

# The Critical Electric Potential Difference for Photophosphorylation

## Its Relation to the Chemiosmotic Hypothesis and to the Triggering Requirements of the ATPase System

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The physical meaning of the observed critical electric potential difference necessary for photophosphorylation is discussed. An interpretation of this phenomenon on the basis of the equilibrium thermodynamic formulation of the chemiosmotic hypothesis requires a reversible ATPase system. Contrary to this, ATP hydrolysis experiments seem to confirm that the  $Mg^{++}$ -dependent ATPase system of class II-chloroplasts is irreversible in the absence of SH-compounds. This sheds doubt on the equilibrium thermodynamic interpretation of the critical electric potential difference for phosphorylation. However it can be shown, that the seeming irreversibility may be due to some trigger requirements of an intrinsically reversible ATPase system.

This leads to an identification of the critical electric potential difference with the triggering level of the enzyme system.

Two operational models for an ATPase system, whose activity is modulated by the electric potential difference, are derived. These account quantitatively for a set of experiments on the critical electric potential difference.

In the preceding paper it has been shown that a certain electric potential difference across the thylakoid membrane is a necessary condition for photophosphorylation in short flash groups. Moreover it has been demonstrated that three protons have to be translocated across their electrochemical potential difference for any molecule of ATP which is synthesized [1]. Both results favour strongly the chemiosmotic hypothesis of Mitchell [2], who has postulated that an electric potential difference provides part of the free energy for the formation of  $ADP \sim P$  bonds.

However, the mechanism which gives rise to a critical level of the electric potential difference has still to be interpreted. An attempt has been made to see whether this phenomenon can be understood from the same role of the electric potential difference, as an energy source for phosphorylation.

It will be demonstrated that, at first sight, the critical electric potential difference can be interpreted from the equilibrium thermodynamic formulation of the chemiosmotic hypothesis. However, an inspection of the basic assumption for this interpretation, the reversibility of the ATPase system, reveals inconsistency with the apparent irreversibility of the enzyme system. The latter has been concluded from hydrolysis experiments on class II-chloroplasts.

This apparent irreversibility deserves a microscopic interpretation. It will be shown that it might reflect the triggering requirements of the ATPase system. This leads to a reinterpretation of the critical electric potential difference involving another role of the electric potential in photophosphorylation: the triggering quality for the enzyme system.

Since the discussion will be based on certain presumptions on the trigger requirements of the ATPase system, it seems necessary to discuss the molecular basis for a triggerable enzyme system in some detail. In the appendix two operational models for a triggerable ATPase system will be derived, which account quantitatively for the experimental results reported in the preceding paper.

### *The Critical Electric Potential Difference and the Chemiosmotic Hypothesis*

Before entering into the discussion, the observed effects and the hypothesis will be summarized briefly.

The time course of the electric potential difference across the thylakoid membrane which is induced by short flash groups under phosphorylating conditions is depicted in the lower part of Fig. 1. (Experimental details and the method for the measurement of the electric potential difference have been specified in

