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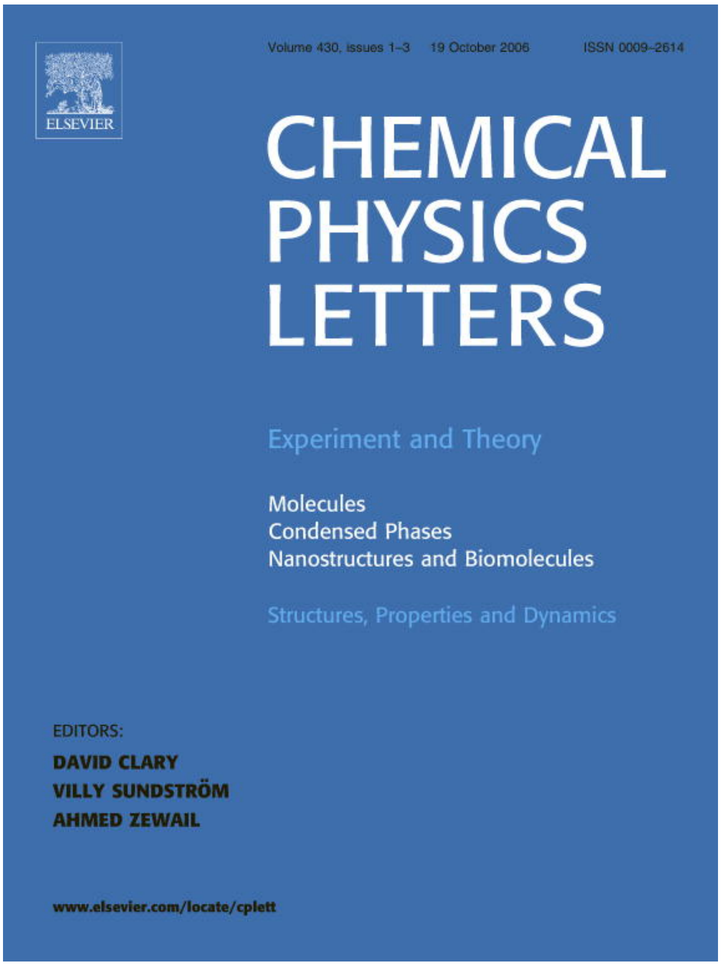
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The functional design of the rotary enzyme ATP synthase is consistent with maximum entropy production

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Abstract

We show that the molecular motor ATP synthase has evolved in accordance with the statistical selection principle of Maximum Shannon Entropy and one of its corollaries, Maximum Entropy Production. These principles predict an optimal angular position for the ATP-binding transition close to the experimental value; an inverse relation between the optimal gearing ratio and the proton motive force (*pmf*); optimal operation at an inflection point in the curve of ATP synthesis rate *versus pmf*, enabling rapid metabolic control; and a high optimal free energy conversion efficiency. Our results suggest a statistical interpretation for the evolutionary optimization of ATP synthase function.

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1. Introduction

Have living organisms evolved to produce entropy at a minimum or a maximum rate? It has been argued that the high efficiency with which living organisms use available free energy implies that they operate in the regime of minimum entropy production, e.g. [1]. But many far-from-equilibrium physical and chemical systems appear to operate at maximum entropy production (MaxEP) [2–7]. Because biological systems also often operate far from equilibrium [8] we might ask: do states of MaxEP also exist in biological systems? And if so, are those MaxEP states compatible with biologically optimal function, including high efficiency? Our objective here is to demonstrate that for at least one important biomolecular motor – the integral membrane protein F_0F_1-ATP synthase (ATPase) – the answer to both questions is 'yes'. Furthermore, by deriving MaxEP from the more fundamental principle of Maximum Shannon Entropy (MaxEnt), we wish to suggest a statistical interpretation for the evolutionary optimization of ATPase function.

