Journal of Bioenergetics and Biomembranes, Vol. 25, No. 3, 1993

Kinetic Models of Coupling between H^+ and Na^+-Translocation and ATP Synthesis/Hydrolysis by F_0F_1-ATPases: Can a Cell Utilize Both $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ for ATP Synthesis under *in vivo* Conditions Using the Same Enzyme?

Boris N. Kholodenko[1]

Received February 19, 1992; accepted September 22, 1992

Kinetic models of the F_0F_1-ATPase able to transport H^+ or/and Na^+ ions are proposed. It is assumed that (i) H^+ and Na^+ compete for the same binding sites, (ii) ion translocation through F_0 is coupled to the rate-limiting step of the F_1-catalyzed reaction. The main characteristics of the dependences of ATP synthesis and hydrolysis rates on $\Delta\varphi$, ΔpH, and ΔpNa are predicted for various versions of the coupling model. The mechanism of the switchover from $\Delta\bar{\mu}_{H^+}$-dependent synthesis to the $\Delta\bar{\mu}_{Na^+}$-dependent one is demonstrated. It is shown that even with a drastic drop in $\Delta\bar{\mu}_{H^+}$, ATP hydrolysis by the proton mode of catalysis can be effectively inhibited by $\Delta\varphi$ and ΔpNa. The results obtained strongly support the possibility that the same F_0F_1-ATPase in bacterial cells can utilize both $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ for ATP synthesis under *in vivo* conditions.

KEY WORDS: F_0F_1-ATPase; coupling; proton and sodium transport; ATP-synthesis/hydrolysis rates.

INTRODUCTION

Starting with the classical work of Mitchell (1961, 1966) the mechanism of coupling between proton translocation and ATP synthesis/hydrolysis has been examined in many theoretical and experimental studies (see, e.g., reviews of Fillingame, 1990; Cross, 1981; Boyer, 1987; and Senior, 1988). A great number of experiments were performed with mitochondria, chloroplasts, and inside-out particles obtained from mitochondria or bacteria. They have demonstrated that for the ATP synthesis by proton F_0F_1-ATPase (ATP synthase) to proceed it is necessary to have a sufficiently great transmembrane gradient of proton electrochemical potential, $\Delta\bar{\mu}_{H^+}$, and not less than two protons should be translocated across the membrane for one ATP molecule synthesized.

There are two different hypotheses describing the coupling between H^+ translocation and ATP synthesis. According to the first one (Mitchell, 1975) $\Delta\bar{\mu}_{H^+}$-driven protons, when reaching the catalytic site of F_1, attack the oxygen atom of phosphate participating in H_2O formation (the direct coupling hypothesis). According to the second hypothesis (Boyer, 1975) $\Delta\bar{\mu}_{H^+}$ is needed to release tightly bound ATP from the catalytic site of F_1 and/or for the binding of ADP and P_i and, probably, for the ATP/ADP + P_i antiport inside the protein globule (Mitchell, 1973). In that case the energy of proton translocation is utilized for a number of successive changes in the enzyme conformation, and H^+ ions transported are not associated with those consumed in the catalytic site of F_1 (the indirect or conformational coupling hypothesis). The reviewer's comment is worth mentioning here that at the $3H^+$/ATP stoichiometry only 2 protons can be used at the F_1 active site, and in this sense, Mitchell's direct model has an indirect component.

It has been discovered recently that F_0F_1-ATPase of strictly anaerobic marine bacterium *Propionigen-*

[1] A. N. Belozersky Institute of Physicochemical Biology, Moscow State University, Moscow 119899, Russia.

0145-479X/93/0600-0285$07.00/0

ium modestum transports Na^+ rather than H^+ at physiological Na^+ and H^+ concentrations (Laubinger and Dimroth, 1987). The sensitivity to inhibitors (DCCD and ventruicidin) and the subunit composition of *P. modestum* ATPase proved to be similar to those of proton F_0F_1-ATPases. This kinship allows one to suggest the similarity between the mechanism of action of Na^+- and H^+-transporting F_0F_1-ATPases. It is clear that the indirect coupling hypothesis does not exclude that Na^+ will be translocated instead of H^+ (or H_3O^+; Boyer, 1989). Moreover, the existence of the bifunctional enzyme transporting both types of ions does not contradict this hypothesis. In fact, it has been reported (Laubinger and Dimroth, 1989) that ATP hydrolysis by *P. modestum* F_0F_1-ATPase, inserted in the liposomes, is accompanied either by Na^+ or H^+ transport depending on the sodium concentration level in the incubation medium. It has been found that Na^+ inhibits the proton transport, and it has been suggested that H^+ and Na^+ ions compete for the same binding sites.

The question arises whether a cell can use the same enzyme, which is capable of utilizing both $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$, for ATP synthesis under *in vivo* conditions. For any such a device, which would be close to the equilibrium, the gist of the problem is that ATP synthesized by the Na^+-dependent way under consumption of $\Delta\bar{\mu}_{Na^+}$ at low $\Delta\bar{\mu}_{H^+}$ would be immediately hydrolyzed by the H^+-dependent way.

Indeed, according to the nonequilibrium thermodynamics, one can write the following linear relationships between the ATP synthesis rate (v_a), the coupled fluxes of H^+ (J_H) and Na^+ (J_{Na}), and the driving forces ("flow-force relationships"; see, e.g., Westerhoff and Van Dam, 1987):

$$\begin{aligned} v_a &= L_H \cdot (n \cdot |\Delta\bar{\mu}_{H^+}| - \Delta G_p) \\ &\quad + L_{Na} \cdot (n \cdot |\Delta\bar{\mu}_{Na^+}| - \Delta G_P) \\ J_H &= n \cdot L_H \cdot (n \cdot |\Delta\bar{\mu}_{H^+}| - \Delta G_P), \\ J_{Na} &= n \cdot L_{Na} \cdot (n \cdot |\Delta\bar{\mu}_{Na^+}| - \Delta G_P) \end{aligned} \quad (1)$$

Both phenomenological coefficients L_H and L_{Na} are proportional to the enzyme concentration, n is the H^+/ATP and Na^+/ATP stoichiometry, $\Delta\bar{\mu}_{Na^+}$ is the tansmembrane gradient of sodium electrochemical potential, ΔG_P is the phosphate potential,

$$\Delta G_P = \Delta G_P^0 + R \cdot T \cdot \ln \frac{ATP}{ADP \cdot P_i} \quad (2)$$

where R and T have their usual thermodynamic meanings, and ΔG_P^0 is the standard free energy of ATP syntehsis ($\Delta G_P^0 \approx 28$ kJ/mol at pH 7.0, $[Mg^{2+}] = 10^{-3}$M, ionic strength 0.1 M, and T = 25°C; (see Rosing and Slater, 1972). It is clear from Eq. (1) that if one of the ion electrochemical potential is high and the other low, e.g., $n \cdot |\Delta\bar{\mu}_{Na^+}| - \Delta G_p > 0$, $n \cdot |\Delta\bar{\mu}_{H^+}| - \Delta G_P < 0$, ATP synthesized at the expense of the first potential will be cleaved by the mode of catalysis depending on the second one. So, only the kinetic characteristics of the hypothetical device can ensure that ATP synthesized by the $\Delta\bar{\mu}_{Na^+}$-dependent mode will not be hydrolyzed when $\Delta\bar{\mu}_{H^+}$ drastically drops.

