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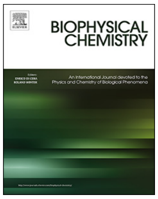

Molecular mechanistic insights into coupling of ion transport to ATP synthesis

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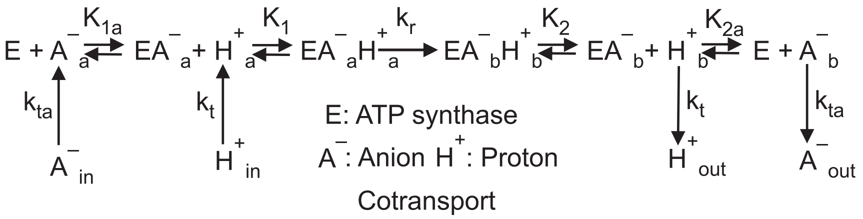
HIGHLIGHTS

- An enzyme kinetic model for ion transport and ATP synthesis in the FO-portion of ATP synthase is developed
- The model explains experimental data on the pH dependence of ATP synthesis over the entire range
- Analysis of the model provides a wealth of kinetic and mechanistic insights into ATP synthesis
- Wyman's linked functions offer a new approach to discriminate between models of energy coupling
- The results are inconsistent with Mitchell's chemiosmotic theory and in agreement with Nath's ATP torsional mechanism

GRAPHICAL ABSTRACT



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ABSTRACT

A wealth of molecular mechanistic insights has been provided into the coupling of ion transport to ATP synthesis based on a two-ion theory of biological energy coupling. A kinetic scheme that considers the mode of functioning of a single F_1F_O-ATP synthase molecule with H^+-A^- cotransport and unidirectional rotation of the c-rotor in the membrane-bound F_O-portion of the enzyme has been developed. Mathematical analysis leads to a detailed enzyme kinetic model applicable to a population of molecules which is compared with experimental data on the pH dependence of ATP synthesis. The model agrees well with the experimental data, and a single equation with a single set of standard enzymological kinetic parameters has been shown to explain the experimental data over the entire range of conditions for the chloroplast ATP synthase. The analysis gives novel insights into kinetic and mechanistic characteristics of ATP synthesis in F_O. These include an order imposed on ion binding and unbinding events in F_O, the essential role of the anion in direct activation of the ATP synthase (in addition to its role as a permeant ion), and the integration in a novel way of the functions of cooperativity and cotransport of dicarboxylic acid anions and protons during physiological ATP synthesis. Further, Wyman's pioneering classical work on the thermodynamics of linked functions has been shown to offer a new approach to distinguish between various models of energy coupling in ATP synthesis. All these results have been found to be inconsistent with Mitchell's chemiosmotic theory and are shown to be in agreement with Nath's torsional mechanism of energy transduction and ATP synthesis.

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1. Introduction

F_1F_O-ATP synthase is the universal enzyme in biological energy conversion located in the coupling membranes of animal mitochondria, plant chloroplasts and bacteria. Most biochemistry textbooks in the past have described the coupling of redox and ATP synthesis chemical reactions using Mitchell's chemiosmotic theory [19, 20]. However, in a recent trilogy of papers in *Biophysical Chemistry* it has been shown conclusively that this theory is false and is beset with a large number of intractable difficulties that cannot be overcome by it because these difficulties are inextricably linked to the fundamental structure of the theory. Twenty years ago, an alternative Nath's torsional mechanism of energy transduction and ATP synthesis ([21–23] and references therein; [27, 28]) was first proposed and developed in conceptual clarity and details over the years to extirpate the above problems. The theory is consistent with the scientific laws and principles as well as with all the accumulated experimental data. This new theory of energy coupling has been described in recent review articles and in new editions of some textbooks by other authors and researchers [1, 3, 7, 11, 13–15, 30, 33, 37, 39].

Several prominent scientists of the 20th century had indeed pointed out several flaws and inconsistencies in Mitchell's chemiosmotic theory ([8, 31, 34–36] among many others). However, despite enormous efforts, an alternative theory to replace Mitchell's chemiosmotic theory was not discovered until the formulation and logical development of Nath's torsional mechanism of energy transduction and ATP synthesis and his two-ion theory of biological energy coupling ([21] and references therein).

In this paper, mechanistic insights into the transport steps in the membrane-bound F_O-portion of ATP synthase are obtained. In earlier work, Jou and colleagues analyzed the action of inhibitors and uncouplers on the F_O-part of ATP synthase by a macroscopic thermodynamic approach [16, 18]. They appear to have been among the first to point out that the quantitative action of couplers and uncouplers could be "dependent also on the transport properties of the membrane" and that their formalism could account also for the functioning of ADP-ATP translocases. Here, following the suggestion of one of the reviewers of [21], an attempt is made to formulate a detailed enzyme kinetic model that mathematically describes the coupling of anion and proton transport steps to ATP synthesis. Development of such a kinetic model also opens up possibilities of comparison of the model with at least some of the available kinetic data on the rate of ATP synthesis [26, 29, 32]. The molecular mechanism of *uncoupling* of ion transport from ATP synthesis is treated in a companion paper.

