ELSEVIER

Biochimica et Biophysica Acta 1187 (1994) 171–176

BBA Biochimica et Biophysica Acta

The H^+-ATPase from chloroplasts: energetics of the catalytic cycle

Peter Gräber *

Institut für Physikalische Chemie, Universität Freiburg, Albertstraße 23a, D-79104 Freiburg, Germany

Received 7 April 1994

Abstract

The H^+-ATPase from chloroplasts couples a transmembrane proton transport with ATP synthesis and ATP hydrolysis. The ability of the enzyme to catalyze this reaction is regulated by a redox-reaction involving HS-groups and by an activation involving protonation/deprotonation reactions. When the enzyme is in the active reduced state the catalytic reaction cycle can be studied. A simple enzyme kinetic model is proposed. In the state E_1 proton binding sites in the CF_0 part are directed to the inside. After protonation ADP and P_i bind in a random sequence to the CF_1 part. The enzyme changes into conformation E_2 where proton binding sites are directed to the outside and enzyme bound ATP is formed. The protons dissociate to the outside, ATP is released and finally a change back to the E_1 state closes the reaction cycle. Literature data are used to construct a Gibbs free enthalpy diagram for the reaction cycle.

Key words: Chloroplast; ATPase, H^+-; Enzyme kinetics

1. Introduction

The H^+-ATPase from chloroplasts is a member of the F-type ATPase family [1]. These enzymes couple a transmembrane proton transport with ATP synthesis and ATP hydrolysis at the plasmamembrane of bacteria, at the thylakoid membrane from chloroplasts and at the inner mitochondrial membrane. Various kinetic models for such a coupling have been previously published [2–10]. Our current working model for the kinetics of the H^+-ATPase from chloroplasts is based on those earlier considerations. Special attention was given to the following points:

(1) A clear distinction is made between the regulation of the activity of CF_0F_1 and the catalytic reaction performed by the enzyme.

(2) The number of intermediates and the number of different enzyme conformations is kept at the minimum in order to allow a simple description.

(3) The model describes the reaction at one catalytic site only; no interactions between different catalytic sites are taken into account. However, I assume that the model can be used to describe the reaction under uni-site and multi-site conditions, the only difference being that the rate constants of some steps might be changed by site-site interactions. The experimental data used in the following refer to multi-site conditions.

2. Regulation

The H^+-ATPase from *E. coli* works in vivo in ATP synthesis and in ATP hydrolysis direction. The enzymes from mitochondria and chloroplasts seem to catalyze under physiological conditions only the ATP synthesis direction. Since the reaction is intrinsically reversible this behaviour requires a regulation of the enzymes: they are allowed to catalyze only when external conditions are such that the synthesis direction is thermodynamically allowed.

This regulation works with the chloroplasts enzyme as shown in Fig. 1 [11]. The enzyme occurs in an oxidized and a reduced state, E^{ox} and E^{red}. The redox state (in particular the redox state of an -S-S-bridge in the γ-subunit [12]) can be changed in vitro by incubation with dithiothreitol [13]; in vivo, thioredoxin reduces CF_0F_1 [14]. The rate of reduction is slow when the membrane is deenergized, it is increased when the membrane is energized. In both redox states the en-

* Corresponding author. Fax: +49 761 2036189.

SSDI 0005-2728(94)00110-Q

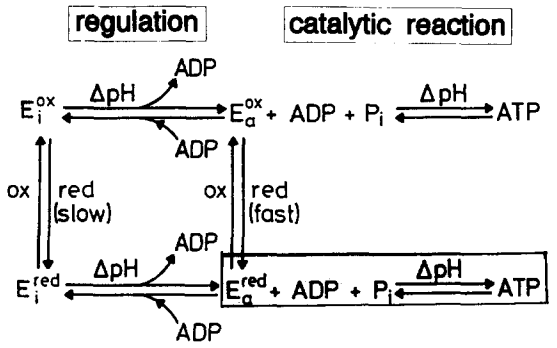

Fig. 1. Relation between regulation of the activity of CF_0F_1 and the catalytic reaction. For details, see text.

zyme is catalytically inactive (E_i^{red} and E_i^{ox} in Fig. 1), i.e., it does not catalyze ATP synthesis and ATP hydrolysis, although the latter is thermodynamically feasible. When the membrane is energized the enzyme is converted into the metastable active enzyme states E_a^{red} or E_a^{ox}.