In the present work at kinetic model of bifunctional, H^+ and Na^+-transporting ATPase [$H^+(Na^+)$-ATPase], capable of utilizing both $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ for ATP synthesis, will be put forward. The dependences of the steady-state fluxes on the electric ($\Delta\varphi$) and concentration (ΔpH and ΔpNa) components of $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ will be analyzed for different models of coupling. The biological relevance of such an enzyme will be discussed.

BASIC PRINCIPLES OF THE COUPLING MODEL

As known from the work of Kagawa and Racker (1971) and Kagawa *et al.* (1979), factor F_0 is not a simple membrane pore but contains binding sites for protons which appear to be involved in the transport. In the absence of ATP synthesis or hydrolysis, the proton pathway in the intact F_0F_1 molecule is in the closed state. For the $H^+(Na^+)$-ATPase model under consideration, inlet and outlet ion channels in F_0 are assumed to be accessible for ions from the aqueous phase at the external and internal sides of the membrane, correspondingly. Ions can only be translocated between the bottoms (X and Y) of these channels in the course of ATP synthesis or hydrolysis.

Suppose that H^+ and/or Na^+ translocation from domain X to Y is coupled to catalytic steps, requiring a substantial input of free energy. We assume that in the reaction of ATP synthesis such a step is transition of F_1 from the state when ATP is tightly bound to the enzyme to the usual state with low affinity for ATP (Senior, 1988),

$$E\text{–}ATP^b \rightleftharpoons E\text{–}ATP \quad (3)$$

Here the superscript b denotes the state of tight binding between the enzyme and ATP. Factor F_1 contains

three catalytic sites, and it was suggested that ATP release from one site is always accompanied by the binding of ADP and P_i at an alternate catalytic site (Cross, 1981; Gresser *et al.*, 1982). Then step (3) should be regarded as coordinated processes taking place simultaneously at all three sites (see models proposed by Senior, 1988; Cox *et al.*, 1986; and Stein and Lauger, 1990). Step (3) may include not only a conformational transition in F_1 but also transport of adenine nucleotides within the enzyme (Mitchell, 1973). Ion translocation would be the driving force for this step, assuming that the standard free energy difference (ΔG_I^0) upon losing ions by X and their binding to Y should be of the same order as the standard free energy difference of step (3).

Ions may be translocated between X and Y owing either to the movement of a part of the F_0F_1 molecule together with bound ions ("protein arm") or to migration ("jumping-over") of the ions themselves. In the former case a single binding site may exist alternating between X and Y, so that simultaneous binding of ions to X and Y is impossible. In the latter case there must be two sites X and Y, and, hence, simultaneous binding can take place. Both H^+ and Na^+ can bind to the same site until the total number of ions reaches n. Then the ion translocation coupled to step (3) can take place (n is the $(H^+ + Na^+)$/ATP stoichiometry). Independent binding of ions with constant pK_H and pK_{Na} is assumed for the sake of simplicity, but pK for the same ions may differ at the different membrane sites. It should be noted that if sites X and Y interact so that binding of ions to one of them precludes binding to the other, the situation will be similar to that in the case of a single site.

In the case of a much lower sodium concentration at both sites of the membrane than corresponding pK_{Na} values, F_0 binding sites may be occupied by protons only, and ATPase operates as a pure H^+-pump. the model of H^+-ATPase, based on similar assumptions, has been recently analyzed (Kholodenko, 1991). It was shown that experimental data available in the literature are described well by the one-site model (or the relatively close model with two interacting sites) and much worse by the two-independent-site model. Keeping this in mind, the models of $H^+(Na^+)$-ATPase will be analyzed in this work with special reference to the one-site model.

Let us designate the following enzyme states as IX and YI. They correspond to one and the same state of the catalytic site, but in the first one (IX) n ions I (I stands for both H^+ and Na^+) are bound in domain X while domain Y is free, whereas in the second one (YI) the situation is opposite. According to our assumption, standard chemical potentials μ_{IX}^0 and μ_{YI}^0 of these states are significantly different:

$$\Delta G_I^0 = \mu_{YI}^0 - \mu_{IX}^0 < 0 \tag{4}$$

The difference in pK_I for domains X and Y can be expressed through ΔG_I^0. In the single site case,

$$pK_I^Y - pK_I^X = (-\Delta G_I^0 + (\mu_{EY}^0 - \mu_{XE}^0))/(n \cdot R \cdot T \cdot \ln 10) \tag{5}$$

where μ_{XE}^0 and μ_{EY}^0 are the standard chemical potentials of the enzyme states when the binding site is free and situated in X or Y, respectively. It should be noted that K_I^X and K_I^Y mean the equilibrium dissociation constants for ions from the bulk aqueous phases. If the pathway of ions from the bulk phases to domains X and Y comprises several steps (jumps along the chains of amino acid residues), the pK values include the products of the equilibrium constants of the corresponding transitions.

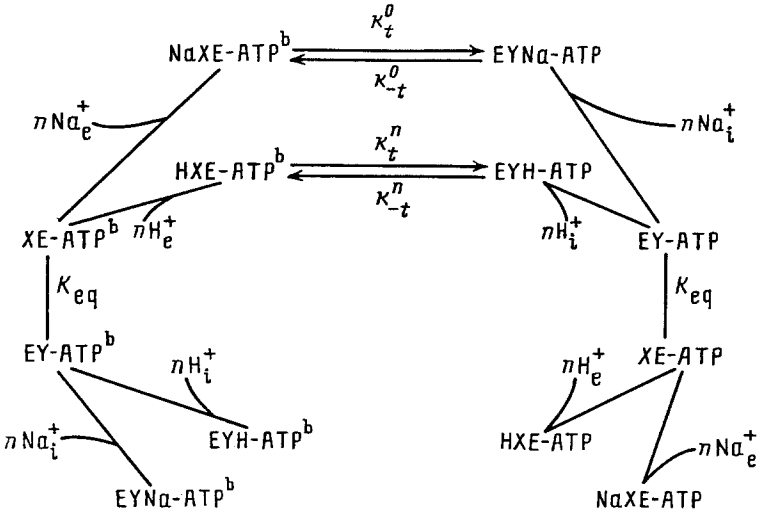
As evident from the equation above, the pK_I^Y and pK_I^X values for the single site may coincide as well as be different (if, for instance, the binding site is surrounded by dissimilar fixed charges in domains X and Y). Coincidence of pK_I^X and pK_I^Y is possible if the initial equilibrium distribution of the enzyme between states XE and EY, differing only in the position of the free binding site, is asymmetrical:

$$\mu_{XE}^0 - \mu_{EY}^0 = R \cdot T \cdot \ln \left(\frac{[EY]}{[XE]} \right)_{rmeq} = R \cdot T \cdot \ln K_{eq} = -\Delta G_I^0 \tag{6}$$

It is clear that for the two-sites case, μ_{XE}^0 and μ_{EY}^0 in Eq. (6) should be identical (since there is only one state in which both sites are free). Consequently, if there are two ion binding sites at the different membrane sides, their pK values will differ irrespective of the nature of their interaction.