Finally, Wyman's pioneering work on the thermodynamics of linked functions [6, 38] is shown to offer a new approach that helps discriminate between the proposed alternative models of biological energy coupling.

2. Experimental kinetic data

Data on ATP synthesis by chloroplasts and purified F_1F_O-ATP synthase reconstituted into liposomes has been collected and analyzed [21, 26, 29, 32]. In the experiments on the reconstituted chloroplast ATP synthase, the system was energized by a succinic acid concentration of 20 mM, and the coupling between ion transport and ATP synthesis was studied at 23 °C. In particular, the dependence of the rate of ATP synthesis was quantified as a function of both pH_{in} from 4.5 to 5.8 and pH_{out} from 7.9 to 9.3 as represented in Fig. 1.

3. Enzyme kinetic model of ATP synthesis

In the third paper [21] of a trilogy of articles in *Biophysical Chemistry* on the thermodynamics and molecular mechanism of ATP synthesis, the extensive proteoliposome data of Soga et al. [32] on reconstituted thermophilic *Bacillus* TF_1F_O was analyzed from first principles. It was shown that the *simplest* model that represents their data set in its entirety was one in which the rate of ATP synthesis in their system varies quadratically with the driving forces of the H^+ *and* K^+ concentration gradients. A second order polynomial fitted to the data of Soga et al. [32] in the region of net ATP synthesis led to an almost perfect fit with a mean R^2 value of 0.995. The important biological implication that an enzyme conformation with *two* different ionic species bound in the F_O portion of ATP synthase – H^+ and the countercation K^+ for the TF_1F_O enzyme system in *Bacillus* – was the only energetically and kinetically competent intermediate in ATP synthesis did not escape notice, and this key aspect was captured by Nath's two-ion theory of biological energy coupling [21].

As summarized above, the heart of the matter concerned the function of the F_1F_O-ATP synthase as a cotransporter and the direct role of the coanion in *activating* the ATP synthase in addition to serving as a permeant ion under physiological conditions. The author's longstanding experimental research program had revealed the identity of the elusive

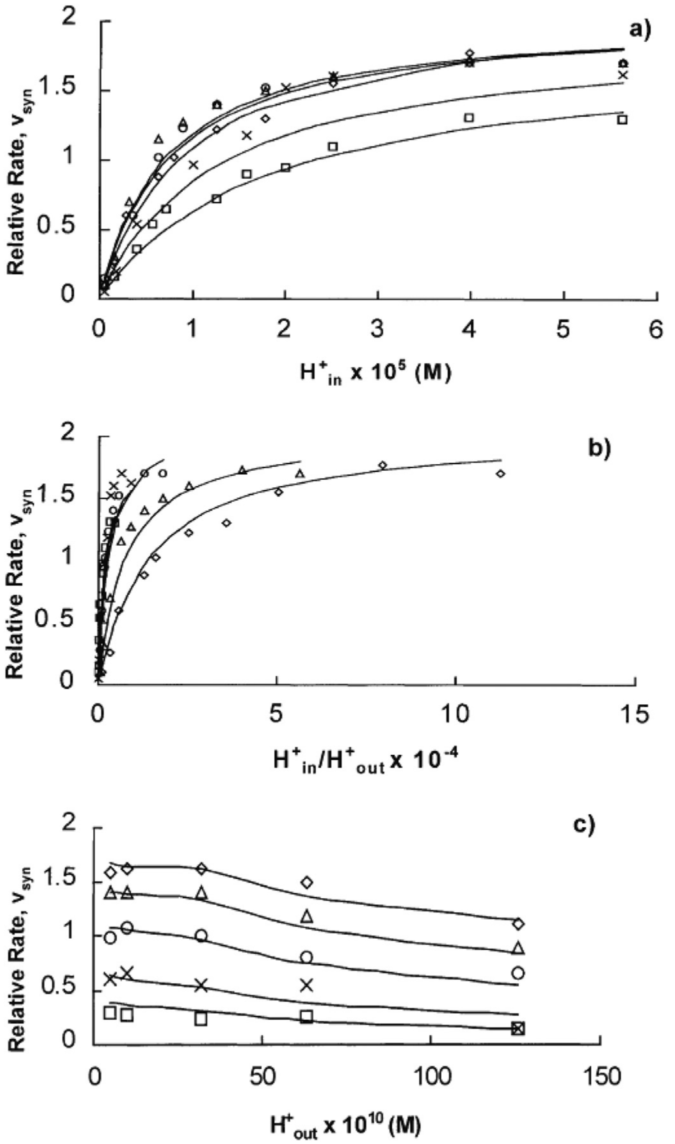

Fig. 1. Relative rates of ATP synthesis for chloroplast ATP synthase (CF_1F_O) as a function of a) H^+_{in}, b) H^+_{in}/H^+_{out} and c) H^+_{out}. Bold curves represent computed rates using Eq. (16) of the enzyme kinetic model in Section 3 with the parameter values tabulated in Table 1. Points represent experimental data at 23 °C. a) and b) pH_{out}: 9.3 (◊); 9.0 (Δ); 8.5 (○); 8.2 (×); 7.9 (□). c) pH_{in}: 4.5 (◊); 4.8 (Δ); 5.1 (○); 5.5 (×); 5.8 (□).