These two enzyme forms can catalyze ATP synthesis and ATP hydrolysis. They differ in two respects.

(1) The inactive-active transition requires a higher ΔpH for the oxidized than for the reduced enzyme. About half of the oxidized enzymes are shifted into the active state at $\Delta pH = 3.4$ [11]. Since the ΔpH required for activation is higher than that for ATP synthesis the enzyme becomes active only when conditions are such that ATP synthesis is feasible. About half of the reduced enzymes are shifted into the active state at ΔpH 2.2 [11]. Recent results indicate that the ΔpH for half maximal activation of E^{red} is 1.6 [15]. In vivo it might be even lower [16]. This ΔpH is lower than that required for the ATP synthesis direction and therefore, some enzymes become active and ATP hydrolysis can be observed. In vivo, the energy stored across the membrane is dissipated in the dark and the reduced enzymes become inactive again. Therefore, in the dark high concentration of ATP (about 1 mM) are found in chloroplasts [17].

(2) The active enzymes E_a^{red} and E_a^{ox} are metastable states. When the membrane is deenergized E_a^{ox} becomes inactive very fast presumably, in the time range below 1 s; E_a^{red} becomes inactive in the time range between seconds and minutes (depending on reaction conditions [23]).

The metastable active state is generated upon energization of the membrane by $\Delta\varphi$ and/or ΔpH. This implies that protons bind from the internal aqueous phase to the F_0-part and subsequently the conformation of the enzyme is changed into the active state. Energization of the membrane leads also to the release of a tightly bound ADP [18,19]. It has been shown that the amount of ADP released in the fast phase gives directly the amount of activated CF_0F_1 [20,21]. Since in the state E_i^{red} the enzyme is catalytically inactive and E_a^{red} catalyzes high rates of ATP hydrolysis the number of activated enzymes can also be measured by the relative rate of ATP hydrolysis. From a kinetic analysis it is found that 2–6 H^+ must bind to transform the enzyme into the active state [5,11,15,22]. It is supposed that these protons do not participate in the catalytic process. Therefore, the enzyme in the metastable state, E_a^{red}, has several bound protons and it has lost its ADP. It contains usually 1–2 ATP on non-catalytic sites [23].

After deenergization, the reduced enzyme remains in this state for some time. When no ADP is present it inactivates slowly to an inactive state without bound

