Kinetic properties of F_0F_1-ATPases

Theoretical predictions from alternating-site models

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ABSTRACT We present an analysis of models based on current structural concepts of the F_0F_1 synthases, accounting for coupling between proton transport and ATP synthesis. It is assumed that each of the three $\alpha\beta$-subunits of the synthase can exist in three different conformational states E, E° and E^*. Proton translocation is coupled to cyclic interconversion of the conformations of the $\alpha\beta$-subunits. The conformational changes of these subunits are assumed to be coordinated so that all three interconvert simultaneously, in a rate-limiting transition. Binding and release of the ligands ATP, ADP, P_i, and protons are assumed to be equilibrium steps. In one family of models, interconversion of the $\alpha\beta$-subunits of F_1 is coupled to the translocation event in F_0 acting as a proton carrier. In a second family of models, protons combine with F_0F_1 and are translocated during the interconversion step in a chemiport. Kinetic tests involving the mutual effects of [ATP], [ADP], $H^{+\prime}$, and $H^{+\prime\prime}$ are described, allowing us to make a distinction between the different models and submodels.

INTRODUCTION

The F_0F_1-type proton-ATPases (ATP synthases) are enzymes catalyzing ATP synthesis driven by an electrochemical proton gradient (Cross, 1981; Fillingame, 1981; Futai and Kanazawa, 1983; Amzel and Pedersen, 1983; Gibson, 1983; Hoppe and Sebald, 1984; Vignais and Satre, 1984; Strotmann and Bickel-Sandkötter, 1984; Hatefi, 1985; Schneider and Altendorf, 1987; Senior, 1988; Penefsky, 1988). F_0F_1-ATPases are found in the inner mitochondrial membrane, in the thylakoid membrane of chloroplasts, and in the plasma membrane of bacteria. In mitochondria and chloroplasts, under physiological conditions, the enzyme seems to operate exclusively in the forward direction, carrying out ATP synthesis. Bacterial F_0F_1-ATPases appear to run backward also as a normal physiological function, hydrolyzing ATP and extruding protons from the cell (Senior, 1988).

The F_0F_1-ATPase is a multisubunit complex (Fig. 1) consisting of the membrane-embedded F_0 part and the F_1 part, which may be detached from F_0 under mild conditions and isolated in water-soluble form. The subunit stoichiometry of F_1 is $\alpha_3\beta_3\gamma\delta\epsilon$, regardless of the organism (Schneider and Altendorf, 1987). In bacteria, F_0 contains three different subunits (a, b, c) with stoichiometry ab_2c_{6-12}, whereas in mitochondria and chloroplasts the composition of F_0 is more complex. Six binding sites for nucleotides (ATP, ADP) are found on F_1. Three sites are located on the β-subunits (one on each subunit) and are thought to be catalytic sites (Senior, 1988; Penefsky, 1988). The other three sites may be located on the α-subunits; their function is unknown.

F_0F_1-ATPases exhibit a strong catalytic site cooperativity. Under conditions where only one site per F_0F_1 is occupied, the reaction rate is low, but increases 10^5–10^6-fold when more than one nucleotide binds to the enzyme (Penefsky, 1985). The low rate of catalysis under single-site conditions results from the extremely high binding affinity for ATP and ADP ($Kd \approx 10^{-12}$ M) which renders product release slow. Binding to a second catalytic site decreases the affinity of the first site by a factor of 10^5 to 10^6.

The detailed mechanism of ATP synthesis is largely unknown, but work in several laboratories has led to the following crucial observations (Senior, 1988; Penefsky, 1988): In ATP synthesis, ADP and P_i bind to the enzyme with high affinity. At the binding site the reaction ADP → ATP proceeds with little change of free energy, i.e., with an equilibrium constant close to unity. The product ATP, however, remains firmly bound to the protein. The energy required for release of the bound ATP is provided by the electrochemical gradient of H^+. Proton flow through the F_0 part of the enzyme is thought to produce a conformational change in the F_0F_1 complex which weakens binding of ATP. Apart from energy input from the proton gradient, release of ATP requires binding of ADP and P_i at a second ("alternating") catalytic site. After release of ATP from the first site, the second site is thought to become a high-affinity binding site at which the reaction ADP → ATP may take place. Different microscopic models which could lead to such an "alternating site" mechanism are feasible (Boyer and Kohl-

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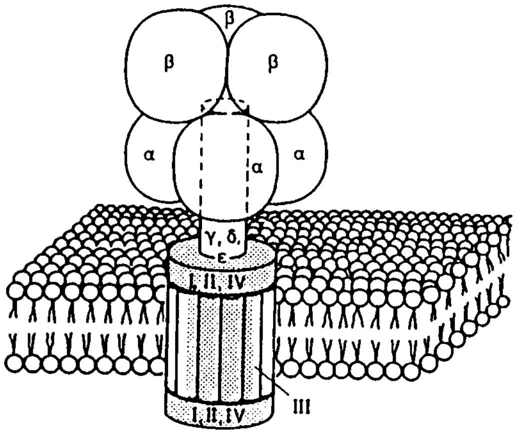

FIGURE 1 Structural model of the F_0F_1-ATPase of chloroplasts, from Boekema et al. (1988).

brenner, 1981; Senior, 1988). An attractive possibility exists in the assumption that each β-subunit can assume three different conformational states and that the three β-subunits move, phase-shifted with respect to each other, through the same cycle of conformational transitions. While this sequential three-site mechanism is hypothetical, the general notion that net ATP synthesis involves cooperation between all three subunits is supported by experiments using chemical modification of β-subunits (Koslov et al., 1985; Melese and Boyer, 1985; Noumi et al., 1986).

A special version of the cyclical three-site mechanism is the rotation model proposed by Cox et al. (1984, 1986) and Gresser et al. (1982). In the model of Cox et al. (1986) for the bacterial F_0F_1-ATPase, it is assumed that proton flow drives rotational motion of an inner core of subunits ($a, b, \gamma, \delta, \epsilon$) with respect to an outer ring of subunits (α, β, c). Other models involving subunit rotation have been discussed by Oosawa and Hayashi (1984) and by Mitchell (1985). The model of Cox et al., in which components of the asymmetric inner core ($a, b, \gamma, \delta, \epsilon$) may be thought to interact sequentially with each of the three β-subunits, could provide a straightforward explanation for the alternating-site behavior of the enzyme. Direct tests of the rotational model are lacking so far. Recent cross-linking experiments have been interpreted as being contrary to (Tozer and Dunn, 1986; Musier and Hammes, 1988) or in favor of the rotation model (Kandpal and Boyer, 1987); see also Senior (1988) for a discussion of rotational catalysis.

In the following section, we describe a theoretical analysis of an alternating-site model of the F_0F_1-ATPase involving cyclic interconversion between three conformational states of the β-subunit. Although the model makes explicit use of the notion of rotation of subunits, it is not restricted to a rotational mechanism, but may be generally applied to the case of out-of-phase conformational transitions in the three catalytic units. As we will show, a rigorous treatment of the model can be carried out, yielding an explicit relation for the dependence of turnover rate on driving force and substrate concentrations. In this way we can derive theoretical predictions that can be experimentally tested and allow us to distinguish between different mechanisms of gradient-driven ATP-synthesis.

ALTERNATING-SITE MODEL WITH INTERCONVERSION OF STATES

Conformational transitions in the catalytic subunits

Following Boyer and Kohlbrenner (1981), we assume that each of the three catalytic units ($\alpha\beta$) of the enzyme can be present in three main conformational states, E, E*, and E° having the following properties: In state E, ADP and P_i are loosely bound, and binding of ADP is preferred over binding of ATP. In state E*, ADP + P_i (and also ATP) are strongly bound; in bound form the substrates undergo a freely reversible reaction, ADP + $P_i \rightleftarrows$ ATP + H_2O. In state E°, ATP is loosely bound and binding of ATP is preferred over binding of ADP.