ATPase is found universally in chloroplasts, bacteria and mitochondria. ATPase couples transmembrane proton translocation to ATP synthesis/hydrolysis. The general nature of the rotary mechanism of energy transduction by ATPase is now well understood [9]: proton-driven rotation of the F_0 motor relative to the $(\alpha\beta)_3$ stator ensemble of F_1 stores torsional elastic energy in the central stalk-like γ axle; 120° rotational relaxation of γ relative to $(\alpha\beta)_3$ induces conformational changes in the substrate binding sites of $(\alpha\beta)_3$ that are coupled to ATP synthesis, each complete revolution of γ yielding three ATP molecules.

We focused on two key functional parameters of ATPase that may have been optimized through natural selection of ATPase structural mutations: the gearing ratio ($g \equiv H^+/\text{ATP}$) [10], and the relative angular position of the catalytic dwell (κ) – a short ($\approx$2 ms) pause in the 120° rotational relaxation of γ during which ATP synthesis/hydrolysis occurs [11]. We examined whether κ and g can be predicted by a principle of MaxEP and, if so, whether the corresponding MaxEP functional state is compatible with biologically optimal function in ATPase.

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2. Methods

2.1. Kinetic model of ATPase

Optimization of κ and g by MaxEP (Section 2.2) was performed using a kinetic model of ATPase in spinach chloroplasts [12,13] in which κ and g were treated as free parameters. The model, which describes the storage and release of torsional elastic energy in the γ axle, serves to define the microscopic functional states of ATPase, analogous to the spectrum of microstates in equilibrium statistical mechanics [14,15].

Substrate exchange occurs at the open binding site of F_1, which can be in one of five functional states (Fig. 1): empty (O:) or binding either ATP (O:ATP), ADP (O:ADP), P_i (O:P), or P_i and ADP together (O:P.ADP). The net rate of the transition O:P.ADP → O:ATP equals the net rate of ATP synthesis (J), with associated forward and backward rate coefficients k_{syn} and k_{hyd}, respectively.

The original kinetic model [13] assumed F_0 contains a ring of 12 c-subunits with an elementary rotation step of 30° for each H^+ translocated, implying $g = 4$ as observed in chloroplasts [10]. However, the relevant functional parameter in the model is N, the mean number of proton-translocating rotary steps of F_0 during one complete revolution, in terms of which the gearing ratio is $g = N/3$. The value of N may be different from the number of c-subunits if, for example, slippage occurs between the proton flux and rotation of the F_0 motor [16]. We did not make any explicit structural assumptions about the number of c-subunits, and instead focused directly on optimization of g.

We extended the original model to arbitrary g as follows. The torsional angle of γ (in radians) is $\varphi = 2\pi j/3g$ where j is the number of elementary rotary steps of F_0. Torsional relaxation of γ by 120° occurs from state j to state $j - g$, in which the catalytic dwell occurs at $j - \kappa g$ ($0 \leqslant \kappa \leqslant 1$). As functions of κ, the rate coefficients k_{syn} and k_{hyd} were then calculated from transition state theory as

$$k_{syn}(\kappa) = k^0_{syn} D(\kappa) \tag{1}$$

$$k_{hyd}(\kappa) = k^0_{hyd} D(\kappa) \exp\left(-\frac{g\Delta\mu_{H^+}}{RT}\right) \tag{2}$$

(k^0_{syn}, k^0_{hyd} = specific binding change rate constants, R = gas constant, T = temperature) where

$$D(\kappa) = \sum_{j=-\infty}^{+\infty} p_j \exp\left[2g\alpha\kappa\left(j - \frac{g}{2}\kappa\right)\Big/RT\right] \tag{3}$$

in which

$$p_j = \frac{\exp[(\Delta\mu_{H^+} j - \alpha j^2)/RT]}{\sum_{j=-\infty}^{+\infty} \exp[(\Delta\mu_{H^+} j - \alpha j^2)/RT]} \tag{4}$$

$$\alpha = \frac{1}{2} N_A M^* \left(\frac{2\pi}{3g}\right)^2 \tag{5}$$

(N_A = Avogadro's number, M^* = torsional rigidity of γ) and $\Delta\mu_{H^+}$ is the transthylakoid proton motive force (*pmf*)

$$\Delta\mu_{H^+} = 2.3RT\Delta\text{pH} - F\Delta\Psi \tag{6}$$

(F = Faraday's constant, ΔpH and $\Delta\Psi$ are the outside-minus-inside differences in pH and electrical potential, respectively). For $g = 4$, Eqs. (1)–(6) reduce to those of the original model [13].