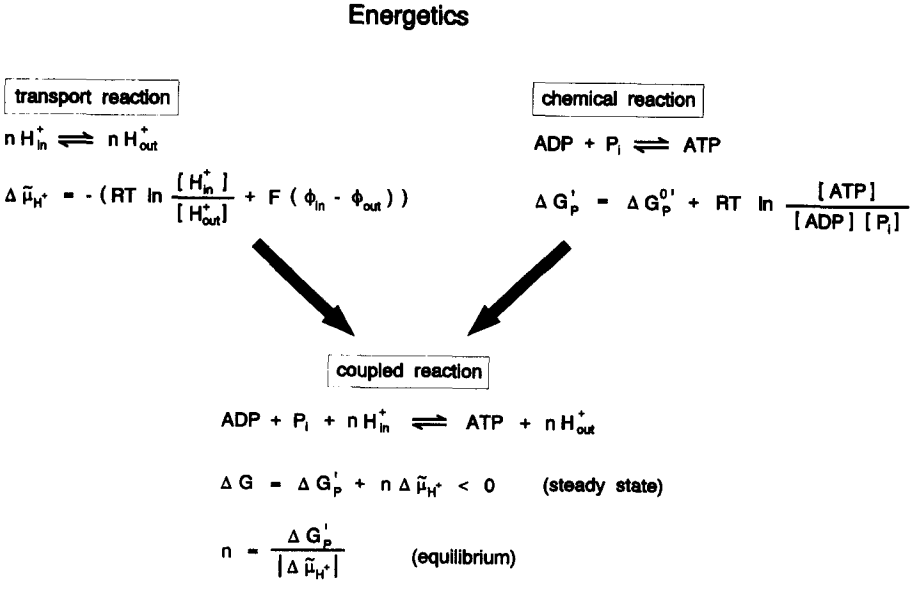

Fig. 2. The energetics of the coupling between proton transport and ATP synthesis/hydrolysis. For details, see text.

ADP (half-lifetime about 10 min). When ADP is present, a rapid binding to the enzyme is observed and an inactive state with a bound ADP is formed [23].

This summary shows that the substrates of the catalytic reaction are also involved in the activation/inactivation of the enzyme. However, for the sake of clarity I assume in the following that activation and catalytic reaction are independent processes: E_a^{red} is generated and this enzyme state then catalyzes ATP synthesis and ATP hydrolysis. In the following, I consider only the reaction catalyzed by E_a^{red} (indicated in the box in Fig. 1) and for convenience I avoid the use of the indices for active and reduced.

3. Energetics

The energetics of proton transport coupled ATP synthesis and ATP hydrolysis is described by the chemiosmotic theory [24]. The relevant equations are summarized in Fig. 2: there is a transport reaction where H^+ are translocated across the membrane from the internal aqueous phase (in) to the external aqueous phase (out) (left). The transmembrane electrochemical potential difference, $\Delta\tilde{\mu}_{H^+}$, is shown below. The chemical reaction and the corresponding free enthalpy change are shown on the right. The H^+-ATPase couples these two processes giving the overall reaction and the corresponding free enthalpy change (center). Great efforts have been made to prove or disprove the predictions of the chemiosmotic theory. The principle of the theory is now widely accepted; however, no general aggreement has been reached about the quantitative aspects. The main experimental problems are the measurements of ΔpH and $\Delta\varphi$ (the components of $\Delta\tilde{\mu}_{H^+}$) and the proper separation of the basal proton transport and the phosphorylation coupled proton transport. For example, it is generally believed that n (the H^+/ATP ratio) is 3. However, recent measurements with the H^+-ATPase from chloroplasts in the state E_a^{red} give $n = 4$ [25,26,27]. Also higher H^+/ATP ratios varying with the experimental conditions have been reported [27].

4. Kinetics

In order to understand how the enzyme couples the vectorial proton translocation with the scalar chemical reaction, it is necessary to formulate a reaction sequence describing the catalytic cycle. Fig. 3 shows the simplest schemes for the transmembrane proton transport (left) and ATP synthesis (right) and their coupling (bottom).

Proton transport across the membrane is described by a four-state model. In state E_1 the proton binding

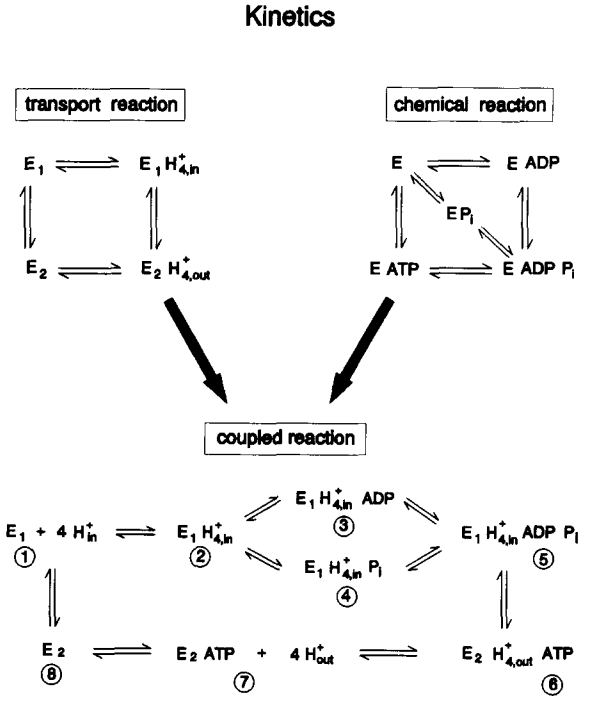

Fig. 3. The kinetics of the coupling between proton transport and ATP synthesis/hydrolysis. For details, see text.