The catalytic cycle connecting states E, E*, and E° may be represented by the reaction scheme of Fig. 2. In forward direction of the cycle (ATP synthesis), binding of P_i and ADP to state E is followed by a transition to state E*, which is associated with a strong increase of binding affinity. In state E*, the reaction ADP $\rightleftarrows$ ATP may take place at the catalytic center with an equilibrium constant close to unity. ATP is released from the enzyme after a transition to state E° with low binding affinity has occurred. The analysis of this reaction scheme may be

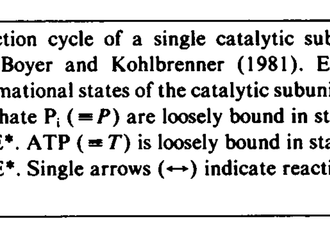

FIGURE 2 Reaction cycle of a single catalytic subunit of the F_0F_1-ATPase, after Boyer and Kohlbrenner (1981). E, E*, and E° are different conformational states of the catalytic subunit. ADP ($\equiv D$) and inorganic phosphate P_i ($\equiv P$) are loosely bound in state E and strongly bound in state E*. ATP ($\equiv T$) is loosely bound in state E° and strongly bound in state E*. Single arrows (↔) indicate reactions assumed to be in equilibrium.

based on the following assumptions:

(*a*) Binding of ATP to state E and binding of ADP + P_i to state E° are neglected.

(*b*) Binding and release of ADP and P_i in state E and of ATP in state E° are always in equilibrium. (In Fig. 2, equilibrium reactions are denoted by single arrows [$\leftrightarrow$].)

(*c*) The reaction ADP + $P_i \rightleftarrows$ ATP between substrates bound to form E* is always in equilibrium. This assumption, although supported by experiments indicating that the actual rate of bond formation (ADP + $P_i \longrightarrow$ ATP) exceeds the rate of net ATP-synthesis (O'Neal and Boyer, 1984), may seem questionable in view of more recent experiments (Matsuno-Yagi and Hatefi, 1986; Stroop and Boyer, 1987). The assumption is introduced here for mathematical simplicity. The form of the derived concentration dependences will remain the same if the contrary assumption is made, although the interpretation of the kinetic parameters will be different, as shown explicitly for the carrier model (Stein, 1986).

(*d*) Each of the three catalytic $\alpha\beta$-units performs the reaction cycle of Fig. 2 phase-shifted with respect to the other two units, as shown in Fig. 3, *A* and *B*. In the scheme of Fig. 3 *A* it has been assumed that the conformational transitions in the $\alpha_3\beta_3$-complex are coupled to rotation of the complex with respect to $\gamma\delta\epsilon$. In this case, state E is always in the 12 o'clock position, state E* in the 4 o'clock position, and state E° in the 8 o'clock position. Fig. 3 *B* depicts the corresponding nonrotational version of the model in which the $\alpha\beta$-units remain fixed with respect to $\gamma\delta\epsilon$, but undergo concerted conformational transitions.

(*e*) Conformational transitions in the forward direction (which may be associated with rotation) are only possible when the $\alpha_3\beta_3$-complex is in state PED/E*T/E° (compare Fig. 3, *A* and *B*). Transitions in backward direction (ATP-hydrolysis) are only possible in state E/PE*D/E°T.

(*f*) The individual $\alpha\beta$-units in the $\alpha_3\beta_3$-complex have identical properties, independent of their position with respect to $\gamma\delta\epsilon$.

According to assumption *f*, the $\alpha_3\beta_3$-complex in Fig. 3, *A* and *B*, has only a single global state (one $\alpha\beta$-unit in state E, one in E*, one in E°). This global state has 4 · 2 · 2 = 16 substates (X, Y, Z) differing in the presence or absence of bound ATP, ADP, and Pi (X = E, PE, ED, PED; Y = PE*D, E*T; Z = E°, E°T). According to assumptions *b* and *c*, all substates are in equilibrium with each other. Using this equilibrium condition, the probabilities $P(X, Y, Z)$ of the 16 states can be easily calculated; $P(X, Y, Z)$ is the probability that the $\alpha_3\beta_3$-complex is in state (X, Y, Z):

$$\sum_{X,Y,Z} P(X, Y, Z) = 1. \tag{1}$$

Introducing the equilibrium dissociation constants K_p, K_D and K_T, as well as the concentrations c_p, c_D and c_T of P_i, ADP and ATP, one obtains:

$$\frac{P(\mathrm{PE}, Y, Z)}{P(\mathrm{E}, Y, Z)} = \frac{P(\mathrm{PED}, Y, Z)}{P(\mathrm{ED}, Y, Z)} = \frac{c_p}{K_p} \equiv p \tag{2}$$

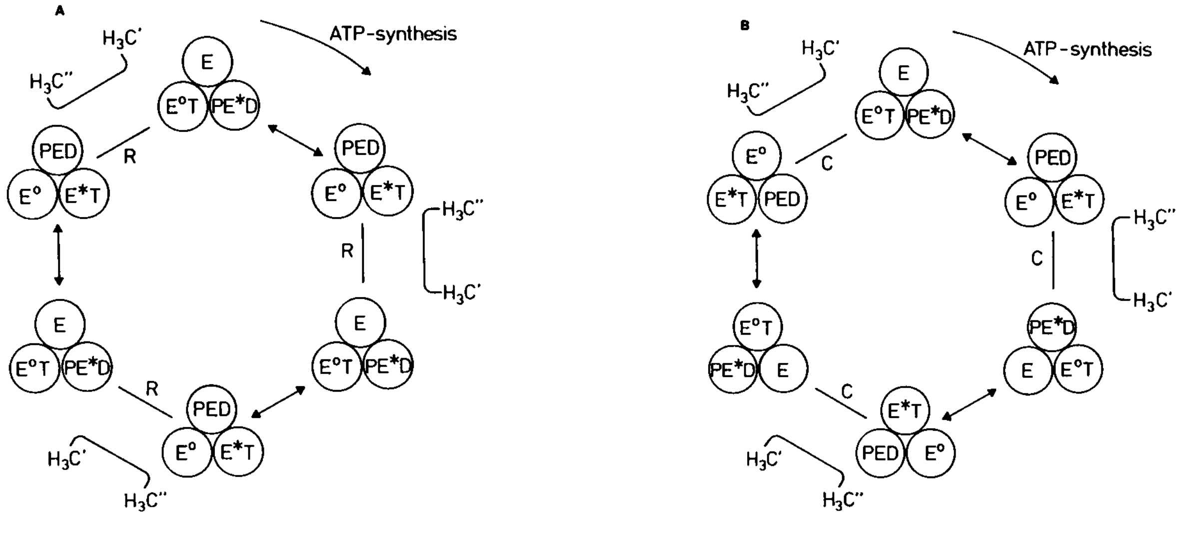

FIGURE 3 Cyclic interconversion of conformational states of the $\alpha_3\beta_3$-complex. Circles denote single $\alpha\beta$-subunits. Arrows indicate equilibrium transitions. H_3C' and $C''H_3$ are states of the proton binding with inward- and outward-facing proton binding sites. (*A*) Rotational mechanism. Conformational transitions (marked by *R*) are assumed to be coupled to rotation of $\alpha_3\beta_3$ by 120° with respect to $\gamma\delta\epsilon$. (*B*) Mechanism with fixed $\alpha\beta$-subunits. Conformational transitions are marked by *C*.

$$\frac{P(\mathrm{ED}, Y, Z)}{P(\mathrm{E}, Y, Z)} = \frac{P(\mathrm{PED}, Y, Z)}{P(\mathrm{PE}, Y, Z)} = \frac{c_\mathrm{D}}{K_\mathrm{D}} \equiv d \tag{3}$$

$$\frac{P(X, Y, \mathrm{E}^{\circ}\mathrm{T})}{P(X, Y, \mathrm{E}^{\circ})} = \frac{c_\mathrm{T}}{K_\mathrm{T}} \equiv t \tag{4}$$

$$\frac{P(X, \mathrm{E^{*}T}, Z)}{P(X, \mathrm{PE^{*}D}, Z)} = S. \tag{5}$$

S is the equilibrium constant of ATP formation at the catalytic site. Implicit in Eqs. 2 and 3 is the assumption that P_i and ADP bind independently to the enzyme.