To a very good approximation the discrete sums in Eqs. (3) and (4) may be replaced by integrals, in which case

$$k_{syn}(\kappa) = k^0_{syn} \exp(\kappa g \Delta\mu_{H^+}/RT) \tag{7}$$

$$k_{hyd}(\kappa) = k^0_{hyd} \exp(-(1-\kappa) g \Delta\mu_{H^+}/RT) \tag{8}$$

These expressions depend on g and $\Delta\mu_{H^+}$ only through $g\Delta\mu_{H^+} \equiv E_{in}/3$ where E_{in} is the free energy input per complete revolution.

For a given value of κ, the steady-state probabilities of the five open states $p(i|\kappa)$ (i = O:, O:ATP, O:ADP, O:P, O:P.ADP) were obtained by numerical solution of the steady-state rate equations (Eqs. (4a)–(4e) in [13]), with k_{syn} and k_{hyd} calculated from Eqs. (1)–(6). An analytical solution may also be obtained using Hill's diagram method [8]. Values for other parameters were based on the controlled laboratory conditions for net positive ATP synthesis imposed in [12,13]. The enzyme is fully activated under continuous illumination in the presence of 1 mM ADP, 1 mM P_i and 10 μM ATP at $T = 293$ K. (Pseudo)-first-order rate constants for substrate association (i.e., second-order rate constants multiplied by substrate concentrations) are $k_{ATP} = 20.8\ \text{s}^{-1}$, $k_{ADP} = 8900\ \text{s}^{-1}$ and $k_P = 810\ \text{s}^{-1}$ for ATP, ADP and P_i binding respectively; corresponding dissociation rates are $k_{\text{-}ATP} = 270\ \text{s}^{-1}$, $k_{\text{-}ADP} = 490\ \text{s}^{-1}$ and $k_{\text{-}P} = 2030\ \text{s}^{-1}$. Other parameters fitted in [13] and considered fixed in our study are $k^0_{syn} = 1.15 \times 10^{-3}\ \text{s}^{-1}$, $k^0_{hyd} = 4.5 \times 10^5\ \text{s}^{-1}$, $M^* = 3.0 \times 10^{-20}$ N m (although under the approximation of Eqs. (7) and (8), k_{syn} and k_{hyd} are independent of M^*).

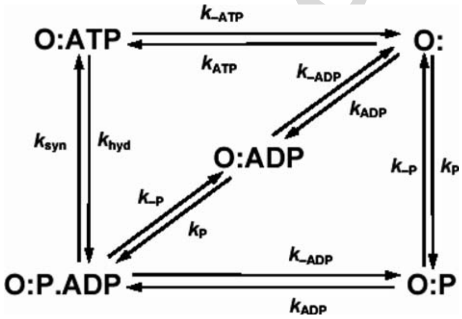

Fig. 1. Transitions between enzyme open states in the kinetic model of F_0F_1-ATP synthase. The five states are empty (O:) and binding either ATP (O:ATP), ADP (O:ADP), P_i (O:P) or ADP and P_i together (O:P.ADP). The transitions O:P.ADP → O:ATP and O:ATP → O:P.ADP correspond to ATP synthesis and hydrolysis, respectively, with associated rate coefficients k_{syn} and k_{hyd} (see Eqs. (1)–(6)). Other rate constants are fixed (see text).

The net ATP synthesis rate (number of ATP molecules produced per enzyme per second) was calculated as

$$J(\kappa) = J_+(\kappa) - J_-(\kappa) \tag{9}$$

where the forward and backward rates are

$$J_+(\kappa) = k_{\text{syn}}(\kappa) p(\text{O} : \text{P.ADP}|\kappa) \tag{10}$$

$$J_-(\kappa) = k_{\text{hyd}}(\kappa) p(\text{O} : \text{ATP}|\kappa) \tag{11}$$

2.2. Maximum Shannon entropy and maximum entropy production

We examined the hypothesis that the evolutionary optimization of κ and g may be described by a statistical selection principle of Maximum Shannon Entropy (MaxEnt) [14,15,17–19]. We applied MaxEnt to ATPase in two equivalent ways. In the first application, the target of selection is the probability distribution $p(\kappa)$ describing the wild type (most probable κ) plus its rarer mutations; in the second it is the net ATP synthesis rate, J. As shown below, the second application leads to the MaxEP principle.

