BREAKTHROUGHS AND VIEWS

Kinetic Modeling of ATP Synthesis by ATP Synthase and Its Mechanistic Implications

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Based on the torsional mechanism of ATP synthesis by ATP synthase, a kinetic scheme has been developed in this work. The scheme considers adenine nucleotide transport, binding of substrates ADP and Pi, unbinding of product ATP, and ATP synthesis. This kinetic scheme has been analyzed mathematically, and a kinetic model has been obtained to explain the experimentally observed hyperbolic Michaelian dependence of the rate of ATP synthesis on the ADP concentration by ATP synthase under physiological steady-state operating conditions. The principal results of the kinetic model have been compared with the experimental data and an estimate of the enzymological kinetic parameters $V_{\text{max}}$, $K_M$, and $K_I$ has been determined. Mechanistic implications arising from further analysis of the kinetic model have been discussed. These biological implications provide deep insight into the sequence of events leading to ATP synthesis.

Key Words: ATP synthase; $F_1F_0$; kinetic model; molecular mechanism; torsional mechanism; kinetic parameters; competitive inhibition; biological implications.

The ultimate goal of metabolism in living cells is the synthesis of ATP from ADP and inorganic phosphate. The ATP is synthesized by the enzyme ATP synthase (or $F_1F_0$ ATPase). This enzyme transforms energy from a transmembrane electrochemical gradient of protons, or, in some cases, Na$^+$ ions, into the chemical energy of ATP. This enzyme consists of two major parts: a membrane-extrinsic, hydrophilic $F_1$ containing three $\alpha$, three $\beta$, and one copy each of $\gamma$, $\delta$, and $\epsilon$ subunits, and a membrane-embedded, hydrophobic $F_0$ composed of 1 $a$, 2 $b$, and 12 $c$ subunits (Fig. 1). The $F_0$ and $F_1$ domains are linked by two slender stalks (1–7). The central stalk is formed by the $\epsilon$ subunit and part of the $\gamma$ subunit, while the peripheral stalk is constituted by the hydrophilic portions of the two $b$ subunits of $F_0$ and the $\delta$ subunit of $F_1$ (Fig. 1). The catalytic binding sites of ATP synthase are predominantly located in the $\beta$ subunits of $F_1$ at the $\alpha-\beta$ interface (1–6). ATP synthesis takes place by conformational changes at the catalytic binding sites. Recent structural (1, 2), biochemical (3, 5, 8), spectroscopic (9, 10), and microscopic (11, 12) studies indicate that these conformational changes arise from rotation of the $\gamma-\epsilon$ subunit in a static barrel of the $\alpha_3\beta_3$ subunits.

Several mechanisms of ATP synthesis in the $F_1$ portion of ATP synthase have been proposed (3, 6, 13–17). According to the binding change mechanism, energy input is needed not for synthesis of ATP at the catalytic site or for $P_i$ binding (which occur spontaneously), but for the release of synthesized ATP from the catalytic site (3). Another central feature of the binding change mechanism is that the catalytic sites interact with each other; i.e., there exists cooperativity among catalytic sites. Thus, substrate binding at one catalytic site promotes and provides part of the driving force for product release from another catalytic site (3). Another tenet is the free rotation of the $\gamma$ subunit in the cavity of the $\alpha_3\beta_3$ barrel in $F_1$. According to the original binding change mechanism, physiological rates of ATP synthesis occur when two of the three catalytic sites contain bound nucleotide. This last tenet was modified by Cross and co-workers, with the other tenets still in place, and in the modified formulation, an intermediate enzyme state containing bound nucleotide in all three catalytic sites was incorporated (6). In a recent proposal, it is asserted that the binding of $P_i$ triggers the relaxation of a high chemical potential form of the ATP synthase molecule to the ground state and that this realized energy drives ATP release from the high

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affinity catalytic site as well as the rotation of the γ subunit (13). Recently, Pedersen and colleagues questioned the existence of cooperativity in ATP synthase and proposed that a state of the enzyme in which all six α–β subunits adopt a similar closed conformation occurs during functioning and argued that this represents the active conformation of the enzyme. In their view, the Walker structure is a non-physiological state induced by crystallization without sufficient total nucleotide present to occupy all three catalytic sites (14). However, none of these mechanisms is complete and detailed, and how exactly proton translocation drives changes in the position of the γ subunit and causes ATP synthesis is not addressed by any one of them. Thus, the most important enigma remains completely unresolved.