According to the assumption *e*, the transition rates in forward and backward direction depend on the probabilities P'' and P' of states (PED, E*T, E°) and (E, PE*D, E°T):

$$P' \equiv P(\mathrm{E}, \mathrm{PE^{*}D}, \mathrm{E^{\circ}T}) \tag{6}$$

$$P'' \equiv \mathrm{P}(\mathrm{PED}, \mathrm{E^{*}T}, \mathrm{E}^{\circ}). \tag{7}$$

P' and P'' are obtained from Eqs. 1–5 in the form

$$\frac{1}{P'} = (1 + S)\left(1 + \frac{1}{t}\right)(1 + p)(1 + d) \tag{8}$$

$$\frac{1}{P''} = \left(1 + \frac{1}{S}\right)(1 + t)\left(1 + \frac{1}{p}\right)\left(1 + \frac{1}{d}\right). \tag{9}$$

Coupling of conformational transitions to proton transport

The catalytic cycle of the F_0F_1-ATPase is driven by transmembrane proton flow. There is now good experimental evidence that after removal of the F_1 part, the membrane-embedded F_0 sector acts as a proton channel (Lill et al., 1987). Proton translocation rates of isolated F_0 channel are high ($\sim 6 \cdot 10^5\ \mathrm{s}^{-1}$ at a driving force of 100 mV), much higher than the maximum rate of ATP synthesis by the intact F_0F_1-ATPase (10^2–$10^3\ \mathrm{s}^{-1}$) (Gräber et al., 1986). How proton flow couples to conformational transitions in the catalytic part of the enzyme is unknown. In the intact F_0F_1-complex, part of the proton pathway may pass through the F_1 sector. It is feasible that the intact proton channel in F_0F_1 exhibits a carrierlike, alternating-access behavior (Läuger, 1980). In such a mechanism, ion flow is obligatorily coupled to conformational transitions of the channel. A structural change in the channel part of F_0F_1 could, in turn, be transmitted to the catalytic subunits (Senior, 1988).

A reaction scheme for a channel undergoing conformational transitions coupled to proton flow is shown in Fig. 4. To account for the experimentally observed coupling ratio of $3H^+$/ATP, it is assumed that the "gating" part of the channel has three binding sites for H^+. The binding sites can be present in an inward-facing configuration (C′) or in an outward-facing configuration (C″). "Inside" is defined here as the side toward which the F_1 sector of the enzyme protrudes and which has, under normal physiological conditions, the lower electrochemical potential of H^+ (in the case of bacteria, the cytoplasm). The analysis of the reaction scheme of Fig. 4 is based on the following assumptions: (*i*) Proton-binding and -release steps are always at equilibrium. (*ii*) Transitions between inward- and outward-facing configurations of the proton-binding sites are possible only in the empty (C′, C″) and in the fully occupied state (H_3C', H_3C''). (*iii*) The transition $H_3C'' \rightarrow H_3C'$ of the channel is strictly coupled to the transition (PED, E*T, E°) → (PE*D, E°, ET) of the $\alpha_3\beta_3$-complex, and vice versa (Fig. 3). Transitions between C′ and C″ are independent of the state of the $a_3\beta_3$-complex.

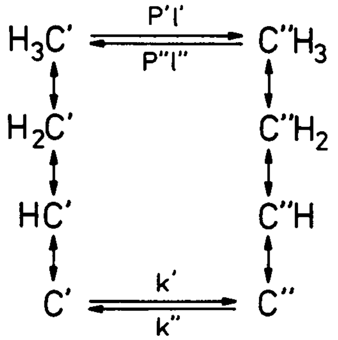

FIGURE 4 Conformational transitions in the H^+-translocating part of F_0F_1-ATPase. The proton-binding sites can alternate between an inward-facing configuration C′ and an outward-facing configuration C″. k', k'', l', and l'' are rate constants. P' and P'' are the probabilities of states (PED, E*T, E°) and (E, PE*D, E°T), respectively, of the $\alpha_3\beta_3$-complex.

Assumption *iii* is essential for the mechanism of coupling between proton flow and ATP synthesis. The assumption is based on the notion that protonation of the binding sites leads to conformational interaction between the channel part of the enzyme and the catalytic sites on F_1. In the deprotonized states C′ and C″, coupling between channel and catalytic subunits is lost, so that transitions C′ ↔ C″ may occur without concomitant change in the $\alpha_3\beta_3$-complex. (The assignment of H_3C'/H_3C'' to the "coupled" and of C′/C″ to the "uncoupled" state is, of course, arbitrary.) Experimental evidence for conformational coupling between F_0 and the *b*-subunits of membrane-bound F_0F_1 has been obtained by Chang and Penefsky (1974) and by Matsuno-Yagi et al. (1985).

For the analysis of the reaction scheme of Fig. 4, we introduce the probability $P(X)$ that the channel is in state X(X = HC′, H_2C', . . .). According to assumption *i*, the following relations hold:

$$\frac{P(\mathrm{HC}')}{P(\mathrm{C}')} = \frac{c'_{\mathrm{H}}}{K'_1} \equiv h'_1 \tag{10}$$

$$\frac{P(\mathrm{H_2C}')}{P(\mathrm{HC}')} = \frac{c'_{\mathrm{H}}}{K'_2} \equiv h'_2 \tag{11}$$

$$\frac{P(\mathrm{H_3C}')}{P(\mathrm{H_2C}')} = \frac{c'_{\mathrm{H}}}{K'_3} \equiv h'_3. \tag{12}$$

Analogous relations are obtained for C", HC", ... c'_{H} and c''_{H} are the proton concentrations in the internal and external aqueous media, respectively. K'_i and K''_i are the equilibrium dissociation constants of $\mathrm{H^+}$ for inward- and outward-facing configuration of the binding sites. The probabilities $P(X)$ of the eight different states X of the channel are connected by

$$\sum_X P(X) = 1. \tag{13}$$

Rate of ATP synthesis

According to the reaction schemes of Fig. 3, *A* and *B*, an ATP molecule is synthesized for every reaction step $\mathrm{H_3C}'' \longrightarrow \mathrm{H_3C}'$. The forward rate of this reaction is equal to $l''P''P(\mathrm{H_3C}'')$, where l'' is the intrinsic rate constant of the process (Fig. 4). The product $P''P(\mathrm{H_3C}'')$ is the probability of finding the $\alpha_3\beta_3$-complex in state (PED, E*T, E°) and, at the same time, the channel in state $\mathrm{H_3C}''$. Correspondingly, the rate of the backward reaction is equal to $l'P'P(\mathrm{H_3C}')$. In the stationary state, the net rate v of ATP synthesis is then given by the difference of forward and backward rates:

$$v = l''P''P(\mathrm{H_3C}'') - l'P'P(\mathrm{H_3C}'). \tag{14}$$

According to the principle of detailed balance, the rate constants k',k'', l',l'' and the equilibrium constants K_{p}, K_{D}, ... are not independent of each other, but connected by the following relation (Appendix A):

$$\frac{k''l'}{k'l''} \cdot \frac{K''_1K''_2K''_3}{K'_1K'_2K'_3} \cdot \frac{K_{\mathrm{p}}K_{\mathrm{D}}}{SK_{\mathrm{T}}\,K_{\mathrm{h}}} = \exp(3u). \tag{15}$$

K_{h} is the equilibrium constant of ATP hydrolysis (bars denote equilibrium concentrations):

$$K_{\mathrm{h}} = \frac{\bar{c}_{\mathrm{P}}\bar{c}_{\mathrm{D}}}{\bar{c}_{\mathrm{T}}}. \tag{16}$$

The transmembrane voltage u is expressed in units of $RT/F \approx 25$ mV (R is the gas constant, T the absolute temperature, and F the Faraday constant):

$$u \equiv \frac{\psi' - \psi''}{RT/F}. \tag{17}$$

ψ' and ψ'' are the electric potentials of the "internal" and "external" aqueous phase, respectively (Fig. 5).