In applying MaxEnt to the selection of $p(\kappa)$, we considered the microscopic functional state of the enzyme as specified by the pair (i, κ), with probability distribution

$$p(i, \kappa) = p(i|\kappa) p(\kappa) \tag{12}$$

where the open state distribution $p(i|\kappa)$ is calculated as in Section 2.1. From Eq. (12) the Shannon entropy of $p(i, \kappa)$ is

$$\begin{aligned} S &\equiv -\sum_{i=1}^{5} \int_0^1 d\kappa p(i, \kappa) \log p(i, \kappa) \\ &= -\int_0^1 d\kappa p(\kappa) \log p(\kappa) + \int_0^1 d\kappa p(\kappa) S_{\text{state}}(\kappa) \end{aligned} \tag{13}$$

where $S_{\text{state}}(\kappa)$, the Shannon entropy of $p(i|\kappa)$, is

$$S_{\text{state}}(\kappa) = -\sum_{i=1}^{5} p(i|\kappa) \log p(i|\kappa) \tag{14}$$

and the summations extend over the 5 open states. We conjectured that evolution has selected that $p(\kappa)$ which maximises S (Eq. (13)), the statistical interpretation being that the resulting $p(\kappa)$ is the most likely one among all those consistent with the steady-state kinetic model [14,15]. Maximization of S subject to normalisation of $p(\kappa)$ yields

$$p(\kappa) \propto \exp(S_{\text{state}}(\kappa)) \tag{15}$$

Thus the most probable value of κ is that for which $S_{\text{state}}(\kappa)$ is maximal.

In applying MaxEnt to the selection of J, we considered the Shannon entropy associated with transitions between open states, following the general formalism in [19] (simplified here). Specifically we first considered $p(i \to j|J)$, the conditional probability per unit time that an enzyme undergoes open state transition $i \to j$, given that the net ATP synthesis rate is J. Thus

$$J = \sum_{i,j=1}^{5} p(i \to j|J) n_{i \to j} \tag{16}$$

where $n_{i\to j} = +1(-1)$ for $i \to j =$ O:P.ADP → O:ATP (O:ATP → O:P.ADP) (Fig. 1) and $n_{i\to j} = 0$ otherwise. The Shannon entropy of $p(i \to j\ |J)$ is

$$S_{\text{trans}}(J) = -\sum_{i,j=1}^{5} p(i \to j|J) \log p(i \to j|J) \tag{17}$$

We maximised $S_{\text{trans}}(J)$ with respect to $p(i \to j|J)$ subject to constraint (16) and normalisation of $p(i \to j|J)$, yielding

$$p(i \to j|J) \propto \exp(n_{i\to j} X(J)/2RT) \tag{18}$$

where $X(J)$ is a Lagrange multiplier associated with Eq. (16) which therefore depends on J, and the factor $2RT$ is introduced for later convenience. Since $n_{j\to i} = -n_{i \to j}$, Eq. (18) implies

$$\frac{n_{i\to j} X(J)}{RT} = \log \frac{p(i \to j|J)}{p(j \to i|J)} \tag{19}$$

and hence

$$X(J) = RT \log \frac{p(\text{O} : \text{P.ADP} \to \text{O} : \text{ATP}|J)}{p(\text{O} : \text{ATP} \to \text{O} : \text{P.ADP}|J)} \tag{20}$$

If we define the entropy production of transition $i \to j$ by

$$\sigma(i \to j|J) \equiv \frac{n_{i\to j} X(J)}{T} \tag{21}$$

then the mean entropy production, given J, is

$$\sigma(J) \equiv \sum_{i,j=1}^{5} p(i \to j|J) \sigma(i \to j|J) = \frac{JX(J)}{T} \tag{22}$$

and so $X(J)$ plays the role of an affinity. Eq. (19) implies the 2^{nd} law like statement $\sigma(J) \geqslant 0$ [17–20]. From Eqs. (9)–(11), (20) and (22), the mean entropy production evaluated under the constraints of the steady-state kinetic model (i.e. by setting $J = J(\kappa)$) is

$$\sigma(\kappa) = RJ(\kappa) \log \left(\frac{J_+(\kappa)}{J_-(\kappa)} \right) \tag{23}$$