To make the analysis of the steady-state kinetics simpler, suppose that the limiting step in the ATP synthesis by F_0F_1-ATPase releases the bound ATP coupled to ion translocation from X to Y. Accordingly, the reverse step is assumed to be rate-limiting

for ATP hydrolysis. The part of the kinetic diagram including the limiting (3) and the ion-binding steps looks as follows:



For the limiting step coupled to the translocation of (different) ions, the direct and the reverse transitions are shown, whereas the other (near-equilibrium) steps are denoted by single lines. The symbols XE- or EY- denote the states where the free site is situated in X or Y and is accessible to ions at the external (e) or internal (i) side of the membrane, respectively. Although, as was stated above, the ion translocation can take place after binding of any n from H^+ and Na^+ ions, for the sake of simplicity only the binding and transitions of n H^+ and n Na^+ are shown. Moreover, in order to simplify the diagram, all n ions are shown to be bound in one step. It is also assumed that the ion dissociation constants are the same in the left and right parts of the diagram.

If the states XE– and EY– in the diagram corresponding to the same states of the catalytic site (i.e., E-ATP^b or E-ATP) are considered identical, it will describe the case of two interacting sites where ions cannot bind simultaneously to X and Y. For the case of two sites with independent pK, the states IXEYI-ATP and IXEYI-ATP^b are to be included in the diagram. They refer to the simultaneous binding of ions to X and Y and do not participate in the ion translocation steps.

ANALYSIS OF THE STEADY-STATE KINETICS

Various versions of the model have their own kinetic characteristics, so the aim of this section is to analyze the corresponding dependences of the steady-state rates of ATP synthesis and hydrolysis on the $\Delta\varphi$, ΔpH, and ΔpNa components of proton ($\Delta\mu_{H^+}$) and sodium ($\Delta\bar{\mu}_{Na^+}$) potentials,

$$\Delta\bar{\mu}_{H^+} = F\cdot\Delta\varphi - R\cdot T\cdot \ln 10\cdot \Delta\mathrm{pH},$$

$$\Delta\bar{\mu}_{Na^+} = F\cdot\Delta\varphi - R\cdot T\ln 10\cdot \Delta\mathrm{pNa},$$

where F is the Faraday constant, $\Delta\mathrm{pH} = \mathrm{pH_i} - \mathrm{pH_e}$, $\Delta\mathrm{pNa} = \mathrm{pNa_i} - \mathrm{pNa_e}$, and $\Delta\varphi = \varphi_i - \varphi_e$.

Any step involving charge movement in the membrane produces electrical work, and the membrane potential ($\Delta\varphi$) affects the rate and equilibrium constants of these steps (Boork and Wennestrom, 1984; Reynolds *et al.*, 1985). Since domains X and Y may be positioned inside the membrane, under the influence of the $\Delta\varphi$ a shift occurs in the apparent values of pK_I^X and pK_I^Y:

$$K_I^X(\Delta\varphi) = K_I^X\cdot\exp(\alpha_X\cdot\phi)$$

$$K_I^Y(\Delta\varphi) = K_I^Y\cdot\exp(-\alpha_Y\cdot\Phi),$$

$$\phi = \Delta\varphi/(R\cdot T/F) \qquad (7)$$

where ϕ is the dimensionless expression of the membrane potential, $R\cdot T/F \approx 26\,\mathrm{mV}$, $\alpha_X\phi$ is the potential difference between the external bulk phase and X, and $\alpha_Y\phi$ is the corresponding difference between y and the internal bulk phase. The α_X and α_Y values are determined by the relative dielectric lengths of the ion semichannels. It is obvious that they obey the inequality

$$\alpha_X + \alpha_Y < 1$$

Besides the pK_I^X and pK_I^Y shifts, the membrane

potential affects the rate constants, k_t^l and k_{-t}^l (see the kinetic diagram, whose upper index l indicates the number of protons among n translocated ions). In the case where the ion translocation proceeds through the movement of the single binding site with m negative charges of its own, one can write

$$k_t^l(\Delta\varphi) = k_t^l \cdot \exp\left(-(n-m)\cdot(\beta-\alpha_X)\cdot\phi\right)$$
$$k_t^l(\Delta\varphi) = k_{-t}^l \cdot \exp\left((n-m)\cdot(1-\beta-\alpha_Y)\cdot\phi\right) \quad (8)$$

where β is a constant that satisfies the double inequality

$$\alpha_X < \beta < 1 - \alpha_Y$$

The equilibrium constant (K_{eq}) for the transition of a free binding site [see Eq. (6)] can also depend on $\Delta\varphi$,

$$K_{eq}(\Delta\varphi) = K_{eq} \cdot \exp\left(m\cdot(1-\alpha_X-\alpha_Y)\cdot\phi\right) \quad (9)$$

Models with two (interacting or independent) binding sites have been defined above to imply the translocation of exactly n charges between X and Y. Then the parameter m in Eq. (8) should be equal to zero.

The limiting-step assumption allows one to obtain the rate equation for ATP synthesis/hydrolysis. The total concentrations of all enzyme states depicted respectively in the left or right hand parts of the kinetic diagram will be designated as [ΣE-ATP^b] and [ΣE-ATP]. Each sum comprises the forms corresponding to various possible states of domains X and Y but to one and the same state of the catalytic site. The steady-state reaction rate (v_a) is equal to the difference between the forward (ATP synthesis, v_a^{synth}) and backward (ATP hydrolysis, v_a^{hydr}) rates:

$$v_a = v_a^{synth} - v_a^{hydr} \quad (10)$$

Obviously, the ATP synthesis and hydrolysis rates are stoichiometrically related to the rates of ion transport,

$$v_a^{synth} = (v_{in}^H + v_{in}^{Na})/n, \qquad v_a^{hydr} = (v_{out}^H + v_{out}^{Na})/n \quad (11)$$

(Note that the F_0F_1-ATPase is assumed to be oriented in the membrane so that the influx of ions (in) corresponds to the ATP synthesis reaction.)