The quantities $P(\mathrm{H_3C}')$ and $P(\mathrm{H_3C}'')$ in Eq. 14 may be evaluated in a straightforward way for the stationary state (Appendix B). This yields, together with Eq. 15, the following expression for the net rate of ATP synthesis:

$$v = A\left[\left(\frac{c''_{\mathrm{H}}}{c'_{\mathrm{H}}}\right)^3 \exp(-3u)\,\frac{c_{\mathrm{P}}c_{\mathrm{D}}/c_{\mathrm{T}}}{K_{\mathrm{h}}} - 1\right] \tag{18}$$

$$A \equiv \frac{P'l'k''h'_1h'_2h'_3}{H'(k'' + P''l''h''_1h''_2h''_3) + H''(k' + P'l'h'_1h'_2h'_3)} \tag{19}$$

$$H' \equiv 1 + h'_1 + h'_1h'_2 + h'_1h'_2h'_3 \tag{20}$$

$$H'' \equiv 1 + h''_1 + h''_1h''_2 + h''_1h''_2h''_3. \tag{21}$$

Eq. 18 describes the dependence of ATP synthesis rate on $\mathrm{H^+}$ concentrations ($c'_{\mathrm{H}}, c''_{\mathrm{H}}$), substrate concentrations ($c_{\mathrm{P}}, c_{\mathrm{D}}, c_{\mathrm{T}}$) and transmembrane voltage u. The expression in the bracket is related to thermodynamic driving force of ATP synthesis; introducing the electrochemical potential difference of protons, $\Delta\tilde{\mu}H = RTln(c''_{\mathrm{H}}/c'_{\mathrm{H}}) - F(\psi' - \psi'')$, and the free energy of ATP hydrolysis, $\Delta G = \Delta G_{\mathrm{o}} + RTln\,(c_{\mathrm{P}}c_{\mathrm{D}}/c_{\mathrm{T}}) = RTln\,(c_{\mathrm{P}}c_{\mathrm{D}}/K_{\mathrm{h}}c_{\mathrm{T}})$, one obtains

$$\left(\frac{c''_{\mathrm{H}}}{c'_{\mathrm{H}}}\right)^3 \exp(-3u) \cdot \frac{c_{\mathrm{P}}c_{\mathrm{D}}/c_{\mathrm{T}}}{K_{\mathrm{h}}} = \exp\left(\frac{3\Delta\tilde{\mu}_{\mathrm{H}} - \Delta G}{RT}\right). \tag{22}$$

For $\Delta\tilde{\mu}H = \Delta G/3$, the system is in thermodynamic equilibrium, and v vanishes, whereas for $\Delta\tilde{\mu}H < \Delta G/3$, hydrolysis of ATP occurs ($v <$ o).

It is pertinent to note that Eq. 18 holds for both the rotational and the nonrotational version of the model, because in the derivation of Eq. 18 the actual mechanism by which the change of binding affinities occurs is immaterial. In fact, it seems impossible distinguishing between both mechanisms on the basis of the concentration dependence of v alone.

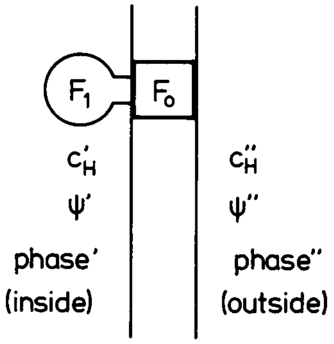

FIGURE 5 Orientation of the $\mathrm{F_oF_1}$-complex in the membrane. c'_{H} and c''_{H} are the proton concentrations, ψ' and ψ'' the electric potentials.

According to Eqs. 18–21, the ATP synthesis rate v is, in general, a complicated function of the concentrations c'_H, c''_H, c_P, c_D, and c_T. (c_P, c_D, and c_T enter into the expression for v via the quantities P' and P'' given by Eqs. 8 and 9.) When all other concentrations are held constant, the dependence of v on ADP concentration c_D may be expressed by

$$v(c_D) = \frac{A_1 + A_2 c_D + A_3 c_D^2}{A_4 + A_5 c_D + A_6 c_D^2}. \tag{23}$$

The parameters A_1–A_6 are independent of c_D. Identical relations (but with different parameters A_1–A_6) are derived for $v(c_P)$ and $v(c_T)$. In certain limiting cases, Eq. 18 considerably simplifies. Maximal rates of ATP synthesis are predicted when the concentrations of ADP and P_i are high and the concentration of ATP is low ($c_P \gg K_P$, $c_D \gg K_D$, $c_T \ll K_T$), and when, in addition, the relations $c'_H \ll K'_i$, $c''_H \gg K''_i$ (i = 1, 2, 3) are fulfilled:

$$v_{\max}^{\text{syn}} = \frac{k'l''}{k' + l''} \cdot \frac{S}{1 + S}. \tag{24}$$

On the other hand, for low concentrations of ADP and P_i and high concentrations of ATP, hydrolysis of ATP coupled to proton extrusion occurs. The hydrolysis rate is maximal under the condition $c_P \ll K_P$, $c_D \ll K_D$, $c_T \gg K_T$, $c'_H \gg K'_i$, $c''_H \ll K''_i$:

$$v_{\max}^{\text{hyd}} = -\frac{k''l'}{k''(1 + S) + l'}. \tag{25}$$

When synthesis of ATP is carried out at $c_T = 0$ and low (rate-limiting) concentrations of ADP and P_i ($c_D \ll K_D$, $c_P \ll K_P$), Eq. 18 reduces to

$$v \approx \frac{S}{1 + S} \cdot \frac{c_P c_D}{K_P K_D} \cdot \frac{k'l''h''_1 h''_2 h''_3}{H'k'' + H''k'}. \tag{26}$$

Conversely, when the enzyme is engaged in ATP hydrolysis at $c_P = c_D = 0$ and rate-limiting concentration of ATP ($C_T \ll K_T$), Eq. 18 assumes the form

$$v \approx \frac{c_T / K_T}{1 + S} \cdot \frac{k''l'h'_1 h'_2 h'_3}{H'k'' + H''k'}. \tag{27}$$

Voltage dependence of turnover rate

The voltage dependence of ATP synthesis rate v is determined by the voltage dependence of the rate constants and equilibrium constants entering into Eq. 18. It seems reasonable to assume that binding of ATP, ADP, and P_i is not affected by the electric field in the membrane. On the other hand, binding of H^+ may be voltage dependent, if part of the membrane potential drops between binding site and adjacent aqueous phase. In this case, the binding site acts as an "ion well" (Mitchell and Moyle, 1974). If $\alpha' u$ is the potential difference between the internal binding sites and aqueous phase' (Fig. 6), and if $\alpha'' u$ is the corresponding quantity for the external sites, the voltage dependence of the equilibrium dissociation constants of H^+ is given by

$$K'_i = \tilde{K}'_i \exp(-\alpha' u) \quad (i = 1, 2, 3) \tag{28}$$

$$K''_i = \tilde{K}''_i \exp(\alpha'' u). \tag{29}$$

$\tilde{K}'_i$ and $\tilde{K}''_i$ are the values of K'_i and K''_i for $u = 0$. Implicit in Eqs. 28 and 29 is the assumption that the three proton-binding sites are equivalent ($\alpha'_i \equiv \alpha'$; $\alpha''_i \equiv \alpha''$).