The above assumes that J is known (*via* κ) when in fact we wish to predict J_C, the most probable value of $J(\kappa)$ that is uniquely selected when κ is allowed to evolve freely under the remaining constraints (C) of the kinetic model. Following [19] we therefore also considered the conditional probability $p(i \to j|C)$ whose Shannon entropy is

$$S_{\text{trans}}(C) = -\sum_{i,j=1}^{5} p(i \to j|C) \log p(i \to j|C) \tag{24}$$

We found that for a given gearing ratio g, $\sigma(\kappa)$ (Eq. (23)) has a unique maximum ($\equiv \sigma_{\max}$) with respect to variations in κ, implying the upper bound constraint

$$\sigma(J_C) = \sum_{i,j=1}^{5} p(i \to j|C) \sigma(i \to j|J_C) \leqslant \sigma_{\max} \tag{25}$$

We then maximised $S_{\mathrm{trans}}(C)$ with respect to $p(i \to j|C)$ subject to constraint (25), the lower bound (2nd law) constraint $\sigma(J_C) \geqslant 0$, and normalisation of $p(i \to j|C)$, giving

$$p(i \to j|C) \propto \exp(\lambda\sigma(i \to j|J_C)) \tag{26}$$

where λ is a Lagrange multiplier associated with constraint (25); $\lambda \neq 0$ if $\sigma(J_C) = \sigma_{\max}$ and $\lambda = 0$ if $\sigma(J_C) < \sigma_{\max}$ [21]. Since J_C is uniquely selected under C, then J_C and C are equally informative about $i \to j$ so that

$$p(i \to j|C) = p(i \to j|J_C) \tag{27}$$

By comparing Eq. (26) and Eq. (18) using Eq. (21), we see that Eq. (27) implies the non-zero value $\lambda = (2R)^{-1}$, so the upper bound on $\sigma(J_C)$ is attained. Thus J_C corresponds to the maximum value of $\sigma(\kappa)$ and MaxEP applies.

In summary, these two applications of MaxEnt lead to the extremal conditions

$$\frac{\partial S_{\mathrm{state}}(\kappa)}{\partial\kappa} = 0 \tag{28a}$$

$$\frac{\partial\sigma(\kappa)}{\partial\kappa} = 0 \tag{28b}$$

We solved for the values of κ and g which simultaneously satisfy Eqs. (28a–b). Because both σ and S_{state} depend on g entirely through the free energy input per revolution

$$E_{\mathrm{in}} = 3g\Delta\mu_{\mathrm{H^+}}, \tag{29}$$

the solution to Eqs. (28a–b) defines optimal values for κ and E_{in}. The optimal efficiency was then calculated as the output/input free energy ratio

$$\eta = \frac{E_{\mathrm{out}}}{E_{\mathrm{in}}} \tag{30}$$

where E_{out} is the free energy output per complete revolution $= 111.3\ \mathrm{kJ\,mol^{-1}}$ under the controlled laboratory conditions of [13], and E_{in} is the optimal free energy input per revolution predicted by Eqs. (28a–b).

The sum of $\sigma(i \to j|J)$ (Eqs. (19) and (21)) over successive transitions $i \to j$ is formally identical to the stochastic path entropy production proposed recently by Seifert for Markov processes [20]. However our study goes one crucial step further by applying the variational principle of MaxEP to the mean entropy production (Eq. (23)). It is also important to note that Eq. (23) is not the total entropy production of the system (which includes *pmf* dissipation, for example) but only the component associated with the open state transitions O:P.ADP ↔ O:ATP that are coupled to ATP synthesis/hydrolysis.