Recently, in a series of papers, we have proposed the torsional mechanism of energy transmission and ATP synthesis (15–17) which addresses the issues of ion-motive torque generation in F0 (15), torque transmission from F0 to F1 (15, 16), energy storage in the enzyme (16), conformational changes in F1 (17), and the catalytic cycle of ATP synthesis (17). We have also treated the thermodynamic aspects of ATP synthesis (18). The salient features of the torsional mechanism are described in brief in the next section. In this work, we have developed a kinetic scheme for ATP synthesis based on the torsional mechanism and have mathematically analyzed it to obtain a kinetic model of ATP synthesis in the F1 portion of ATP synthase. The principal equation arising from our kinetic model has been compared to experimental data for the rate of ATP synthesis as a function of ADP concentration (19) and the values of the kinetic parameters have been estimated. The kinetic model provides a deep insight into the sequence of events leading to ATP synthesis.

FIG. 1. Schematic diagram of the Escherichia coli ATP synthase enzyme.

The torsional mechanism of ATP synthesis

A central feature of the torsional mechanism (15–17) is the development of a torsional strain in the γ subunit due to rotation of the bottom of the central stalk of ATP synthase (caused by the transmembrane electrochemical potential gradient (15)) while the top of the γ subunit remains stationary due to its interactions with the catalytic sites. The developed torsional strain is responsible for storage of torsional energy in the γ subunit and causes conformational changes at the catalytic sites (16, 17). Upon rotation of the bottom of the central stalk (consisting of the ε subunit and the lower portion of the γ subunit) by 30° driven by the proton-motive force, Mg2+ interacts with its ligands in the open conformation (βE) and creates a site with the correct conformation for ADP binding. The substrate MgADP binds and causes the catalytic site to adopt a closed conformation (βC). Rotation of the top of the γ subunit by 120° causes change in the conformation of βC to the loose conformation βTP which enables binding of P_i in βTP (17). Upon another 120° rotation of the top of the γ subunit, the γ–βTP interactions break, leading to the establishment of the tight conformation, βDP resulting in synthesis of MgATP by nucleophilic attack involving ADP-O and P_i (17). A further rotation and interaction of the ε subunit with the catalytic site leads to opening of the catalytic site (βE) and release of the bound MgATP. The energy for release of preformed MgATP is provided by the rotation of the top of the γ subunit by 120° as a consequence of relaxation of the γ subunit due to breaking of the constraints at the top of the ‘shaft’ upon accumulation of torsional energy (15–17).

Kinetic scheme for the torsional mechanism

The ATP synthase is present in the mitochondria of animal cells as well as in the chloroplasts of plant cells. In the ATP synthesis mode, the ADP present in the
cytoplasm of the cells is transported into the organelle (and the ATP synthesized in the organelle is transported out into the cytoplasm) by the adenine nucleotide transporter. The ADP concentration in the cytoplasm, ADP$_{cy}$, is higher than the ADP concentration inside the organelle, ADP$_{or}$. This concentration gradient drives the transport of ADP from the cytoplasm into the organelle. In the presence of Mg$^{2+}$, the substrate MgADP binds to the $\beta_\varepsilon$ catalytic site of ATP synthase according to the molecular mechanism detailed in ref. 17 and attains the closed conformation, $\beta_\varepsilon$. Due to change in interactions between $\beta_\varepsilon$ and the $\gamma$ subunit, the catalytic site is altered to the loose conformation $\beta_{TP}$ in order to affect $P_i$ binding to the catalytic site. A further change in $\gamma$–$\beta$ interactions leads to the tight conformation, $\beta_{DP}$; enzyme-bound ATP is synthesized during this step. The synthesized ATP is released into the matrix of the organelle (with an ATP concentration of ATP$_{or}$). The ATP concentration in the organelle, ATP$_{or}$, is higher than the ATP concentration in the cytoplasm, ATP$_{cy}$, and this concentration gradient transports ATP from the organelle into the cytoplasm. When the above molecular mechanism (17) is expressed in the form of a sequence, we arrive at the kinetic scheme depicted in Fig. 2.

In this kinetic scheme, $E$ represents the ATP synthase enzyme molecule in the open conformation, E.ADP the enzyme–ADP complex in the closed conformation, E.ADP.$P_i$ the enzyme–ADP–inorganic phosphate complex in the loose conformation, and E.ATP the enzyme–ATP complex in the tight conformation. $k_1$ and $k_2$ denote the dissociation constants of the corresponding elementary steps (Fig. 2). $k_r$ denotes the rate constant for conversion of E.ADP to E.ADP.$P_i$ and $k_r'$ the rate constant for conversion of E.ADP.$P_i$ to E.ATP. $k_1$ stands for the constant of proportionality relating the rate of transport of adenine nucleotides by the adenine nucleotide transporter to the corresponding adenine nucleotide concentration gradients (Fig. 2).