sites are directed to the inside; after proton binding, a conformational change into state E_2 occurs. In E_2 the proton binding sites are directed to the outside, the protons dissociate to the outside and finally the conformation changes back to E_1.

This scheme describes a carrier type and also channel type translocator. If the F_0 part acts as a proton channel [28], then the channel must provide alternating access to both sides of the membrane, since otherwise no useful work can be obtained. The two alternating conformations are represented by E_1 and E_2.

The chemical reaction is described by a five-state model: ADP binds to the enzyme, followed by P_i binding or P_i binds first followed by ADP binding. The enzyme-bound ADP P_i reacts to enzyme bound ATP and finally ATP is released and the free enzyme is regenerated.

There are of course different ways in which both the reactions may be coupled [2–10]. In accordance with recent experimental results I use the following reaction sequence. In the state E_1 the proton binding sites are directed to the inside and the enzyme is protonated. After protonation it can bind first ADP and then P_i or first P_i and then ADP. After this binding there is a conformational change into the state E_2, where proton binding sites are directed to the outside and concomitantly enzyme-bound ATP is formed. The protons dissociate to the outside, ATP is released and finally the enzyme changes back into the state E_1, thereby closing the reaction cycle.

This sequence is based on the following observations: ADP binding to the enzyme is slow without energization. When the membrane is energized ADP binding is fast. Therefore, proton binding to the enzyme must occur first and then either ADP or P_i is bound. Presumably, ADP and P_i bind in a random order [26,29]. When all substrates are bound (H^+_{in}, ADP, P_i) the enzyme changes its conformation and the proton binding sites are now directed to the outside; concomitantly enzyme bound ATP is formed. This assignment is not based on experimental results. The argument behind this step is the following: protonation from the inside shifts the enzyme into the high energy conformation, E_1, and the catalytic site for ADP and P_i opens. After binding of the substrates the enzyme relaxes to the low-energy state, E_2, and this transition provides the energy for synthesis of enzyme bound ATP. After this step, protons are released to the outside, followed by the release of ATP; however, the latter sequence is not exactly known. The change of the enzyme conformation into the state E_1 closes the cycle.

The above scheme is of course greatly simplified.

(1) Protonation/deprotonation reactions are assumed to occur in one step. This is presumably not correct. For details see Ref. [10].

(2) The scheme describes the reaction at only one catalytic site. However, most experimental evidence indicates two or three catalytic sites involved in the reaction (for review see Ref. [30]). I have assumed here that under uni-site and under multi-site conditions the same reaction sequence occurs. The cooperativity between sites is assumed to change only the rate constants for some steps. In the following, I use constants obtained under multi-site conditions, i.e., the resulting $\Delta G^{\circ\prime}$ diagram includes the site-site interaction.

(3) Presumably, the enzyme runs through several different conformations during the reaction cycle: the two conformations proposed in the model are minimally required for coupling.

5. Energetics of the reaction cycle

Based on this reaction scheme a $\Delta G^{\circ\prime}$ diagram can be constructed using literature data for the different equilibrium constants. The dissociation constants for protonation outside and inside result from measurements of the rate of ATP synthesis as a function of pH_{out} and pH_{in} with CF_0F_1 proteoliposomes [10]. The data ($K_{in} = 1.6 \cdot 10^{-6}$ M, $K_{out} = 1.6 \cdot 10^{-8}$ M) are converted into the biochemical standard state (pH 7) and written in ATP synthesis direction. With the nomenclature in Fig. 3 results: $K'_{12} = (K'_{in})^{-1} = 0.0625\ M^{-1}$ and $K'_{67} = K'_{out} = 0.16$ M. I assume that 4 H^+ are bound and translocated per ATP synthesized and obtain finally $\Delta G^{\circ\prime} = -4\ RT\ \ln K'_{12}$ (see Table 1).