3. Results and discussion

3.1. Optimal values of $\boldsymbol{\kappa}$ and $\boldsymbol{E_{in}}$

We found that Eqs. (28a–b) have the unique solution $\kappa = 0.598$, $E_{\mathrm{in}} = 161.4\ \mathrm{kJ\ mol^{-1}}$. Only for these parameter values do S_{state} (κ) and $\sigma(\kappa)$ have coincident maxima with respect to κ. This is illustrated in Fig. 2a for a *pmf* of $\Delta\mu_{\mathrm{H^+}} = 13.4\ \mathrm{kJ\ mol^{-1}}$ (2.4 pH difference equivalent) which, from Eq. (29) and $E_{\mathrm{in}} = 161.4\ \mathrm{kJ\ mol^{-1}}$, corresponds to an optimal gearing ratio equal to the observed value $g = 4$ in chloroplasts [10]. The maximum in σ reflects a trade-off between increasing flux J and decreasing affinity X (Fig. 2a). The maximum value of $S_{\mathrm{state}} = 1.168$ corresponds to the most uniform steady-state population distribution ($p_{\mathrm{O:}}$, $p_{\mathrm{O:ATP}}$, $p_{\mathrm{O:ADP}}$, $p_{\mathrm{O:P}}$, $p_{\mathrm{O:P.ADP}}$) = (0.04, 0.30, 0.49, 0.01, 0.16) consistent with the constraints (C) set by the steady-state kinetic model.

These results pertain to an ATP concentration of 10 μM favouring net ATP synthesis ($J > 0$). At an ATP concentration of 3 mM favouring net ATP hydrolysis ($J < 0$), we found that σ is maximized at the value $\kappa = 0.602$ (data not shown), very close to the value $\kappa = 0.598$ that maximizes σ when $J > 0$.

3.2. Is the functional behaviour predicted by MaxEnt biologically realistic?

We discuss five features of the MaxEnt functional state found above (solution of Eqs. (28a–b)). First, both values

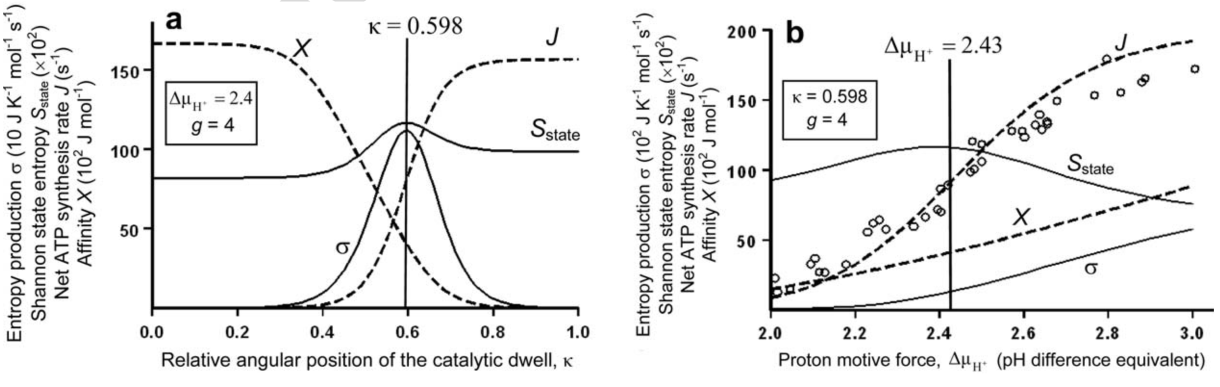

Fig. 2. Dependence of steady-state σ, S_{state}, J and X on (a) κ at $\Delta\mu_{\mathrm{H^+}} = 13.4\ \mathrm{kJ\ mol^{-1}}$ (2.4 pH difference equivalent), $g = 4$; (b) $\Delta\mu_{\mathrm{H^+}}$ at $\kappa = 0.598$, $g = 4$. (a) shows that σ and S_{state} have coincident maxima with respect to κ for $\kappa = 0.598$ and $E_{\mathrm{in}} = 3g\Delta\mu_{\mathrm{H^+}} = 161.4\ \mathrm{kJ\ mol^{-1}}$. In (b) the J–$\Delta\mu_{\mathrm{H^+}}$ curve has an inflection point at $\Delta\mu_{\mathrm{H^+}} = 2.43$ (pH difference equivalent), close to the value $\Delta\mu_{\mathrm{H^+}} = 2.4$ for which σ and S_{state} have coincident maxima with respect to κ. Open circles: measured ATP synthesis rates [12,13].