Ion fluxes in the single-site model are determined by the following equations:

$$v_{in}^H = [\Sigma E{-}ATP^b] \cdot \sum_{l=0}^{n} l\cdot k_t^l(\Delta\varphi)\cdot C_n^l\cdot(h_e)^l\cdot(\nu_e)^{n-1}/D$$
$$v_{in}^{Na} = [\Sigma E{-}ATP^b] \cdot \sum_{l=0}^{n} (n-1)\cdot k_t^l(\Delta\varphi)\cdot C_n^l\cdot(h_e)^l\cdot(\nu_e)^{n-1}/D \quad (12)$$

$$v_{out}^H = [\Sigma E{-}ATP]\cdot K_{eq}(\Delta\varphi) \cdot \sum_{l=0}^{n} l\cdot k_{-t}^l(\Delta\varphi)\cdot C_n^l\cdot(h_i)^l\cdot(\nu_i)^{n-l}/D$$
$$v_{out}^{Na} = [\Sigma E{-}ATP]\cdot K_{eq}(\Delta\varphi) \cdot \sum_{l=0}^{n} (n-l)\cdot k_{-t}^l(\Delta\varphi)\cdot C_n^l\cdot(h_i)^l\cdot(\nu_i)^{n-l}/D \quad (13)$$

$$C_n^l = n!/(l!\cdot(n-l)!)$$
$$D = (1+h_e+\nu_e)^n + k_{eq}(\Delta\varphi)\cdot(1+h_i+\nu_i)^n$$

Here,

$$h_e = H_e/K_H^X(\Delta\varphi), \qquad \nu_e = Na_e/K_{Na}^X(\Delta\varphi)$$
$$h_i = H_i/K_H^Y(\Delta\varphi), \qquad \nu_i = Na_i/K_{Na}^Y(\Delta\varphi)$$

where H_e, Na_e and H_i, Na_i are the ion concentrations in the aqueous phases outside and inside, respectively.

In the case where the rate constants k_t^l and k_{-t}^l do not depend on l, the rate equations can be simplified. It should be noted that, in this case, according to the principle of detailed balance, the difference in pK is to be equal for H^+ and Na^+ ions, i.e., $K_H^X/K_H^Y = K_{Na}^X/K_{Na}^Y$. It follows from Eqs. (11)–(13) that in this case the ATP synthesis and hydrolysis rates can be expressed as

$$v_a^{synth} = [\Sigma E{-}ATP^b]\cdot k_t^l(\Delta\varphi)\cdot(h_e+\nu_e)^n/D \quad (14)$$

$$v_a^{hydr} = [\Sigma E{-}ATP]\cdot K_{eq}(\Delta\varphi)\cdot k_{-t}^l(\Delta\varphi)\cdot(h_i+\nu_i)^n/D \quad (15)$$

For the two-site model the rate equations can be obtained from Eqs. (12) and (13) by substituting 1 for $K_{eq}(\Delta\varphi)$ and replacing the denominator D by

$$D = (1+h_e+\nu_e+h_i+\nu_i)^n$$

for the case of interacating sites, and by

$$D = (1 + h_e + \nu_e)^n \cdot (1 + h_i + \nu_i)^n$$

for the case of independent sites.

The total concentrations, [ΣE−ATP^b] and [ΣE−ATP], depend on the concentrations of adenylates and phosphate and, generally speaking, may depend on $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$. Nevertheless, under the specially designed experimental conditions where the reaction is far from equilibrium, Eqs. (11)–(15) unequivocally determine the dependences of the ATP synthesis and hydrolysis rates on the components of proton and sodium potentials. Indeed, let us assume that in the *in vitro* experiment the substrates for synthesis, ADP and P_i, are available in excess while the ATP formed is captured by the hexokinase trap. Then, since step (3) is rate limiting, [ΣE−ATP^b] under such condition will be approximately equal to the total enzyme concentration, whereas [ΣE−ATP] will be negligibly small. The rate of ATP synthesis will be determined by Eq. (12) where [ΣE−ATP^b] is replaced by a constant equal to the total enzyme concentration. Similarly, in the case of hydrolysis, with ATP in excess, and the produced ADP trapped by pyruvate kinase, the rate can be given by Eq. (13) where the total enzyme concentration should be substituted for [ΣE−ATP].

In order to find the reaction rate at any adenylate and phosphate concentrations, additional assumptions must be made. For example, it is easy to obtain the rate equation assuming the usual Michaelis mechanism of the reaction (Boyer and Kohlbrenner, 1982; Stein and Lauger, 1990):

$$v_a/V = \Big\{ d \cdot p \cdot \sum_{l=0}^{n} k_t^l(\Delta\varphi) \cdot C_n^l \cdot (h_e)^l \cdot (\nu_e)^{n-l} - t \cdot K_{eq}(\Delta\varphi) \cdot \sum_{l=0}^{n} k_{-t}^l(\Delta\varphi) \cdot C_n^l \cdot (h_i)^l \cdot (\nu_i)^{n-l} \Big\} / \{(l + d \cdot p + t) \cdot D\} \qquad (16)$$

$$d = [\mathrm{ADP}]/K_D, \qquad t = [\mathrm{ATP}]/K_T, \qquad p = [P_i]/K_P$$

here constant V is proportional to the total enzyme concentration, and the other constants are connected by the Haldane relation:

$$\frac{K_D \cdot K_P \cdot K_{eq} \cdot k_{-t}^l}{K_T \cdot k_t^l} \cdot \begin{pmatrix} K_H^X \\ \text{----} \\ K_H^Y \end{pmatrix}^l \cdot \begin{pmatrix} K_{Na}^X \\ \text{- - - -} \\ K_{Na}^Y \end{pmatrix}^{n-l} = \exp(\Delta G_p^0/R \cdot T)$$

where the values of the constants are taken at $\Delta\varphi = 0$, and ΔG_p^0 is the standard free energy of ATP synthesis [cf. Eq. (2)].

Since the actual catalytic mechanism executed by F_1 may be more complicated (Matsuno-Yagi and Hatefi, 1990) than that assumed for deriving Eq. (16), the latter can only serve as an illustration. However, Eqs. (11)–(15) allow one, without any additional assumptions about the catalytic mechanism, to analyze the kinetics of ATP synthesis and hydrolysis under conditions far from the thermodynamic equilibrium.

It is evident from the flux equations that the ranges of pH and pNa in the medium in which $H^+(Na^+)$-ATPase operates as a proton or sodium pump depend on the parameters pK_H and pK_{Na}. We have chosen such values so that at saturating Na^+ concentrations in the medium and at low pH < 7 the ATP synthesis was coupled mainly with H^+ transport, and at high pH > 8 with Na^+ transport.

The dependences of the ATP synthesis rate and the coupled ion fluxes on ΔpH and ΔpNa at $\Delta\varphi = 0$ are shown in Fig. 1 (all the rates are normalized to the corresponding maximal rates). One can see the synergism of the effects of ΔpH and ΔpNa: An increase in one of these gradients increases the ATP synthesis rate, which can be reached at the expense of the other. Obviously, such an effect would be absent for purely H^+- or Na^+-ATPases. At $\Delta\varphi = 0$ the ATP hydrolysis rate is completely inhibited only by the joint action of ΔpH and ΔpNa (cf. Figs. 2b and 2c). Certainly, both in the case of hydrolysis and synthesis, the synergism of ΔpH and ΔpNa is not observed at extremely low pH ≤ 5.5 or extremely high pH ≥ 10.5.

