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Research paper

Optimality principle for the coupled chemical reactions of ATP synthesis and its molecular interpretation

Sunil Nath

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

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ABSTRACT

Metabolic energy obtained from the coupled chemical reactions of oxidative phosphorylation (OX PHOS) is harnessed in the form of ATP by cells. We experimentally measured thermodynamic forces and fluxes during ATP synthesis, and calculated the thermodynamic efficiency, η and the rate of free energy dissipation, Φ. We show that the OX PHOS system is tuned such that the coupled nonequilibrium processes operate at optimal η. This state does not coincide with the state of minimum Φ but is compatible with maximum Φ under the imposed constraints. Conditions that must hold for species concentration in order to satisfy the principle of optimal efficiency are derived analytically and a molecular explanation based on Nath's torsional mechanism of energy transduction and ATP synthesis is suggested. Differences of the proposed principle with Prigogine's principle are discussed.

1. Introduction

Living systems are governed by physical and chemical processes that operate in a nonequilibrium steady state [1]. Attempts to describe the steady state and their transitions upon changes in external conditions have been analyzed based on nonequilibrium thermodynamics and kinetics [2–9]. This has led to characterization of the nonequilibrium steady state as one of minimum entropy production [10] or maximum entropy production or free energy dissipation [11,12], among other classifications [13,14], although no consensus could be reached [15,16] given the complexity of small systems and the inherent conceptual difficulties [17–19]. It was recommended for progress and practical usefulness that "someone probably from outside the field would need to identify a complex system and visualize fairly easily the physics so that the results could be generally understood without relying entirely on some abstruse mathematical proof" [15]. However, exact calculations of the energy conversion efficiencies and the free energy dissipation that could shed light on this aspect and stemmed *directly* from consolidated experimental measurements of both the relevant generalized flows/reaction rates and their generalized thermodynamic driving forces for a molecular biological process were unavailable, to the best of the author's knowledge. It is the purpose of this work to provide such new information embedded within a general physico-chemical framework that enables a quantitative analysis of biological energy transduction and suggests a molecular explanation of the results.

Demirel and Sandler reviewed the application of nonequilibrium thermodynamics to a host of physical, chemical and biological problems in an attempt to stimulate research in this field [20,21]. A generalized kinetic approach has also been developed to explore nonequilibrium steady states [22]. It is hoped that a generalized thermodynamic framework will offer a complementary systematic approach using thermodynamics to characterize the behavior and properties of nonequilibrium steady states. Related thermodynamic issues have also been addressed previously in *Chemical Physics Letters* [23] and elsewhere [24,25].

E-mail address: sunil_nath_iit@yahoo.com

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2. Experimental methods

Given that the fundamental coupled nonequilibrium processes of ATP synthesis by the central oxidative phosphorylation (OX PHOS) pathway are responsible for generating > 90% of the active (state 3) energy demand of ATP in cells, and also contribute significantly to the resting (state 4) metabolic rate, we chose to experimentally probe the mitochondrial state 3 to state 4 transition in OX PHOS (Fig. 1) by methods described earlier [26]. Briefly, rat liver mitochondria were incubated at 25 °C at a concentration of ~1 mg protein/mL. The reaction mixture contained 50 mM KCl, 50 mM Tris chloride buffer, pH 7.5, 2.5 mM EDTA, 7.5 mM succinate, varying concentrations of Mg^{2+} and Pi (5–10 mM), and 0.2–0.3 μg rotenone mg^{-1} protein. A low constant rate of oxygen consumption developed that corresponded to the resting steady state of static head called state 4. On addition of ADP (0.4 mM), the ADP began to phosphorylate to ATP, and due to the phenomenon of respiratory control arising from the coupling of the process of ATP synthesis to oxidation, the steady state respiration rate increased sharply (state 3). Similar experiments were performed in the presence of 7.5 mM ATP in the external medium. Aliquots were deproteinized by addition of phenol and adenine nucleotides and inorganic phosphate Pi were determined enzymatically [27,28] and the amount of oxygen consumed and ADP phosphorylated with time was quantitated. Upon exhaustion of the plug of ADP, the respiration returned to its idling mode of the state 4 resting rate (Fig. 1).