of κ (0.598 and 0.602) predicted by MaxEnt are consistent with the empirical estimate $\kappa = 0.6$ (angular position 72°) obtained by a visual fit of the kinetic model to measured ATP synthesis/hydrolysis rates in spinach chloroplasts over the range $\Delta\mu_{\mathrm{H}^+} = 2.0$–$3.0$ (pH difference equivalent) with $g = 4$ fixed [13].

Second, the transthylakoid *pmf* $\Delta\mu_{\mathrm{H}^+} = 13.4\ \mathrm{kJ\ mol^{-1}}$ (2.4 pH difference equivalent) for which MaxEnt predicts the observed gearing ratio $g = 4$ in chloroplasts [10] is consistent with *in vivo* estimates indicating a transthylakoid pH difference of about 2 (moderately acidic lumen) and an electrical potential difference representing 8–33% of the total *pmf* [22].

Third, from Eq. (30) MaxEnt predicts an optimal efficiency of $\eta = 0.69$ for all gearing ratios g. Thus, while here the ATPase efficiency is being optimized and not maximized, a high optimal efficiency is nevertheless predicted by MaxEnt. A similar conclusion applies to the initial reactions of bacterial photosynthesis [23]. We are unaware of independent experimental estimates of the efficiency defined by Eq. (30); our prediction that $\eta = 0.69$ for different g is thus open to further experimental testing.

Fourth, Eq. (29) and the unique value $E_{\mathrm{in}} = 161.4$ kJ mol^{-1} predicted by MaxEnt imply an inverse relationship between the optimal gearing ratio and the transthylakoid *pmf*:

$$g_{\mathrm{opt}} = \frac{161.4\ \mathrm{kJ\ mol^{-1}}}{3\Delta\mu_{\mathrm{H}^+}} \tag{31}$$

It is unlikely that g would adjust in this way to short-term fluctuations in $\Delta\mu_{\mathrm{H}^+}$. Rather we suggest that $\Delta\mu_{\mathrm{H}^+}$ might be interpreted here as the long-term average *pmf* under which g has evolved. That g and $\Delta\mu_{\mathrm{H}^+}$ are inversely related appears biologically reasonable, the system consuming less free energy per revolution (E_{in}) than it would if g were fixed as $\Delta\mu_{\mathrm{H}^+}$ increases. Eq. (31) represents another testable prediction of our approach.

Fifth, we found that the optimal value $E_{\mathrm{in}} = 161.4$ kJ mol^{-1} predicted by MaxEnt lies close to the value $E_{\mathrm{in}} = 164$ kJ mol^{-1} at which the curve of the ATP synthesis rate $J(\kappa = 0.598,\ E_{\mathrm{in}})$ *versus* E_{in} has an inflection point. From Eq. (29) it follows that, for a given gearing ratio g (and $\kappa = 0.598$), an approximately linear flux–force relation between J and $\Delta\mu_{\mathrm{H}^+}$ holds over an extended range of $\Delta\mu_{\mathrm{H}^+}$ values in the vicinity of the MaxEnt solution. At the inflection point J is maximally sensitive to $\Delta\mu_{\mathrm{H}^+}$, thus enabling rapid regulation of J under short-term fluctuations in *pmf*. In other words, the gearing ratio adjusts to the mean *pmf* (Eq. (31)) such that optimal metabolic control of J is achieved. This feature is illustrated in Fig. 2b for the case $g = 4$, where the inflection point occurs at $\Delta\mu_{\mathrm{H}^+} = 2.43$ (pH difference equivalent), very close to the value $\Delta\mu_{\mathrm{H}^+} = 2.4$ of the corresponding MaxEnt state (Fig. 2a). This prediction is also open to further experimental tests.