The curves depicted in the figures are of apparently threshold character. It is clear that this is a purely kinetic phenomenon. In fact, the equations used here describe the synthesis and hydrolysis rates under conditions that are far from thermodynamic equilibrium, when at any ΔpH and ΔpNa considered either ATP synthesis or hydrolysis is thermodynamically advantageous. As follows from the rate equations, the "kinetic" threshold is determined by the difference between pK^X and pK^Y and by the value of K_{eq} or, in other words, by the standard free energy change, ΔG_I^0.

Before investigating the kinetics further, we should focus on the following important problem: whether the influences of the electric ($\Delta\varphi$) and chemical components (ΔpH and ΔpNa) of the driving

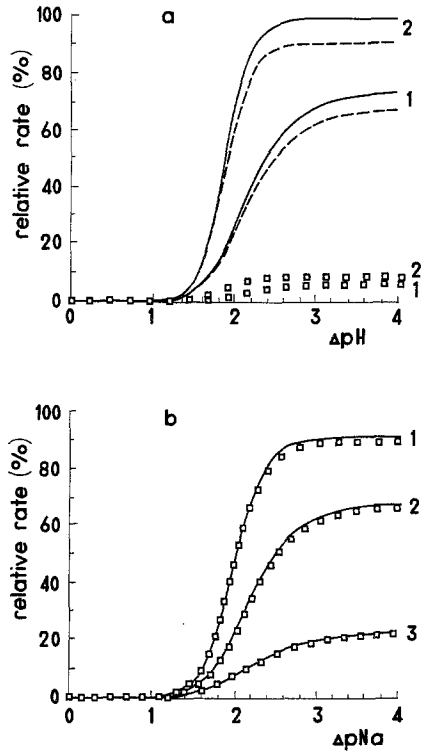


Fig. 1. Dependences of the relative rates of ATP synthesis (solid lines) and coupled fluxes of H^+ (dashed lines) and Na^+ ions (squares) on ΔpH (a) and ΔpNa (b) at $\Delta\varphi = 0$. The curves were calculated using Eqs. (11)–(14), and all the rates were normalized to the corresponding maximal rates. (a) medium pH 6.5, ΔpNa = 1 (curve 1) and 2 (curve 2); (b) pH 9.3, ΔpH = 2 (curve 1), 0.4 (curve 2), and 0 (curve 3). The Na^+ concentration in the medium is 0.3 M, and the model parameters are: $n = 3$, $pK_H^X = 9,1$, $pK_H^Y = 10,1$, $pK_{Na}^X = 2,1$, $pK_{Na}^Y = 3,1$, $pK_{eq} = 2,7$, $\alpha_X = 0,1$, and $\alpha_Y = 0.6$.

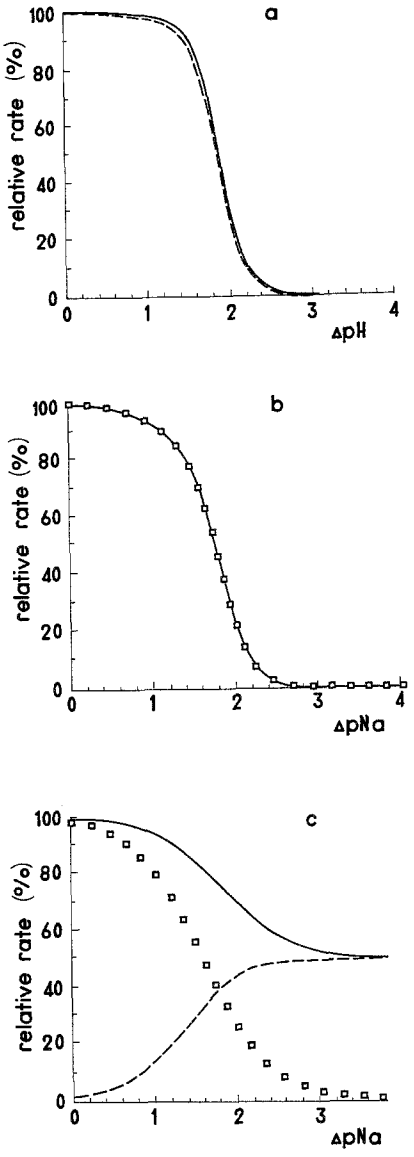


Fig. 2. Dependences of the relative rates of ATP hydrolysis (solid lines) and coupled fluxes of H^+ (dashed lines) and Na^+ ions (squares) on ΔpH (a) and ΔpNa (b, c) at $\Delta\varphi = 0$. (a) medium pH 6.5, ΔpNa = 2; (b) pH 9.3, ΔpH = 2; (c) pH 9.3, ΔpH = 0. The Na^+ concentrations in the medium and model parameters are as in the legend to Fig. 1.

forces on the ATP synthesis and hydrolysis rates are equivalent. Let us begin with the analysis of a situation with $h_e \gg \nu_e$, $h_i \gg \nu_i$, and the enzyme operating as a proton pump [see Eqs. (12) and (13)]. As evident from the rate equations, at a fixed H_e concentration the effect of ΔpH can be described as a shift in $-pK_H^Y$ by ΔpH (with $H_i = H_e$ preserved), and at a fixed H_i concentration as a shift in pK_H^X. On the other hand, analyzing the effect of the electric field one can see that for the single-site model $\Delta\varphi$ produces: (i) a shift in pK described by Eq. (7), (ii) a change in K_{eq} which may be approximated (if $h_e > 1$, $h_i > 1$) as an additional shift in pK_H^Y, so that the combined shift in $(pK_H^X - pK_H^Y)$ is $\{(\alpha_X + \alpha_Y) + (m/n) \cdot (1 - \alpha_X - \alpha_Y)\} \cdot \Delta\varphi/\zeta$, $\zeta = \ln 10 \cdot R \cdot T/F \approx 60\,\text{mV}$, and (iii) changes in k_t^l and k_{-t}^l corresponding to the changes in the maximal rates of synthesis and hydolysis given by Eq. (8). This means that for the two forms of energy (ΔpH and $\Delta\varphi/\zeta$) to be equivalent, it is necessary that at least one of the following conditions hold:

$$m = n \tag{18}$$

i.e., the positive charge of ions translocated between X and Y is completely compensated by the negative charges of the binding site, or

$$\alpha_X + \alpha_Y = 1 \tag{19}$$

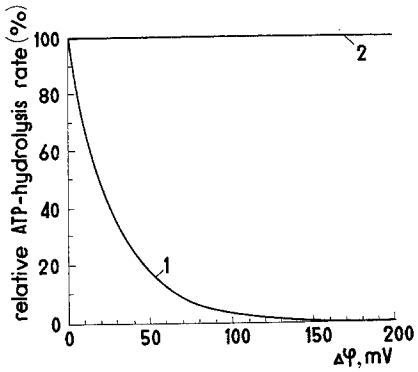


Fig. 3. Dependences of the relative rate of ATP hydrolysis at zero $\Delta\bar{\mu}_{H^+}$ on $\Delta\varphi$ (the pH gradient is opposite to the membrane potential in this case). (1) "Nonequivalent" model, $m = 0$; (2) "equivalent" model, $m = n$. Medium pH 7.5, $\Delta pNa = 2$; under such conditions ATP hydrolysis is almost completely coupled to H^+ transport. Other parameter values are as in the legend to Fig. 1.

the latter condition means that ions must be translocated between X and Y along an equipotential surface, i.e., the sum of the lengths of the inlet and outlet ion channels must be equal to the total membrane thickness. This finding closely resembles a tacit Mitchell's assumption in his definition of a "proton well" (Mitchell, 1969 1977; Mitchell and Moyle, 1974).