**MATHEMATICAL ANALYSIS OF THE KINETIC SCHEME**

For steady-state operation, the rates of transport, binding, and dissociation of ADP and ATP are equal. Thus, $v_{syn}$, the rate of ATP synthesis can be written as

$$v_{syn} = k_t (ADP_{cy} - ADP_{or}). \quad [1]$$

and, because $P_i$ is in excess, its activity can be taken as 1. Therefore,

$$v_{syn} = k_r E.ADP \quad [2]$$

$$v_{syn} = k_r' E.ADP.P_i \quad [3]$$

and

$$v_{syn} = k_t (ATP_{or} - ATP_{cy}). \quad [4]$$

From the material balance on $E$, we get

$$E_0 = E + E.ADP + E.ADP.P_i + E.ATP, \quad [5]$$

where $E_0$ represents the total enzyme concentration. Thus,

$$E_0 = E + E(ADP_{or})/K_1 + (E(ADP_{or})/K_1)(k_r/k_r') + E(ATP_{or})/K_2 \quad [6]$$

i.e.

$$E = E_0/[1 + ADP_{or}(1 + k_r/k_r')/K_1 + ATP_{or}/K_2] \quad [7]$$

Combining Eqs. [2] and [7], we have

$$v_{syn} = k_r E_0 ADP_{or}/(K_1 + ADP_{or}(1 + k_r/k_r') + ATP_{or}(K_1/K_2)) \quad [8]$$

Writing ADP$_{or}$ and ATP$_{or}$ in terms of ADP$_{cy}$ and ATP$_{cy}$ using Eqs. [1] and [4] yields

$$v_{syn} = k_r E_0 (ADP_{cy} - v_{syn}/k_1)/(K_1 + ADP_{cy}(1 + k_r/k_r')) + ATP_{cy}(K_1/K_2) + v_{syn}(K_1/K_2 - 1 - k_r/k_r')/k_1] \quad [9]$$
For very fast diffusion of adenine nucleotides into and from the F_1 portion of ATP synthase by the adenine nucleotide transporter, i.e., for very large k_t, we obtain

\[
v_{\text{syn}} = \left\{ \frac{k_t E_0}{1 + k_t/k'_t} \right\} \frac{\text{ADP}_c}{[\text{ADP}_c + \text{ATP}_c \left( \frac{K_i}{(1 + k_t/k'_t) K_2} \right) + (K_1 + k_t E_0/k_i)/(1 + k_t/k'_t)}] \quad [10]
\]

or

\[
v_{\text{syn}} = V_{\text{max}} \frac{\text{ADP}_c}{[\text{ADP}_c + K_M(1 + \text{ATP}_c/K_i)]}, \quad [11]
\]

where

\[
V_{\text{max}} = \frac{k_t E_0}{1 + k_t/k'_t}
\]

\[
K_M = \left( \frac{K_1 + k_t E_0/k_i}{1 + k_t/k'_t} \right)
\]

and

\[
K_i = \left( \frac{K_2 + K_2 k_t E_0}{K_1 k_i} \right)
\]


**BIOLOGICAL AND FUNCTIONAL IMPLICATIONS**

In the physiological mode of operation, ATP synthesis takes place under steady-state conditions. This implies that the rate of transport of adenine nucleotides into and out of the organelle, the rate of inorganic phosphate binding, and the rate of ATP synthesis are equal (see Eqs. [1]-[4]). From our kinetic model we find that the rate of ATP synthesis is Michaelian with respect to substrate ADP, as clearly indicated by Eqs. [10] and [11]. This prediction is in complete agreement with experimental data on thiol-modulated and activated CF_0F_1-ATP synthase from spinach (19) which showed hyperbolic Michaelis-Menten kinetics with respect to ADP in the synthesis mode at different ATP concentrations in the medium. In fact, the principal equations of our kinetic model clearly demonstrate that the system is characterized by three basic, enzymological kinetic parameters, \( V_{\text{max}} \), \( K_M \), and \( K_i \), which can all be experimentally determined by enzymological experiments. Our kinetic model of ATP synthase was compared to the available experimental data (19) to obtain an estimate of these kinetic parameters. In carrying out this analysis, we have only used experimental data on ATP synthesis taken at low ATP concentrations compared to ADP concentrations in the medium (ATP concentration 0.01 mM, maximum ADP concentration 1 mM). Data was also available at an ATP concentration of 3 mM; however this was not employed because it corresponds to an ATP concentration several fold greater than physiological ATP concentrations in the synthesis mode. Rewriting Eq. [11], we have

\[
v_{\text{syn}} = \frac{V_{\text{max}} \text{ADP}_c}{[\text{ADP}_c + K_M \left( 1 + \text{ATP}_c/K_i \right)]} \quad [12]
\]

where the apparent \( K_M, K_M' = K_M \left( 1 + \text{ATP}_c/K_i \right) \).