A priori one expects far-from-equilibrium flux–force relations to be highly non-linear [8]. Surprisingly, flux–force linearity has been observed in a number of far-from-equilibrium biological systems, and operation in the vicinity of inflection points has been proposed as a possible phenomenological explanation [24]. The close proximity we found between the MaxEnt state and the J–$\Delta\mu_{\mathrm{H}^+}$ inflection point suggests that MaxEnt may be the fundamental statistical principle which underpins this explanation.

In physics, MaxEnt provides a statistical interpretation of evolution as natural selection of the most probable system behaviour under given constraints [14,15,17–19]. In biology, MaxEnt might seem incompatible with macromolecular evolution which is traditionally viewed as leading to low probability structures (low configurational entropy) [1]. However, our results suggest that when it is applied to the probabilities of macromolecular functional states, MaxEnt is compatible with evolutionary optimization and so offers a common statistical interpretation of evolution in physics and biology.

4. Conclusion

A state of MaxEP exists with respect to variation in the functional design of ATPase. The MaxEP state is consistent with the observed design of spinach chloroplast ATPase and compatible with its optimal biological function, including high efficiency. The theoretical basis of our results in MaxEnt suggests that the evolutionary optimization of ATPase may be interpreted statistically as selection of the most probable functional design within the constraints of the model considered here. While our study is confined to one model of one biomolecular motor under controlled laboratory conditions, we suggest that MaxEnt may describe the functional design of biomacromolecules more generally.

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References

[1] T.G. Dewey, M.D. Donne, J. Theor. Biol. 193 (1998) 593.
[2] G.W. Paltridge, Q.J.R. Meteorol. Soc. 104 (1978) 927.
[3] A. Hill, Nature 348 (1990) 426.
[4] R.D. Lorenz, J.I. Lunine, P.G. Withers, C.P. McKay, Geophys. Rev. Lett. 28 (2001) 415.
[5] A. Kleidon, R.D. Lorenz (Eds.), Non-Equilibrium Thermodynamics and Entropy Production: Life, Earth and Beyond, Springer, Heidelberg, 2004.
[6] J. Whitfield, Nature 436 (2005) 905.
[7] P. Županović, D. Juretić, S. Botrić, Phys. Rev. E 70 (2004) 056108.
[8] T.L. Hill, Free Energy Transduction in Biology, Academic Press, New York, 1977.
[9] P.D. Boyer, Ann. Rev. Biochem. 66 (1997) 717.

[10] P. Turina, D. Samoray, P. Gräber, Eur. Mol. Biol. Org. J. 22 (2003) 418.

[11] H. Ueno, T. Suzuki, K. Kinosita Jr., M. Yoshida, Proc. Natl. Acad. Sci. USA 102 (2005) 1333.

[12] O. Pänke, B. Rumberg, FEBS Lett. 383 (1996) 196.

[13] O. Pänke, B. Rumberg, Biochim. Biophys. Acta 1412 (1999) 118.

[14] E.T. Jaynes, in: R.D. Levine, M. Tribus (Eds.), The Maximum Entropy Principle, MIT, Cambridge, MA, 1979, p. 15.

[15] E.T. Jaynes, Probability Theory: The Logic of Science, Cambridge University Press, Cambridge, 2003.

[16] G. Groth, W. Junge, Biochemistry 32 (1993) 8103.

[17] R.C. Dewar, J. Phys. A: Math. Gen. 36 (2003) 631.

[18] R.C. Dewar, in: A. Kleidon, R.D. Lorenz (Eds.), Non-Equilibrium Thermodynamics and Entropy Production: Life, Earth and Beyond, Springer, Heidelberg, 2004, p. 41.

[19] R.C. Dewar, J. Phys. A: Math. Gen. 38 (2005) L371.

[20] U. Seifert, Phys. Rev. Lett. 95 (2005) 040602.

[21] P. Ishwar, P. Moulin, IEEE Trans. Info. Theory 51 (2005) 3322.

[22] D. Kramer, C.A. Sacksteder, J.A. Cruz, Photosynth. Res. 60 (1999) 151.

[23] D. Juretić, P. Županović, Comp. Biol. Chem. 27 (2003) 541.

[24] K.J. Rothschild, S.A. Ellias, A. Essig, H.E. Stanley, Biophys. J. 30 (1981) 209.