthase can be interpreted as selection of the most probable functional design within the constraints considered here.

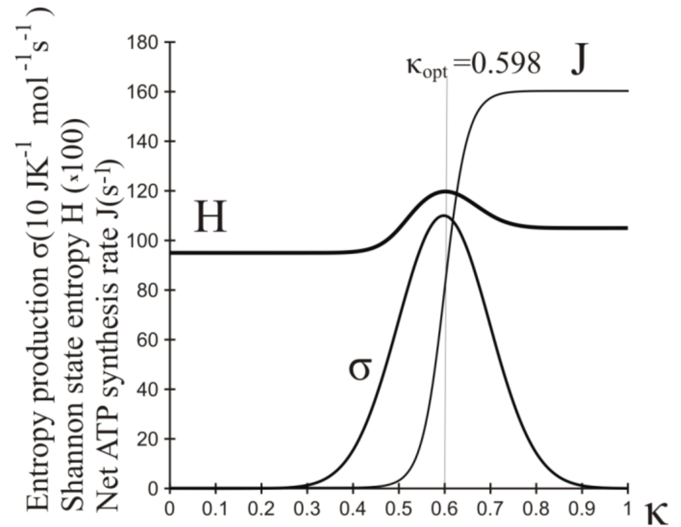

Figure 5. Information entropy of state probabilities, entropy production and net ATP synthesis rate as a function of relative angular position of catalytic dwell at optimal input free energy E_{opt}=161.4 kJmol^{-1}.

NOMENCLATURE

Symbol	Quantity	SI Unit
σ	Entropy production	$JK^{-1}s^{-1}$
J	Flux	s^{-1}
X	Thermodynamic force	J
[E]	Concentration of E species	m^{-3}
$k_i^{+(-)}$	Forward (backward) kinetic constant of ith transition	s^{-1}
A	Affinity	J
H	Information entropy	
p_i	Probability	
E	Free energy	J
g	Gearing ratio	
$\Delta\mu_{H^+}$	Proton motive force	J
F	Faraday constant	C mol^{-1}
κ	Relative angular position of catalytic dwell	

REFERENCES

[1] A. Dobovišek, P. Županović, M. Brumen, Ž. Bonačić-Lošić, D. Kuić and D.Juretić, Enzyme kinetics and the maximum entropy production principle. *Biophys. Chem.,* vol. 154, pp.49-55, 2011.

[2] A. Dobovišek, P. Županović, M. Brumen and D.Juretić, Maximum entropy production and maximum Shannon entropy as germane principles for the evolution of enzyme, in R. Dewar, R. Niven and K. Regenauer (eds.), Beyond the Second Law: Entropy Production and Non-Equilibrium Systems, Springer Verlag, to be published.

[3] R.C. Dewar, D. Juretić and P. Županović,The functional design of the rotary enzyme ATP synthase is consistent with maximum entropy production. *Chem. Phys. Lett*., vol 430, pp. 177–182, 2006.

[4] P. Glansdorff and I. Prigogine, Thermodynamic theory of structure, stability and fluctuations. John Wiley & Sons, New York, 1971.

[5] L.M. Martyushev and V.D. Seleznev, Maximum entropy production principle in physics, chemistry and biology, *Phys. Rep.*, vol 426, pp. 1-45, 2006.

[6] W.J. Albery and J.R. Knowles, Evolution of enzyme function and the development of catalytic efficiency *Biochemistry,* vol 15, pp. 5631–5640, 1976.

[7] P.D. Boyer, The ATP synthase –a splendid molecular machine. *Ann. Rev. Biochem*, vol 66, pp. 717-749, 1997

[8] O. Pänke and B. Rumberg, Kinetic modeling of rotary CF0F1-ATP synthase: storage of elastic energy during energy transduction. *Biochim. Biophys. Acta*, vol 1412, pp. 118-128, 1999.

[9] T. Hill, Free Energy Transduction and Biochemical Cycle Kinetics, Dover Publications, Inc. New York, 2005.

[10] O. Pänke and B. Rumberg, Kinetic modelling of the proton translocating CF0CF1-ATP synthase from spinach. *FEBS Lett.*, vol 383, pp. 196-200, 1996.

[11] R.C. Dewar, D. Juretić and P. Županović, The functional design of the rotary enzyme ATP synthase is consistent with maximum entropy production. *Chem. Phys. Lett.*, vol 430, pp. 177–182, 2006.

[12] P. Turina, D. Samoray and P. Graeber, H+/ATP ratio of proton transport-coupled ATP synthesis and hydrolysis catalysed by CF0F1-liposomes. *Eur. Mol. Biol. Org. J.*, vol 22, pp. 418-426, 2003.

[13] E.T.Jaynes, Information theory and statistical mechanics. *Phys. Rev*., vol 106, p. 620–630, 1957.

[14] E.T.Jaynes, Information theory and statistical mechanics II. *Phys. Rev*., vol 108, pp. 171–190, 1957.