In the transition $C' \longrightarrow C''$, the binding sites, in general, move over a certain distance. Depending on the electric charge of the empty and occupied sites, the rate constants of the processes $C' \leftrightarrow C''$ and $H_3C' \leftrightarrow H_3C''$ become functions of voltage. If z_L is the charge of the proton-binding ligand system (e_0 is the elementary charge), and if β is the relative dielectric distance over which the binding sites move (Fig. 6), the voltage dependence of the rate constants is approximately given by (Läuger, 1984):

$$k' = \tilde{k}' \exp[(z_L \beta + \eta)u/2] \tag{30}$$

$$k'' = \tilde{k}'' \exp[-(z_L \beta + \eta)u/2] \tag{31}$$

$$l' = \tilde{l}' \exp\{[(z_L + 3)\beta + \eta]u/2\} \tag{32}$$

$$l'' = \tilde{l}'' \exp\{-[(z_L + 3)\beta + \eta]u/2\} \tag{33}$$

$$\alpha' + \alpha'' + \beta = 1. \tag{34}$$

$\tilde{k}'$, $\tilde{k}''$, $\tilde{l}'$, and $\tilde{l}''$ are the values of k', k'', l', and l'' at zero voltage. The parameter η accounts for any intrinsic charge translocations other than the movement of binding sites (Läuger, 1984).

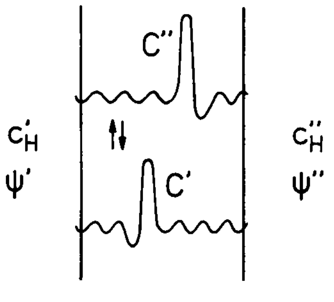

FIGURE 6 Potential-energy profile of H^+ along the transport pathway. In state C' of the channel, proton-binding sites are accessible from the lefthand aqueous medium (phase'), but separated from the righthand medium by a high energy barrier. In state C'' the binding sites are accesible from phase ''. α', α'', and β are relative dielectric distances.

Testing and characterizing a co-transport ("chemiport") model for coupling on the F_0F_1 ATPase

We have thought it worthwhile to consider some kinetic tests of a second class of models for coupling on the F_0F_1 ATPase. In this second class, the complex F_0 is an integral part of the F_0F_1 ATPase functionally as well as structurally. The whole F_0F_1 ATPase acts formally as cotransporter (see Stein, 1986) in which the substrate is transported across the membrane during the progress of the chemical reaction. It acts, therefore, as a "chemiporter." This definition of a "chemiporter" does not imply direct intervention of the transported protons in the chemical reaction. Throughout this section of the discussion we will again assume that the steps involved in the debinding of the substrates ATP, ADP ($+P_i$), and the transported substrate H are rapid in comparison with the rates of the conformation changes that interchange E^o, E, and E^* forms, i.e., that these forms and their ligands are in equilibrium. To simplify the discussion, we will consider that the transported substrate is H, a single uncharged molecule rather than the three protons that stoichiometry demands. The generalization of the subsequent treatment to take into account the triple stoichiometry of proton binding and the transmembrane potential would proceed exactly along the lines of the preceding discussion of the "carrier" model for the F_0F_1 ATPase.

We retain the designation of the three conformations of the F_1 subunits of the F_0F_1 ATPase as E^o for the form which has a preferentially higher affinity for ATP, E for the form with a preferentially higher affinity for ADP ($+P_i$), and E^* for the form which binds both ATP and ADP + P_i very strongly while they undergo the chemical transformation of bound ADP + P_i into bound ATP.

The main difference between the model in this and the previous section is that in the latter there is kinetic path allowing the interconversion of the two forms of the unloaded carrier (C′ and C″ in Fig. 4). This kinetic path ensures that the equilibria between ligands and binding site in one conformation have a kinetic influence on the equilibria in the other. This makes the two classes of models quite distinct kinetically.

F_0 as a classical "chemiport"

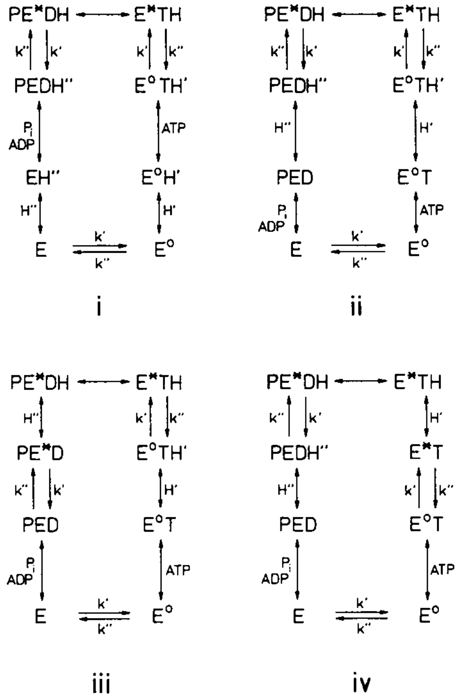

FIGURE 7 Four forms of the "chemiport" model for the action of the F_0F_1 ATPase. In each model form E, E^o, and E^* are states of the F_0F_1 ATPase as defined in the text. In the four reaction schemes, the order of binding of the transportable substrate H and of the phospholigands, ATP, ADP, and P_i varies. In schemes *i* and *ii*, all ligands bind and debind in different order but between the same pair of conformation changes, whereas in *iii* and *iv* these ligands bind/debind after different conformation changes. Protons bound at sites facing phase′ and phase″ are denoted by H′ and H″, respectively; occluded protons are denoted by H.

Fig. 7 depicts some possible cases of the "chemiport" model for the F_0F_1 ATPase. The ligands, H″, H′, ATP, ADP, and Pi bind and debind to the F_0F_1 ATPase at various steps around the chemiport cycle. On the models drawn, binding/debinding is ordered but we will also take into account in what follows the possibility that these events may be random. The double arrows in the diagram represent the conformation changes or rotation events which interconvert the states of the subunits of F_1 and, on the "chemiport" model, bring about that conformation change which carries the substrate across the membrane. Because we assume that the whole F_0F_1 ATPase molecule acts in a coordinated fashion, the conformation changes/rotations are allowed only when the F_0F_1 ATPase is in one of the two forms competent to undergo the conformational change that transfers substrate across the membrane, namely E^o.PED.E^*T or E^oT.E.PE^*D. H can bind to or debind from the F_0F_1 ATPase during any or all of the steps during which the ligands ATP, ADP, and Pi bind or debind, or during the steps in which the chemical reaction of ATP bound to E^* breaks down to ADP + Pi bound to E^*, or during the corresponding synthesis of ATP on E^*.

Two features of the "chemiport" model should be noted. First, the diagrams of Fig. 7 are all isomorphous with the diagram of Fig. 2 except for the introduction of the steps for the binding/debinding of the transportable substrate, H. This means that all the mathematical considerations derived for the earlier model apply also to the version that we are presently considering, as far as the equilibria with regard to the phospholigands are concerned. Second, the cases depicted in Fig. 7, *i* and *ii*, are perfectly isomorphous with the conventional model for the action of the sodium pump and the calcium pump of animal cell membranes (see Stein, 1986). To see this, write Na_{cyt} for H', Na_{ex} for H'', in the figure and E_1 for E°, E_2 for E, with finally, E_{occ} for E* (where Na_{cyt} is the sodium concentration within the cell, Na_{ex} that at the cell exterior, and E_1, E_2, and E_{occ} are, respectively, the forms of the sodium pump with sodium-binding sites that face the cytoplasm, that face the exterior solution, or are the so-called "occluded" form of the pump, in which sodium ions are unavailable at either face of the membrane). It is important, then, that both the E_1E_2 and F_oF_1 models can be written in the same form, emphasising the essential unity of the coupling of transport with chemical reactions in these cases. Perhaps all these systems should be considered, indeed, as $E_1E_2E_3$ systems! (In the sodium and calcium pumps, of course, the pump enzyme is phosphorylated during the process in which the cation is carried across the membrane, whereas in the F_oF_1 ATPase, no phosphorylation takes place of the enzyme itself, but rather the ATP [and the ADP] is very tightly bound).