For two-interacting-site model (simultaneous binding of ions to both sites is impossible), the equivalence of ΔpH and $\Delta\varphi$ may be achieved if condition (19) is fulfilled. For the two-independent-site model, such an equivalence is impossible.

At very high pH, when $\nu_e \gg h_e$, $\gg h_i$, and the enzyme operates as a sodium pump, conditions (18) or (19) ensure the equivalence of ΔpNa and $\Delta\varphi$. In the pH range where the probabilities of binding of protons and sodium ions with the enzyme are comparable, the membrane potential may be replaced only by two equal gradients ΔpH and ΔpNa. Reviewing the reasoning above, one may conclude that the key condition of equivalence of different forms of driving forces is that the electrogenic steps in the charge translocation cycle be not coupled to the limiting step of catalysis by F_1 (Maloney and Schattschneider, 1980; Maloney and Hansen, 1982).

Let us compare the kinetic characteristics of the "equivalent" [one of the conditions (18) or (19) is fulfilled] and "nonequivalent" models. Considering the ATP hydrolysis rate at zero $\Delta\bar{\mu}_{H^+}$ (e.g., when a protonophore is present), one can see that it does not depend on concrete $\Delta\varphi$ and ΔpH values in the equivalent model and may be high at any $\Delta\varphi$ (Fig.

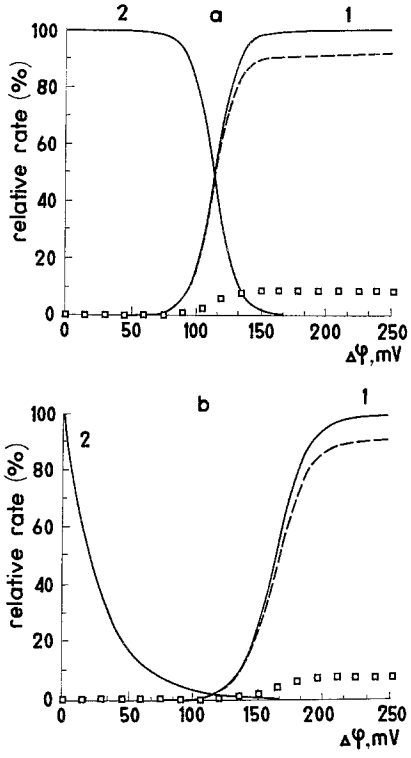


Fig. 4. Dependences of the relative rates of ATP synthesis (solid lines, curve1) and hydrolysis (solid lines, curve 2) on $\Delta\varphi$ for the "equivalent" (a) and "nonequivalent" (b) models. The fluxes of H^+ (dashed lines) and Na^+ ions (squares) coupled to ATP synthesis are shown. Medium pH 6.5, $\Delta pH = 0$, $\Delta pNa = 2$; other parameters are as in the legend to Fig. 3.

3; note that the pH gradient is opposite to the membrane potential in this case). However, for the nonequivalent model it is markedly inhibited by $\Delta\varphi$ (due to the electrogenicnature of the rate-limiting step) also at zero $\Delta\bar{\mu}_{H^+}$ (Fig. 3).

The rate of the enzyme reaction does not grow infinitely with rise in substrate concentrations, but the saturation effect takes place. In the models considered, the ATP synthesis rate reaches a plateau with increase in ΔpH or ΔpNa (fig. 1). In the equivalent model, a similar effect occurs, obviously, also for the dependence on $\Delta\varphi$ (Fig. 4a). At the same time, for the nonequivalent model the presence or absence of $\Delta\varphi$ saturation of the synthesis rate allows one to distinguish different versions of the model. The saturation is observed when $\beta = \alpha_X$. Then $\Delta\varphi$ does not enhance the maximal rate of ATP synthesis and only reduces the maximal rate of hydrolysis [Eq. (8)]; see Fig. 4b. If $\beta > \alpha_X$, the maximal rate of synthesis rises exponentially with increase in $\Delta\varphi$.

The dependences of the ATP synthesis rate on

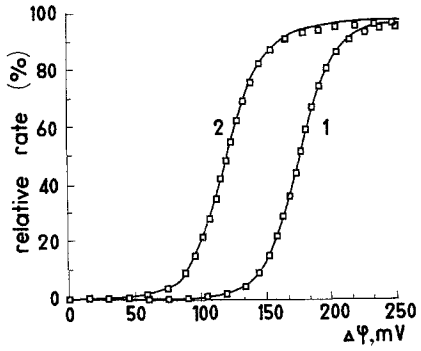


Fig. 5. Dependences of the relative rates of ATP synthesis (solid lines) and coupled Na^+ flux (squares) on $\Delta\varphi$ at inverse ΔpH. Alkaline pH 9.6, $\Delta pH = -1.5$ $\Delta pNa = 0$ (curve 1) or 2 (curve 2), $m = 0$; other parameters are as in the legend to Fig. 1.

$\Delta\varphi$ are also threshold (Fig. 4). It is clear that for the nonequivalent model this threshold is more pronounced if saturation with respect to $\Delta\varphi$ takes place. At the same time, the dependences of the hydrolysis rate on $\Delta\varphi$ are threshold only for the equivalent model (Fig. 4; cf. the different curves in Fig. 3).

Increasing medium pH leads to switchover from the $\Delta\bar{\mu}_{H^+}$-driven synthesis to the $\Delta\bar{\mu}_{Na^+}$-driven one (Figs. 1 and 5). Moreover, synthesis coupled with Na^+ transport is possible even at inverse (negative) ΔpH (Fig. 5). Under these conditions ATP hydrolysis will be coupled with H^+ transport if there is marked ΔpNa, and with Na^+ transport otherwise (Fig. 6).

All the types of kinetic dependences described are characteristic of the models involving one or two interacting sites. In the two-independent-site model the ATP synthesis and hydrolysis rates drop drastically when the NA^+ and H^+ concentrations become higher than their equilibrium dissociation constants K_{Na}^X and K_H^Y, respectively. Therefore the model versions treated above seem to be more plausible.