The dissociation constants for ADP and P_i have been obtained recently from an analysis of the rate of ATP synthesis as a function of P_i and ADP concentration at constant ΔpH [29]. These dissociation constants depend on ΔpH, whereas the corresponding K_M values do not. Nevertheless, the dissociation constants, K_D, and the Michaelis-Menten constants, K_M, have the same order of magnitude. The dissociation constant of ATP was estimated from K_M values. Several data are collected in Table 1 and I have chosen a mean value for calculation of the $\Delta G^{\circ\prime}$.

The equilibrium constant between enzyme-bound substrates and products, K_{eq}, has been measured under uni-site conditions [31]. This produced $K_{eq} = 0.5$,

Table 1
Equilibrium constants for the different steps of the reaction cycle

The literature data are converted into the biochemical standard state, written in ATP synthesis direction and $\Delta G^{\circ\prime}$ is calculated. For details, see text.

Equilibrium constant	Literature	Used in this work	ATP synthesis direction	$\Delta G^{\circ\prime}$ (kJ/mol)
K_D (H^+_{in})	$1.6 \cdot 10^{-6}$ M [10]	$1.6 \cdot 10^{-6}$ M	$K_{12} = 6.25 \cdot 10^5\ M^{-1}$	
	$4 \cdot 10^{-6}$ M [7]		$K'_{12} = 6.25 \cdot 10^{-2}\ M^{-1}$	+27.5
K_D (ADP)	$(1.5–6) \cdot 10^{-5}$ M [29]	$2 \cdot 10^{-5}$ M	$K'_{23} = 5 \cdot 10^4\ M^{-1}$	−26.9
	$4 \cdot 10^{-6}$ M [7]			
K_M (ADP)	$4.5 \cdot 10^{-5}$ M [29]			
	$3.2 \cdot 10^{-5}$ M [34]			
K_D (P_i)	$(3–8) \cdot 10^{-4}$ M [29]	$4 \cdot 10^{-4}$ M	$K'_{35} = 2.5 \cdot 10^3\ M^{-1}$	−19.4
	$4 \cdot 10^{-3}$ M [7]			
K_M (P_i)	$8.3 \cdot 10^{-4}$ M [29]			
K_{eq}	0.4–0.6 [31]	0.5	$K'_{56} = 2$	−1.7
K_D (H^+_{out})	$1.6 \cdot 10^{-8}$ M [10]	$1.6 \cdot 10^{-8}$ M	$K_{67} = 1.6 \cdot 10^{-8}$ M	
	$4 \cdot 10^{-9}$ M [7]		$K'_{67} = 0.16$ M	+18.2
K_D (ATP)	$3 \cdot 10^{-4}$ M [7]	$1 \cdot 10^{-4}$ M	$K'_{78} = 1 \cdot 10^{-4}$ M	+22.8
K_M (ADP)	$7.5 \cdot 10^{-5}$ M [29]			
	$2.2 \cdot 10^{-5}$ M [23]			
K_E			$K_{81} = 7.3 \cdot 10^{-3}$	+12.7