Because the F_oF_1 ATPase is here considered to act in concerted fashion there is only one rate of reaction for the conformation change or rotation that moves substrate from side '' to side ', namely *k''* as in Fig. 7, and only one rate, *k'*, for the reverse reaction. In addition, since forms such as E°, E, and E* necessarily interconvert, the total amount present of each of those forms is always equal and is one-third of the total amount of F_oF_1 ATPase present. To calculate the rate of the chemical reaction and the coupled transport event we need calculate only the concentration of that form of each of the subunits of the F_oF_1 ATPase that is involved in the reaction in that direction. This will be the concentration of E°T.E.PE*D, for the transport reaction in the direction '' to ', and E°.PED.E*T, for the transport from ' to ''. This enables us to write two "master" equations for these transport events, equations which do not yet take into account the binding of the transported substrate and thus have to be modified for each binding case, in turn:

$$v_{''\rightarrow'} = \frac{k''dpS}{(1+S)(1+d)(1+p)(1+t)} \tag{35}$$

and

$$v_{'\rightarrow''} = \frac{k't}{(1+S)(1+d)(1+p)(1+t)}, \tag{36}$$

where v in each case refers to a single unit of F_oF_1 and v (of Eq. 14) $= v_{''\rightarrow'} - v_{'\rightarrow''}$, and S, p, d, and t are defined in Eqs. 2–5. We modify the "master" equations as follows: Take first the case depicted as Fig. 7 *i*, where H'' binds and debinds during the binding of ADP + Pi on subunit E, and H' binds and debinds during the binding of ATP on subunit E°. Take the case where the transportable substrate H binds to E at side '' and this binding reaction is followed by the binding of ADP and Pi (the two latter in random order). We have, then, the sequence E --- EH'' ----- PEDH''. This is an "ordered" reaction in which H'' binds first. Substituting the appropriate equations for the dissociation constants for H'', ADP, and Pi, we obtain, for the concentration of the transporting form PEDH'': PEDH'' $= pdh''/\{1 + h''(1+p)(1+d)\}$, where h' and h'' in this and subsequent equations are given by $[H' + SP']/KH'$ and $[H'' + SP'']/KH''$, respectively. Next take the case (again, that depicted in Fig. 7 *i*) where the ligand ATP debinds from E°TH' at side ', and this debinding reaction is followed by the debinding of H'. We have, then, the sequence E°TH' --- E°H' ----- E°. This is an "ordered" reaction in which H' debinds second. Again, substituting the appropriate equations for the dissociation constants we obtain, for the concentration of the transporting form E°: E° $= 1/(1 + h' + h't)$. The concentration of the form E*TH is simply $S/(1+S)$.

Finally, putting all this together, we have for the transport rate, given by the concentration of the form E°.PEDH''.E*TH.

$$v_{''\rightarrow'} = \frac{k''h''dpS}{(1+S)[1+h''(1+p)(1+d)](1+h'+h't)}. \tag{37}$$

Consider, now, another case (that of Fig. 7 *iii*), where H binds during the chemical reaction ATP → ADP + Pi on subunit E*. Take the case where the substrate binds to PE*D at side '' and this binding reaction is followed by the chemical reaction on E*. We have, then, the sequence: PE*D --- PE*DH'' ----- E*TH. This is an "ordered" reaction in which H'' binds first. Substituting the appropriate equations for the dissociation constants we obtain, for the concentration of the transporting form E*TH: E*TH $= S/(1 + S + Sh'')$. Next take the case (also that of Fig. 7 *iii*), where the substrate debinds from E°T at side ' and this debinding reaction precedes the debinding of ATP to yield E°. We have, then, the sequence E°TH' --- E°T ---- E°. This is an "ordered" reaction in which H' binds first. Again, substituting the appropriate equations for the dissociation constants, we obtain, for the concentration of the transporting form E°: E° $= 1/(1 + t + h't)$. The concentration of PED is simply given by $1/(1+p)(1+d)$ because, by hypothesis, no binding or debinding of H takes place during the interaction with ADP and Pi.

Finally, putting all this together, we have for the transport rate, given by the concentration of the form E°.PED.E*TH:

$$v_{''\rightarrow'} = \frac{k''h''dpS}{(1+S+Sh'')(1+p)(1+d)(1+t+th')}. \tag{38}$$

These are only two of a possible 12 different schemes in which H″ binds during one or all of the three ligand binding/debinding steps or transformations in such ordered reactions, with H′ debinding before or after the second ligand or reaction. The complete set of such forms is listed in Table 1. There are also corresponding schemes in which the binding and/or debinding of the transportable substrates and the ligands or the occurrence of the chemical reaction follow a random order. We proceed to consider these various cases on the basis of Table 1.

TABLE 1 Kinetic equations for the "chemiport" form of the coupling of transport and ATP synthesis on the F_0F_1 ATPase

All equations have the general form $v_{\rightarrow} = k'' h''dpS/\mathrm{DENOMINATOR}$, while $v = v_{\rightarrow} - v_{\leftarrow} = [(C_{H''}C_PC_D)/(C_{H'}C_TK_h)] - 1/(\mathrm{DENOMINATOR})/k'ht$, where DENOMINATOR is $(1 + \text{term in } S)(1 + \text{term, in } p, d)(1 + \text{term in } t)$ as follows (refer to Fig. 2 for a depiction of the reactions).

(*a*) Ordered reactions with H′ off first, H″ on second with respect to the ligand reactions

Case*	Appropriate DENOMINATOR*
H″ on at E^0, or its derivative:	
Case 1: H′ off at E	$(1 + S)[(1 + p)(1 + d) + h'](1 + t + h'')$
Case 2: H′ off at PE*D	$(1 + S + h')[(1 + p)(1 + d)](1 + t + h'')$
H″ on at E, or its derivative:	
Case 3: H′ off at PE*D	$(1 + S + h')\{(1 + p)(1 + d) + pdh''\}(1 + t)$
Case 4: H′ off at E^0T	$(1 + S)\{(1 + p)(1 + d) + pdh''\}(1 + t + h'T)$
H″ on at E*, or its derivative:	
Case 5: H′ off at E	$(1 + S + Sh'')\{(1 + p)(1 + d) + h'\}(1 + t)$
Case 6: H′ off at E^0T	$(1 + S + Sh'')(1 + p)(1 + d)(1 + t + h't)$

(*b*) Ordered reactions with H′ off first, H″ on first with respect to the ligand reactions

To obtain the appropriate equation, in group *a* above replace:

$(1 + t + h'')$	with	$(h'' + t + h''t)$
$\{(1 + p)(1 + d) + pdh''D\}$	with	$\{1 + h''(1 + p)(1 + d)\}$
$(1 + S + h''S)$	with	$(1 + h'' + h''S)$

(*c*) Ordered reactions with H′ off second, H″ on first with respect to the ligand reactions

To obtain the appropriate equation, in group *a* above replace:

$(1 + t + h'')$	with	$(h'' + t + h''t)$
$(1 + t + h't)$	with	$(1 + h' + h't)$
$(1 + S + h')$	with	$(S + h'S + h')$
$(1 + S + h''S)$	with	$(1 + h'' + h''S)$
$\{(1 + p)(1 + d) + h'\}$	with	$[pd + h'(1 + p)(1 + d)]$
$\{(1 + p)(1 + d) + pdh''\}$	with	$[1 + h''(1 + p)(1 + d)]$

(*d*) Ordered reactions with H′ off second, H″ on second with respect to the ligand reactions

TABLE 1 (continued)

To obtain the appropriate equation, in group *a* above replace:

$(1 + t + h't)$	with	$(1 + h' + h't)$
$(1 + S + h')$	with	$(S + h'S + h')$
$[(1 + p)(1 + d) + h']$	with	$[pd + h'(1 + p)(1 + d)]$

(*e*) Random order of binding/debinding of substrates and ligands (or occurrence of the chemical transformation)

To obtain the appropriate equation, in all cases above replace:

t and *h*′ terms	with	$(1 + t)(1 + h')$
t and *h*″ terms	with	$(1 + t)(1 + h'')$
S and *h*′ terms	with	$(1 + S)(1 + h')$
S and *h*″ terms	with	$(1 + S)(1 + h'')$
p, *d*, and *h*′ terms	with	$\{(1 + p)(1 + d)\}(1 + h')$
p, *d*, and *h*″ terms	with	$\{(1 + p)(1 + d)\}(1 + h'')$

*Refers to all groups *a–e* in table.