DISCUSSION

In the present paper we propose kinetic models of F_0F_1-ATPase capable of synthesizing ATP at the expense of both $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$. Structurally, these models imply the existence of two ion semichannels accessible for competing H^+ and Na^+ ions from the bulk phases. Ion translocation between membrane domains X and Y, lying at the bottoms of the inlet and outlet channels, is possible only in certain states of the catalytic site of F_1. For the sake of definiteness it is presupposed that the catalytic step coupled with

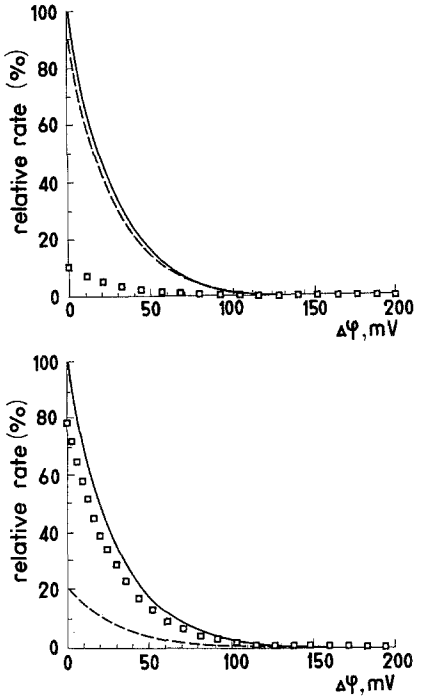


Fig. 6. Dependences of the relative states of ATP hydrolysis (solid lines) and coupled fluxes of H^+ (dashed lines) and Na^+ ions (squares) on $\Delta\varphi$ at inverse ΔpH. Alkaline pH 9.6, $\Delta pH = -1.5$. (a) $\Delta pNa = 1.5$; (b) $\Delta pNa = 0$. Other parameter values are as in the legend to Fig. 5.

ion translocation is the release of tightly bound ATP (step 1) which requires an input of free energy.

Most of the kinetic models of proton ATPases put forward earlier (see, e.g., Kamp *et al.*, 1988; Westerhoff and Van Dam, 1987) postulate that the free energy of $\Delta\bar{\mu}_{H^+}$ is transduced to the enzyme only at that step of the catalytic cycle which is coupled with proton translocation. The cardinal difference of the models treated here is that the high-energy state (E^*) may arise in any or in numerous states of F_1 (at least for E-ATP[b] and ATP). This occurs through ion binding to X or conformational transition alternating with the orientation of the binding site. At the step of the catalytic cycle coupled with ion translocation between X and Y the energy of E^* relaxation is used for the release of bound ATP.

It is not clear whether $\Delta\bar{\mu}_{H^+}$- and $\Delta\bar{\mu}_{Na^+}$-driven ATP synthesis can really be performed in a cell by the same enzyme under physiological conditions. Although it was reported (Laubinger and Dimroth, 1989) that in proteoliposomes the same *P. modestum* ATPase transports either Na^+ or H^+, depending on the $[Na^+]$ level in the medium, *in vivo* it operates only

as a Na^+-pump. A number of observations indicate that ATP synthesis can be driven by $\Delta\bar{\mu}_{Na^+}$, which is generated by Na^+-motive respiratory pumps (Skulachev, 1992). At the same time, Na^+- and H^+-motive respiratory chains can co-exist (Tokuda and Unemoto, 1982, 1983), and, apparently, cells are able to generate $\Delta\bar{\mu}_{H^+}$ or $\Delta\mu_{na^+}$ under different conditions.

Bacteria are known to efficiently maintain the intracellular pH (Slonczewski *et al.*, 1981; Padan *et al.*, 1981). At acid or neutral pH of the medium, ΔpH and $\Delta\varphi$ generated by the respiratory chain are of the same direction (more acid and more positive outside), and high $\Delta\bar{\mu}_{H^+}$, sufficient for ATP synthesis, is generated. At alkaline pH the sign of $\Delta\varphi$ is the same (more positive outside), but that of ΔpH is inverse (due to the homeostasis of the intracellular pH, more acid pH is found inside). Therefore, $\Delta\bar{\mu}_{H^+}$ drops drastically at very high pH. It is clear that under such conditons the respiratory phosphorylation of ATP could proceed only at the expense of $\Delta\bar{\mu}_{Na^+}$.

In order to survive at both acidic and high alkaline pH, bacteria need either two different H^+- and Na^+-ATPases or single $H^+(Na^+)$-ATPase similar to that treated in this work. Obviously, in the former cse, at high alkaline pH and low $\Delta\bar{\mu}_{H^+}$ H^+-ATPase should be completely arrested to avoid hydrolysis of the ATP synthesized by Na^+-ATPase. In the latter case, $H^+(Na^+)$-ATPase automatically switches over from the ATP synthesis at the expense of $\Delta\bar{\mu}_{H^+}$ to the $\Delta\bar{\mu}_{Na^+}$-driven one (Figs. 1 and 5). We emphasize that the synthesis may proceed with a high rate even at inverse ΔpH (fig. 5).

In the case where both ion potentials are greater than ΔG_p [see Eq. (1)], kinetic support of ATP synthesis by $\Delta\bar{\mu}_{H^+}$ and $\Delta\bar{\mu}_{Na^+}$ takes place. At the same time, at low $\Delta\mu_{H^+}$ ATP hydrolysis by the proton mode of catalysis can be efficiently inhibited by $\Delta\bar{\mu}^+_{Na}$. At extemely high pH > 10.5, proton hydrolysis can be suppressed by ΔpNa alone; at medium pH within the range 9.5–10.5 it can be arrested if a membrane potential (even not large, subthreshold) is present together with ΔpNa. At the same time, at neutral or weakly alkaline pH and zero $\Delta\bar{\mu}_{H^+}$ proton hydrolysis can be suppressed only by $\Delta\varphi$ and only in the case of the nonequivalent model (Fig. 3). For the model with equivalent electric and concentration forms of driving forces, the rate of the futile cycle of synthesis—hydrolysis (coupled with Na^+ and H^+ transport, respectively)—cannot be controlled by $\Delta\varphi$.

The question whether a cell can synthesize ATP, consuming $\Delta\bar{\mu}_{H^+}$ or $\Delta\bar{\mu}_{Na^+}$ by the same enzyme, has proved to be especially intriguing in connection with the investigation of the energetics of marine alkalotolerant bacterium *Vibrio alginolyticus*. These cells live in mats of algae where strong pH oscillations occur due to the photosynthetic activity: pH, which is neutral in the morning, shifts to high values in the evening. It has been found that the *V. alginolyticus* respiratory chain includes both $\Delta\bar{\mu}_{H^+}$- and $\Delta\bar{\mu}_{Na^+}$-generators (Na^+ -motive NADH-quinone reductase and H^+ -motive terminal oxidase) (Tokuda and Unemoto, 1982; Skulachev, 1988). Although both the $\Delta\bar{\mu}_{H^+}$- and $\Delta\bar{\mu}_{Na^+}$-driven ATP syntheses were observed (Skulachev, 1988; Dibrov *et al.*, 1986) only one type of F_0F_1 -ATPase was revealed in the *V. alginolyticus* membrane (Dmitriev *et al.*, 1989).