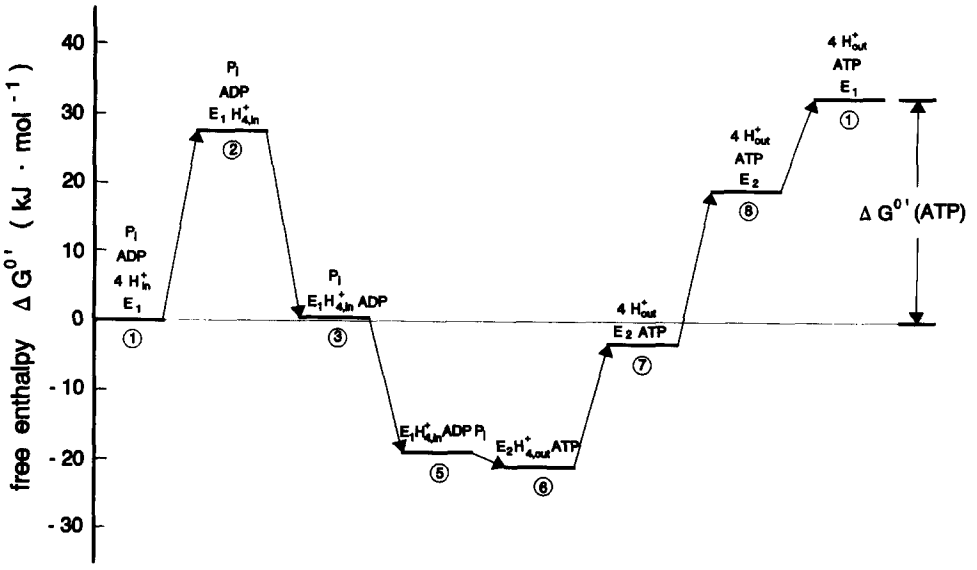

Fig. 4. Standard free enthalpy changes during the reaction cycle. The free enthalpy of the state E_1, 4 H^+_{in}, ADP, P_i is arbitrarily defined as zero. Data for the ΔG^{α} of the different steps are collected in Table 1. For details, see text.

and the constant does not change significantly under multisite conditions [32].

In the last step the reaction cycle is closed by the reaction $E_2 \rightarrow E_1$. There is as yet no experimental evidence for this step. Nevertheless, it must exist and its ΔG^{α} can be calculated from the energy balance of the cycle. After one turnover of the cycle, 1 mol ATP is formed from ADP and P_i under standard conditions. The corresponding free enthalpy change is ΔG^{α} (ATP) = 32.8 kJ mol^{-1} (at pH 8, 1 mM $MgCl_2$, ionic strength 0.1 M) [33].

It follows then for the step 8 → 1

$$\Delta G^{\alpha}_{81} = \Delta G^{\alpha}(ATP) - \sum_i \Delta G^{\alpha}_i$$

where ΔG^{α} is the free enthalpy change of step i. From ΔG^{α}_{81} the equilibrium constant is calculated $K'_{81} = 7.3 \cdot 10^{-3}$. This means that under standard conditions a fraction of about 1% of the enzymes is in the state E_1. All these data are collected in Table 1 and the resulting free enthalpy diagram is shown in Fig. 4.

The proposed scheme describes a proton induced binding change mechanism: protonation from the inside favours the enzyme conformation E_1 and only this conformation binds ADP and P_i but not ATP. When the enzyme is in the conformation E_2 it binds ATP but not ADP and P_i. The substrate specifity changes drastically from E_1 to E_2 and this change is connected with the protonation/deprotonation reaction. It is required that the protonation state of the groups in the F_0 part is transmitted to the F_1 part where the nucleotide binding sites are located so that both events are coupled by far-reaching conformational interactions.

Of course, there might be also ATP binding to the state E_1, as well as ADP and P_i binding to E_2. However, the corresponding affinities are much lower. These reactions are side-reactions and they are not considered to be essential for the mechanism of coupling between proton transport and ATP synthesis.

Acknowledgment

I thank Susanne Fischer for the graphs. Our work was supported by the Deutsche Forschungsgemeinschaft and the European Union (BIO 2-CT93–0078).