The most important feature of Table 1 is that in all cases, the terms in *S*, *p*, *d*, and *t* appear separately in the DENOMINATOR term. Hence, when the various equations are solved to extract the relevant Michaelis parameters, K_m, no mutual effects of *S*, *p*, *d*, and *t* on their respective Michaelis parameters will be found. This is in complete contrast with the "carrier" model of the previous subsection, and allows us to propose a kinetic test to distinguish between these two classes of coupling models: Measure the effect of ATP on the transport of substrate from one side of the membrane to the other. If the half-saturation concentration for this effect of ATP in stimulating or inhibiting the reaction is unaffected by the concentration of ADP (and Pi), the "carrier" model can be excluded. If this Michaelis parameter is, rather, affected by ADP (or Pi), the "chemiport" model is excluded. Likewise, if it is the ADP concentration or that of Pi that is being varied to extract the relevant Michaelis parameter, no effect of ATP concentration on this parameter will be found if the "chemiport" model is valid, whereas an effect will be found if, rather, the "carrier" model is appropriate.

If, indeed, it is the "carrier" model that is appropriate, no further analysis need be made because there is only one form of kinetics for this model (Eqs. 18 and 19). If, however, it is found that the "chemiport" model is valid, then Table 1 allows a large number of tests to be applied to establish the order of binding/debinding of the substrates and ligands. The tests follow simply from the table. Measure the Michaelis parameter for the effect of ATP or ADP (and Pi) on the reaction rate. We have already found this to be unaffected by the ADP (or Pi) and ATP concentrations, respectively, but now measure these parameters as a function of the substrate concentrations H″ and H′.

If any of the Michaelis parameters is affected in a predictable way (see next paragraph), by altering the substrate concentration one can begin to suspect that the substrate binds to, or debinds from, the F_oF_1 ATPase during the step of binding or debinding of that particular ligand, ATP, or ADP (or Pi).

Take, for instance, case 1 in Table 1, where the DENOMINATOR contains the terms $[(1 + p)(1 + d) + h']$ and $(1 + t + h'')$. Then the Michaelis parameter for the effect of ADP (and Pi) on the rate of the reaction is given by $K_m = K_D/(1 + h')$. This decreases as H′ increases from a value of K_D at H′ = 0, to $K_m = 0$, as H′ approaches infinity. However, K_m for ADP is independent of the concentration of ATP. Similarly, the parameter for the effect of ATP in inhibiting the rate of the reaction is given by $K_I = K_T/(1 + h'')$, which decreases from K_T to zero as H″ increases from zero to approach infinity, but is again independent of the concentration of ADP (or Pi). We can set up Table 2 which records the predictions of the various subcases of the "chemiport" model in group *a* of Table 1, as far as the effect of varying the substrate concentration on the Michaelis parameters is concerned. (The other groups *b–d* of Table 1 can readily be shown to give the same predictions.)

As can be seen from the Table each case in group *a* of Table 1 predicts a different spectrum of effects of altering the substrate concentrations of H″ and H′ on the Michaelis parameters for the activating or inhibiting actions of ATP and ADP (or Pi).

To distinguish between the various groups of Table 1, we proceed as follows: The decrease with H″ of K_m for ADP (and Pi) proceeds from a value equal to K_D down to zero if the binding of H″ is ordered and the binding of ADP (or Pi) takes place before H″ binds, whereas it is from a value of infinity down to K_D if ligand binding takes place after H″ binds. Thus the order of binding/debinding of substrates and of ADP (or Pi) can be determined. Similarly, the increase of K_m for ADP (and Pi) with H″, and the decrease of K_I for ATP with H′ and its increase with H″ give information about the order of binding of ADP (and Pi), ATP, H″, and H′, respectively. This approach is summarized in Table 3.

By using these tables, then, we can distinguish many of the different cases recorded in Table 1. Where one does run into difficulties is when one finds that the Michaelis parameter for one of the ligands is unaffected by the concentrations of substrates H″ or H′. We cannot determine whether this independence results from the fact that the ligand and substrate do not bind and/or debind at the same step, or whether they do bind but in random order. Whenever, on the other hand, an effect of H″ or H′ on the K_m for ADP or the K_I for ATP is found, we can make valid

TABLE 2 Effects of varying the concentration of H″ and/or H′ on the Michaelis parameters for the binding/debinding of ATP and ADP (or Pi)

	K_m for *D*		K_I for *T*	
Case No.*	Decreases with H″	Increases with H′	Increases with H″	Decreases with H′
1	–	+	+	–
2	–	–	+	–
3	+	–	–	–
4	+	–	–	+
5	–	+	–	–
6	–	–	–	+

*Numbering same as in Table 1.

TABLE 3 Order of binding/debinding of substrates and ligands as determined by detailed effects of substrate concentrations on K_m for ADP (or Pi) and K_I for ATP

Effect on K_m for ADP (or Pi)	Conclusion
Raising H″ decreases K_m:	
from K_D to zero	H″ binds after ADP (+Pi)
from infinity to K_D	H″ binds before ADP (+Pi)
Raising H″ has no effect	ADP (+Pi) are not involved when H″ binds, or the binding reactions take place in random order
Raising H′ increases K_m:	
from K_D to infinity	H′ debinds before ADP (+Pi) binds
from zero to K_D	H′ debinds after ADP (+Pi) binds
Raising H″ has no effect	ADP (+Pi) are not involved when H′ debinds, or the binding reactions take place in random order
Effect on K_I for ATP:	
Raising H′ decreases K_I:	
from K_T to zero	H′ debinds before ATP binds
from infinity to K_T	H′ debinds after ATP binds
Raising H′ has no effect	ATP is not involved when H′ debinds, or the binding reactions take place in random order
Raising H″ increases K_I: from K_T to infinity	H″ binds after ATP debinds
from zero to K_T	H″ binds before ATP debinds
Raising H″ has no effect	ATP is not involved when H″ binds, or the binding reactions take place in random order

statements about the order of addition of the substrate and ligand.

We can ask what the conditions are for the equilibrium distribution of substrates and ligands in this "chemiport" formulation of the kinetics of the F_0F_1 ATPase. We equate the numerator of the equation describing transport in the $''$ to $'$ direction to the numerator of the corresponding equation for $'$ to $''$ direction. On simplifying, we obtain $k''h''pdS = k'h't$. Writing out in full the terms in p, d, t, and h we obtain:

$$\frac{[H']}{[H'']} = \frac{k''K_{H'}K_T S[ADP]\,[Pi]}{k'K_{H''}K_D K_P[ATP]}. \tag{40}$$

This equation must also be true when $[H'] = [H'']$, when $[ADP] \cdot [Pi]/[ATP]$ is equal to the equilibrium constant for ATP hydrolysis, in the absence of coupling, which we write as K_h.

It follows, therefore, that the kinetic parameters of the model, as found in Eq. 40 are related by:

$$S\,K_T K_h/K_D K_P = k'K_{H''}/k''\,K_{H'}, \tag{41}$$

an equation which is, of course, exactly parallel with Eq. 15, found for the "carrier" model of the action of the F_0F_1 ATPase.