The existence of two different H^+- and Na^+ -ATPases was suggested by Tsuchiya's group (Sakai *et al.*, 1989). They selected a mutant of *Vibrio parahaemolyticus*, a close relative of *V. alginolyticus*, with greatly lowered levels of α and β subunits of factor F_1. Data on the $\Delta\bar{\mu}_{H^+}$- and $\delta\bar{\mu}_{Na^+}$ -driven ATP synthesis rates in the mutant and in the wild type were interpreted as an indication that *V. parahaemolyticus* Na^+ -ATPase is not a F_0F_1 -pump. However, in a recent publication (Sakai-tomita *et al.*, 1991), the authors noted that their mutant probably "possesses altered F_0F_1 and lost the ability to utilize H^+ as a coupling ion."

An alternative possibility of $H^+(Na^+)$-ATPase, which is H^+- or Na^+-motive at high or low pH, respectively, seems to be quite reasonable for *V. alginolyticus* cells (Skulachev, 1992). In this case a shift of environmental pH to high alkaline values, accompanied by a drop in $\Delta\bar{\mu}_{H^+}$, will led to $H^+(Na^+)$-ATPase switchover from the H^+ to Na^+ mode of ATP synthesis. As was shown, there is no danger of ATP hydrolysis at low $\Delta\bar{\mu}_{H^+}$ and sufficiently high $\Delta\bar{\mu}_{Na^+}$ (or $\Delta\varphi$).

ACKNOWLEDGMENT

The author is indebted to Prof. V. P. Skulachev and Dr. B. V. Chernyak for stimulating and fruitful discussions.

REFERENCES

Boork, J., and Wennestrom, H. (1984). *Biochim. Biophys. Acta* **767,** 314–320.
Boyer, P. D. (1975). *FEBS Lett.* **58,** 1–6.
Boyer, P. D. (1987). *Biochemistry* **26,** 8503–8507.

Boyer, P. D. (1989). *FASEB J.* **3,** 2164–2178.
Boyer, P. D., and Kohlbrenner, W. E., (1981). The present status of the binding-change mechanism and its relation to ATP formation in chloroplasts. In: *Energy coupling in Photosynthesis*, (Selman, B. R., and Selman-Reimer, S. eds.), Elsevier, Amsterdam, pp. 231–240.
Cox, G. B., Fimmel, A. L., Gibson, F., and Hatch, L. (1986). *Biochim. Biophys. Acta* **849,** 62-69.
Cross, R. L. (1981). *Annu. Rev. Biochem.* **50,** 681–714.
Dibrov, P. A., Lazarova, R. L., Skulachev, V. P., and Verkhovska, M. L. (1986). *Biochim. Biophys. Acta* **850,** 458–465.
Dmitriev O., Grinkevich V. A., and Skulachev V. P. (1989). *FEBS Lett.* **258,** 219–222.
Fillinghame, R. H. (1990). Molecular mechanics of ATP synthesis by F_0F_1 -type H^+ -transporting ATP synthases. In: *The Bacteria* (Krulwich, T. A., ed.), Academic Press, San Diego, Vol. 12, pp, 345–391.
Gresser, M. J., Myers J. A., and Boyer, P. D. (1982). *J. Biol. Chem.* **257,** 12030–12038.
Kagawa Y., and Racker, E. (1971). *J. Biol. Chem.* **246,** 5477–5487.
Kagawa, Y., Sone, N. Hirata, H., and Yoshida, M. (1979). *J. Bioenerg. Biomembr.* **11,** 39–78.
Kamp, F., Welch, G. R., and Westerhoff, H. V. (1988). *Cell Biophys.* **12,** 201–236.
Kholodenko, B. N. (1991) *J. Chem. Biochem. Kinet.* accepted.
Laubinger, W., and Dimroth, P. (1987). *Eur. J. Biochem.* **168,** 475–480.
Laubinger, W., and Dimroth, P. (1989). *Biochemistry* **28,** 7194–7198.
Maloney, P. C. and Hansen, F. C. (1982). *J. Memb. Biol.* **66,** 63–75.
Maloney, P. C., and Schattschneider, S. (1980). *FEBS Lett.* **110,** 337–340.
Matsuno-Yagi, A., and Hatefi, Y. (1990). *J. Biol. Chem.* **265,** 82–88.
Mitchell, P. (1961). *Nature (London)* **191,** 144–148.
Mitchell, P. (1966). *Biol. Rev.* **41,** 445–502.
Mitchell, P. (1969). *Theor. Exp. Biophys.* **2,** 159–216.
Mitchell, P., (1973). *FEBS Lett.* **33,** 267–274.
Mitchell, P. (1975). *FEBS Lett.* **50,** 95–97.
Mitchell, P. (1977). *Symp. Soc. Gen. Microbiol.* **27,** 383–423.
Mitchell, P., and Moyle, J. (1974). *Biochem. Soc. Spec. Suppl.* **4,** 91–111.
Padan, E., Zilberstein, D., and Schuldiner, S. (1981). *Biochim. Biophys. Acta* **650,** 151–166.
Penefsky, H. S. (1985). *J. Biol. Chem.* **260,** 13728–13734.
Reynolds, J. A., Johnson, E. A., and Tanford, C. (1985). *Proc. Natl. Acad. Sci. USA* **82,** 6869–6873.
Rosing, J., and Slater E. C. (1972). *Biochim. Biophys. Acta.* **267,** 275–290.
Sakai, Y., Moritani, C., Tsuda, M., and Tsuchiya, T. (1989). *Biochim. Biophys. Acta* **973,** 450–456.
Sakai-Tomita, Y., Tsuda, M., and Tsuchiya, T. (1991). *Biochem. Biophys. Rs. Commun.* **179,** 224–228.
Senior, A. E. (1988). *Physiol. Rev.* **68,** 177–231.
Skulachev, V. P. (1988). *Membrane Bioenergetics*, Springer-Verlag, Berlin.
Skulachev, V. P. (1992). Chemiosmotic systems and the basic principles of cell energetics. In: *New Comprehensive Biochemistry. Molecular Bioenergetics,* (Ernster, L. ed.), Elsevier, Amsterdam.
Slonczewski, L. L., Rosen, B. P., Alger, J. R., and Macnab, R. M. (1981). *Proc. Natl. Acad. Sci. USA* **78,** 6271–6275.
Stein, W. D., and Lauger, P. (1990). *Biophys. J.* **57,** 255–267.
Tokuda, H., and Unemoto, t. (1982). *J. Biol. Chem.* **257,** 10007–10014.
Tokuda, H., and Unemoto, T. J. (1983). *J. Bacteriol.* **156,** 636–643.
Unemoto, T., and Hayashi M. (1979). *J. Biochem.* **85,** 1461–1467.
Westerhoff, H. V., and Van Dam, K. (1987). *Thermodynamics and Control of Biological Free-energy Transduction.* Elsevier, Amsterdam, New York, Oxford.