References

[1] Pedersen, P.L. and Carafoli, E. (1987) Trends. Biochem. Sci. 12, 146–150.
[2] Pietrobon, D. and Caplan, S.R. (1985) Biochemistry 24, 5764–5776.
[3] Junge, W. (1970) Eur. J. Biochem. 14, 582–592.
[4] Rumberg, B. und Heinze, T. (1980) Ber. Bunsenges. Phys. Chem. 84, 1055–1059.
[5] Gräber, P. und Schlodder, E. (1981) in Photosynthesis (Akoyunoglou, G., ed.), Vol. II, pp. 867–879, Balaban, Philadelphia.
[6] Dewey, T.G. and Hammes, G.G. (1981) J. Biol. Chem. 256, 8941–8946.
[7] Strelow, F., Tran-Anh, T. and Rumberg, B. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. III. 9, pp. 157–160, Kluwer, Dordrecht.
[8] Jencks, W.P. (1989) Methods Enzymol. 171, 1896–1904.
[9] Stein, W.D. and Läuger, P. (1990) Biophys. J. 57, 255–267.
[10] Possmayer, F.E. and Gräber, P. (1994) J. Biol. Chem. 269, 1896–1904.
[11] Junesch, U. and Gräber, P. (1987) Biochim. Biophys. Acta 893, 275–288.
[12] Nalin, C.M. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7275–7280.
[13] Bakker-Grunwald, T. and Van Dam, K. (1976) Biochim. Biophys. Acta 347, 290–298.

[14] Mills, J.D., Mitchell, P. and Schürmann, P. (1980) FEBS Lett. 112, 173–177.
[15] Strelow, F. and Rumberg, B. (1993) FEBS Lett. 323, 19–22.
[16] Kramer, D.M. and Crofts, A.R. (1989) Biochim. Biophys. Acta 967, 28–41.
[17] Giersch, C. Heber, U., Kobayashi, Y., Inoue, Y., Shibata, K. and Heldt, H.W. (1980) Biochim. Biophys. Acta 590, 59–73.
[18] Harris, D.A. and Slater, E.C. (1975) Biochim. Biophys. Acta 387, 335–348.
[19] Strotmann, H., Bickel, S. and Huchzermeyer, B. (1976) FEBS Lett. 61, 194–198.
[20] Gräber, P., Schlodder, E. and Witt, H.T. (1977) Biochim. Biophys. Acta 461, 426–440.
[21] Strotmann, H. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. II.6. 477–484, Martinus Nijhoff/Dr. W. Junk, The Hague.
[22] Mills, J.D. and Mitchell, P. (1984) Biochim. Biophys. Acta 764, 93–104.
[23] Labahn, A. and Gräber, P. (1993) Biochim. Biophys. Acta 1141, 288–296.
[24] Mitchell, P. (1961) Nature 191, 144–148.
[25] Gräber, P., Junesch, U. and Thulke, G. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. III. 2, pp. 177–184, M. Nijhoff, Dordrecht.
[26] Rumberg, B., Schubert, K., Strelow, F. and Tran-Anh, T. (1990) in Current Research Photosynthesis (Baltscheffsky, M. ed.), Vol. III. 9. pp. 125–130, Kluwer, Dordrecht.
[27] Van Walraven, H.S., Scholts, M.J.C., Koppenaal, F., Bakels, R.H. and Krab, K. (1990) Biochim. Biophys. Acta 1015, 425–434.
[28] Lill, H., Althoff, G. and Junge, W. (1987) J. Membr. Biol. 98, 69–78.
[29] Kothen, G., Schwarz, O. and Strotmann, H. (1992) in Research in Photosynthesis (Murata, N., ed.), II, 645–652, Kluwer, Dordrecht.
[30] Boyer, P.D. (1993) Biochim. Biophys. Acta 1140, 215–250.
[31] Labahn, A. and Gräber,P. (1992) FEBS Lett. 313, 177–180.
[32] Seybold, A. and Gräber,P. (1994) 8th EBEC Conference.
[33] Rosing, J. and Slater, E.C. (1972) Biochim. Biophys. Acta 267, 275–290.
[34] Labahn, A. and Gräber, P. (1993) Biochim. Biophys. Acta 1144, 170–176.