From the experimental data, we obtained \( V_{\text{max}} \) and \( K_M' \) directly; these values are tabulated in Table 1. The \( K_M \) value (in the absence of ATP) has been reported to be 30 \( \mu \text{M} \) in the synthesis mode (20, 21). For ATP_c as 0.01 mM, the value of \( K_i \) was calculated from Eq. [12] and is shown in Table 1. Thus, the kinetic parameters could be estimated; enzymological experiments to evaluate these kinetic parameters accurately are currently in progress in our laboratory.

The principal results of our kinetic model (Eqs. [10], [11]) being Michaelian in nature indicate the absence of cooperativity among catalytic sites in the physiological mode of operation of ATP synthase. It is possible that simultaneous negative cooperativity of binding and positive cooperativity of catalysis may lead to hyperbolic kinetics. However, recently, it has been experimentally demonstrated that negative cooperativity of binding and positive catalytic cooperativity need not occur simultaneously (22). The absence of cooperativity in ATP synthase has also been proposed by other investigators (17, 23, 24). Moreover, the negative binding "cooperativity" occurs only during unsteady state filling of the catalytic sites during enzyme start-up and not concomitantly with positive catalytic "cooperativity" (17). Another implication of the torsional mechanism is that the differential affinity of nucleotide binding to the three catalytic sites in ATP synthase does not arise from negative cooperativity of binding during steady state operation (as proposed in current mechanisms) but is due to asymmetric interactions of the catalytic sites with the \( \gamma \) and \( \epsilon \) subunits. Further, the rate enhancement on transition from unisite to trisite catalysis is caused, not by positive cooperativity among catalytic sites, but is a consequence of the mode of functioning of the enzyme itself (17). Key sets of experiments to unambiguously distinguish between these possible mechanisms need to be systematically designed and conducted.

**TABLE 1**


<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Estimated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{max}} )</td>
<td>200 s^{-1}</td>
</tr>
<tr>
<td>( K_M )</td>
<td>( 4.2 \times 10^{-3} ) M</td>
</tr>
<tr>
<td>( K_M' )</td>
<td>( 3.0 \times 10^{-3} ) M</td>
</tr>
<tr>
<td>( K_i )</td>
<td>( 2.5 \times 10^{-3} ) M</td>
</tr>
</tbody>
</table>
Equations [10]-[12] of our kinetic model suggest the occurrence of competitive inhibition of ATP synthase by ATP as the inhibitor in the synthesis mode. This implies that the ATP competes with ADP, or the bound ATP changes the conformation of the site meant for ADP binding, thereby not allowing the ADP to bind to the catalytic site. Hence, unless product ATP is released from the catalytic site, binding of substrate ADP is not possible. Thus, for the physiological mode of steady state ATP synthesis, product release must precede substrate binding. As the analysis is based on a general kinetic scheme which is applicable to all mechanisms, the above implication is valid irrespective of the specific mechanism.

CONCLUSIONS

Based on the torsional mechanism of ATP synthesis, a kinetic model has been developed, mathematically analyzed and compared with experimental data on the substrate ADP concentration dependence of the rate of ATP synthesis by CF₁CF₀-ATP synthase from spinach. The kinetic scheme considers the physiological steady state mode of operation of ATP synthase which implies that the rates of adenine nucleotide transport, binding of substrates, and synthesis of ATP are equal. The kinetic model predicts a hyperbolic Michaelis–Menten kinetics for the rate of ATP synthesis with respect to the substrate ADP concentration, which agrees well with the experimental data. An estimate of the enzymological kinetic parameters were obtained by comparison of the principal results of our kinetic model with the experimental observations. The Michaellian nature of the results of our model indicates the absence of site-site cooperativity during steady state ATP synthesis. Our results also show the presence of competitive inhibition of ATP synthase by product ATP as inhibitor. An important consequence of the competitive product inhibition is the order imposed on binding and release events, i.e., product ATP release must precede substrate ADP binding. These biological and functional implications should prove helpful in understanding the mechanistic aspects of ATP synthesis.

REFERENCES