3. Results

The input reaction in the ADP-induced oxygen jump process shown in Fig. 1 is that of oxidation (O) of respiratory substrate (e.g. succinate or NADH + H^+) that runs downhill, while the output reaction consists of the "uphill" phosphorylation (P) of ADP to produce ATP. Here we are concerned with the evaluation of the P to O ratio, the efficiency of the coupled energy transduction process, η, and the dissipation function, Φ which is the product of the absolute temperature and the rate of entropy production. The input oxidation reaction occurs at a rate J_O along its generalized thermodynamic driving force of the reaction affinity of oxidation (A_O, taken as positive) while the output phosphorylation reaction takes place at rate J_P against its phosphorylation affinity (A_P, taken as negative), which is equal in magnitude but opposite in sign to the Gibbs energy of phosphorylation, ΔG_P. With these considerations, the percent efficiency of the coupled oxidative phosphorylation process is given by the equation

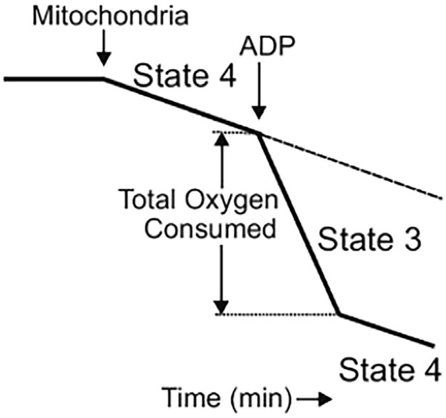

Fig. 1. Schematic of the state 3 to state 4 transition in ATP synthesis by oxidative phosphorylation (OX PHOS).

$$\eta = -\frac{J_P A_P}{J_O A_O} \times 100 \tag{1}$$

If the system is in a nonequilibrium steady state, and the processes are highly coupled (as is generally the case in physiological OX PHOS, with degree of coupling, q > 0.95) [1,6] such that other side reactions and transmembrane leaks are negligible, then the rate of free energy dissipation is governed by the dissipation function, Φ and is given by the equation

$$\Phi = T\sigma = J_O A_O + J_P A_P \tag{2}$$

where σ is the rate of entropy production within the system. The second law of thermodynamics dictates that η < 1 and Φ > 0 [6]. Combining Eqs. (1) and (2), the output phosphorylation reaction can be eliminated, and the following relationship is obtained between the thermodynamic efficiency and the dissipation function

$$\eta = 1 - \frac{\Phi}{J_O A_O} \tag{3}$$

The affinity of the oxidation reaction can be taken as constant under the operating conditions (155 kJ/mol for succinate as substrate). The intuitive result of some workers that a decreasing Φ should correspond to an increasing η [29] would indeed occur if J_O is also taken as constant, as can be clearly seen from Eq. (3). However, there is no reason to expect this constancy of reaction rate in a typical oxygen-jump experiment involving respiratory control in ATP synthesis by OX PHOS (Fig. 1). In fact, the extent to which the rate of the input oxidation reaction, J_O increases with increase in the rate of free energy dissipation Φ will determine whether η increases or decreases as Φ is increased. A test of this aspect requires measurement of both thermodynamic flows and forces in the model OX PHOS system described above.

Attempts to record flow-force values during the state 3 to state 4 transition [30] were rendered difficult by the rapid reaction rate that consumed the ADP within seconds, and the system showed two-state behavior between state 3 and state 4. However, the addition of external ATP enabled measurement by slowing the kinetics and Fig. 2a and b show the results of experiments in the presence of 7.5 mM ATP. The phosphorylation affinity was calculated by the equation

$$A_P = A_P^0 + RT\ln\frac{[ATP]}{[ADP][Pi]} \tag{4}$$

using the standard free energy change found earlier [31]. Fig. 2a shows the measured values of the P to O ratio as a function of the affinity ratio during the state 3 to state 4 transition with succinate as respiratory substrate. Values of η using the experimentally measured P/O and A_P/A_O were calculated from Eq. (1) and are shown in Fig. 2b. Values of Φ using Eq. (2) are also plotted in Fig. 2b. Consensus values of the state 3 P/O and η for succinate as substrate are indicated by a bold circle in Fig. 2. Results from a linear nonequilibrium thermodynamics (LNEQT) analysis using experimental values of the various conductances and Onsager coefficients for the redox and ATPase proton pumps [24] with ~10% slip are also shown in Fig. 2a. The overall efficiencies calculated using LNEQT as the P/O times the affinity ratio are also plotted as a dashed curve in Fig. 2b. The LNEQT analysis with q = 0.963 agrees well with the experimental values in the region around state 3 but deviates from experiment as the system is displaced towards state 4. Addition of leak conductances or alteration of the values of Onsager phenomenological coefficients (L_{ij}) of the system within the experimental range given by Jou and Ferrer [24] did not change the lack of complete agreement between theory and experiment.

The results shown in Fig. 2 reveal that the experimentally determined ratio of reaction rates J_P/J_O as well as the efficiency, η fall