COMPARISON WITH EXPERIMENTAL RESULTS

We have not been able to find even a nearly complete study of the kinetics of the F_0F_1 ATPase, along the lines suggested by the preceding analysis. The technical basis is now, however, established for such an analysis. The work of Gräber and his colleagues (reviewed in Gräber, 1987) shows how measurements can now be made of the rate of ATP synthesis as a function of pH in intact chloroplasts. The pH of the chloroplast interior is established by an initial equilibration (e.g., pH 4–7) and the reaction of ATP synthesis started when the organelles are injected rapidly into solutions of a different, defined (external) pH (generally 8.2). The transmembrane potential can also be controlled using a valinomycin/potassium system. The experiments are presently conducted at an external concentration of 5 mM Pi and 100 μM ADP, but they could obviously be performed over a wide range of these effectors (and of ATP as well). Under the just-mentioned conditions, ATP synthesis has a maximum turnover of some 410 mol ATP per mol F_0F_1 ATPase per second and is a smooth function of pH, fitting a curve with a pK (internal) of 5.9 ± 0.25, and a stoichiometry for protons of 1.7. (This is at zero transmembrane potential.) A positive inside potential shifts the curve towards higher internal pK values consistent with the effect of potential on the electrochemical gradient, on the model of there being no potential well. In such experiments, there is always the danger that an observed effect of pH may be regulatory rather than being the contribution of protons as transportable substrates. This, indeed, seems to be the case for the F_0F_1 ATPase of the chloroplast. Treatment of a suspension of chloroplasts with dithiothreitol converts them into an activated form where the pH curve is shifted some 0.7 U more acid, and the dependence of activity on pH is now steeper, approaching a stoichiometry of 3. Presumably under these conditions one is seeing the catalytic, rather than the regulatory, effect of protons. Were such studies to be performed as a function of ADP, ATP, and Pi concentration, and of the pH on both sides of the thylakoid membrane, one would have available the data for the full kinetic analysis described in the earlier sections of this paper.

The F_0 portion of the F_0F_1 ATPase has been studied separately from the intact enzyme as far as its ability to transport protons is concerned. Here, too, techniques are now available not, of course, for the full kinetic analysis, but for studying the mutual effects of internal and external pH on proton transport. A flash spectrophotometric technique applied to the F_0 complex (derived from thylakoid membranes) reconstituted into liposomes gave single-channel conductances of some 1 pS, equivalent to a turnover of 6×10^5 protons per "channel" per second, at room temperature, with a transmembrane applied potential of 100 mV (Lill et al., 1987). At a stoichiometry of 3 protons to 1 mol ATP synthesized, this is 500 times larger than the maximum turnover of the intact F_0F_1 ATPase, consistent with the repeated finding that subunits of the F_1 portion drastically reduce the proton permeability of the F_0 portion (Gao and Baeuerlein, 1988). The flash spectrophotometric studies of reconstituted F_0 subunits has not yet been extended to an investigation of the dependence of rate on the concentrations of internal and external proton. It will be interesting to see which portions of F_1 mediate the interaction of phospholigands and protons on the F_0F_1 ATPase. (It is perhaps worth mentioning that in an early study, using F_0 subunits derived from the F_0F_1 ATPase of a thermophilic bacterium, where Sone et al. (1981) reported maximum turnover numbers as low as 47 protons/mole $F_0 \cdot s$, a pH dependence of proton conductance with a pK in the range 6.9–7.5 could be demonstrated. Chemical modification of a tyrosine residue shifted this pK ~ 0.9 pH units more acid.)

Refined studies with the techniques now available should allow the testing and characterizing of the kinetic models discussed in the preceding portions of the present paper.

APPENDIX A

Derivation of Eq. 15

To prove Eq. 15, we start with the identity

$$\frac{P(\mathrm{H_3C'})}{P(\mathrm{H_2C'})}\cdot\frac{P(\mathrm{H_2C'})}{P(\mathrm{HC'})}\cdot\frac{P(\mathrm{HC'})}{P(\mathrm{C'})}\cdot\frac{P(\mathrm{C'})}{P(\mathrm{C''})}\cdot\frac{P(\mathrm{C''})}{P(\mathrm{HC''})}\cdot\frac{P(\mathrm{HC''})}{P(\mathrm{H_2C''})}\cdot\frac{P(\mathrm{H_2C''})}{P(\mathrm{H_3C''})}\cdot\frac{P(\mathrm{H_3C''})}{P(\mathrm{H_3C'})}\equiv 1. \quad \text{(A1)}$$

We apply this equation to an equilibrium situation in which the following relations hold (bars denote equilibrium concentrations):

$$\frac{P(\mathrm{H_iC'})}{P(\mathrm{H_{i-1}\,C'})}=\frac{c\mathrm{H'}}{K'_{\mathrm{i}}};\quad \frac{P(\mathrm{H_iC''})}{P(\mathrm{H_{i-1}C''})}=\frac{c\mathrm{H''}}{K''_{\mathrm{i}}} \quad \text{(A2)}$$

$$(\mathrm{i} = 1, 2, 3)$$

$$\frac{P(\mathrm{C'})}{P(\mathrm{C''})}=\frac{k''}{k'};\quad \frac{P(\mathrm{H_3C'})}{P(\mathrm{H_3C''})}=\frac{P''l''}{P'l'} \quad \text{(A3)}$$

$$\frac{P''}{P'}=S\cdot\frac{K_{\mathrm{T}}}{K_{\mathrm{P}}K_{\mathrm{D}}}\cdot\frac{\bar{c}_{\mathrm{P}}\bar{c}_{\mathrm{D}}}{\bar{c}_{\mathrm{T}}} \quad \text{(A4)}$$

$$\frac{\bar{c}''_{\mathrm{H}}}{\bar{c}'_{\mathrm{H}}}=\exp(u). \quad \text{(A5)}$$

Eq. A3 expresses the fact that under equilibrium conditions the net rate of the reactions C′ → C″ and $\mathrm{H_3C'} \rightarrow \mathrm{H_3C''}$ vanish. Eq. A4 follows directly from Eqs. 2–5, 8, and 9. The dimensionless voltage u is defined by Eq. 17. Because in equilibrium any voltage u can be generated by a suitable choice of c'_{H} and c''_{H}, and because the rate constants and equilibrium constants in Eq. 15 are independent of c'_{H} and c''_{H}, Eq. 15 holds for arbitrary voltages u.

APPENDIX B

Derivation of Eqs. 18–21

In the stationary state, the net frequency of transitions C′ → C″ must be equal to the net frequency of transitions $\mathrm{H_3C''} \rightarrow \mathrm{H_3C'}$:

$$k'P(\mathrm{C'}) - k''P(\mathrm{C''}) = l''P''P(\mathrm{H_3C''}) - l'P'P(\mathrm{H_3C'}). \quad \text{(B1)}$$

From Eq. B1 together with Eqs. 10–13 (and the corresponding relations for h''_1, h''_2, h''_3), the eight probabilities $P(X)$ may be evaluated. This yields:

$$P(\mathrm{H_3C'})=\frac{h'}{D}(k''+P''l''h'') \quad \text{(B2)}$$

$$P(\mathrm{H_3C''})=\frac{h''}{D}(k'+P'l'h') \quad \text{(B3)}$$

$$h'\equiv h'_1h'_2h'_3;\quad h''\equiv h''_1h''_2h''_3 \quad \text{(B4)}$$

$$D\equiv \mathrm{H'}(k''+P''l''h'')+\mathrm{H''}(k'+P'l'h'). \quad \text{(B5)}$$

H′ and H″ are defined by Eqs. 20 and 21. Introduction of Eqs. B2–B4 and 15 into Eq. 14 yields Eq. 18.

Received for publication 8 May 1989 and in final form 2 October 1989.

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