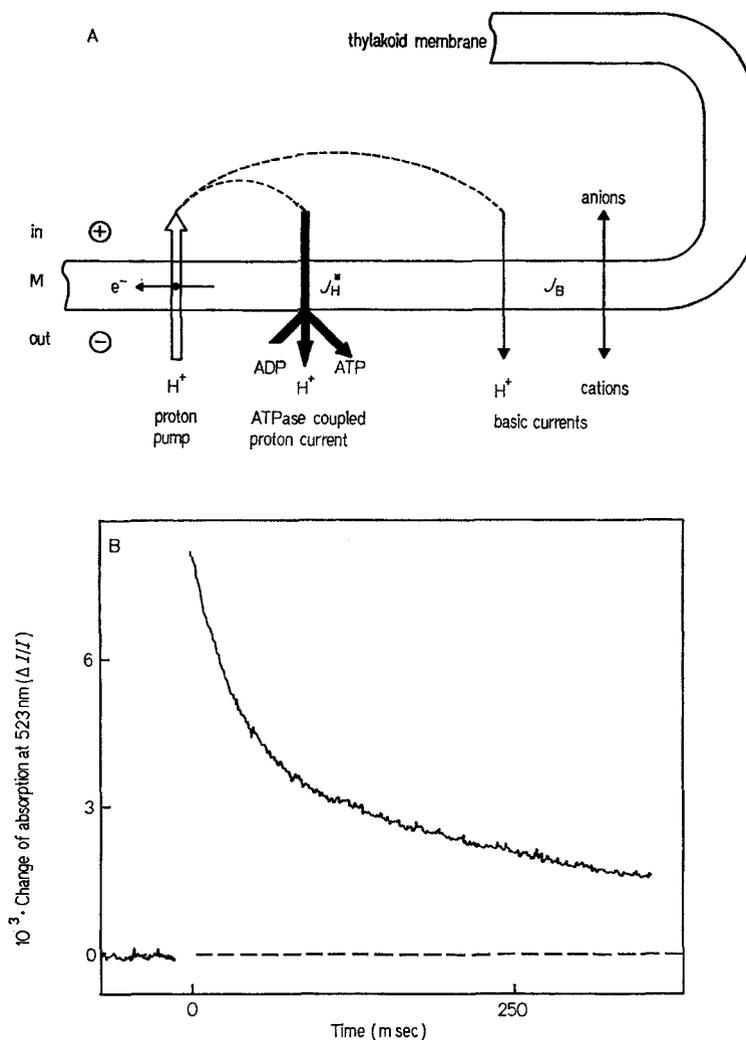


Fig. 1. A. Simplified model of the electric phenomena at the thylakoid membrane. B. Time course of the electric potential difference across the thylakoid membrane under phosphorylating conditions, indicated by the electrochromic absorption change at 523 nm

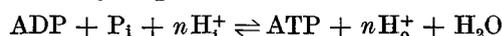
the preceding paper.) A model for the understanding of this time course is depicted in the upper part of Fig. 1. This model is based on a series of previous papers which have been cited in the preceding one (see [7, 15–17, 20, 21] in the preceding paper [1]).

The electric potential difference is indicated by the absorption change at 523 nm. The rise of absorption indicates the onset of the electric potential difference induced by a short flash group. The decay of the electric potential difference is biphasic. The rapid decay is observed under phosphorylating conditions only. It is due to an additional proton conductivity. A stoichiometric coupling between the additional proton current and the ATP-synthesis has been demonstrated in the preceding paper. The slow decay has a first order rate constant which is

practically equal to the rate constant observed under non-phosphorylating conditions. This slow decay is ascribed to the rather low basic conductivity of the membrane. As demonstrated in the preceding paper, the ATP-synthesis and the rapid proton current coupled to it, both stop at a critical potential difference.

How can the observed critical potential difference be understood from the chemiosmotic hypothesis?

It has been postulated by Mitchell that the free energy which is necessary for the synthesis of ATP is derived from an electrochemical potential difference across a membrane by a reversible proton translocating ATPase [2]. The concept can be summarized in the following simplified reaction scheme:



where  $n$  stands for the  $H^+$ /ATP-stoichiometric ratio ( $n = 3$  as shown in the preceding paper) and where  $H_i^+$  and  $H_o^+$  stand for the inner and the outer protons, respectively. In the thermodynamic equilibrium the following relation between the electrochemical potential difference of the proton and the ATP/ADP-ratio has been postulated:

$$n(\mathcal{F}\Delta\psi + RT\Delta pH) = \Delta F^\circ + RT \ln \frac{[ATP]}{[ADP]} \quad (1)$$

Following a proposal of Mitchell the expression  $(\mathcal{F}\Delta\psi + RT\Delta pH)$  will be named proton motive force and abbreviated pmf. Most recent determinations of the standard free energy  $\Delta F^\circ$  have yielded a value of about 13.8 kcal/mole ( $[P_i] = 5$  mM, pH 8) [4].

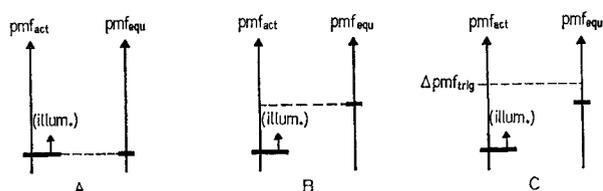


Fig. 2. Illustration of the energetics of a chemiosmotic phosphorylation mechanism.  $pmf_{act}$  = actual proton motive force across the membrane;  $pmf_{equ}$  = estimated proton motive force corresponding to the actual ATP/ADP-ratio in the suspension;  $pmf_{trig}$  = proton motive force necessary to trigger the ATPase system

A basic assumption of the chemiosmotic hypothesis is the reversibility of the ATPase. Although the reversibility of the ATPase on isolated chloroplasts requires further discussion (see below), the critical electric potential difference shall be interpreted, first, under the assumption of a perfectly reversible ATPase. The assumptions used and the complications occurring will be discussed later.

The arguments can be illustrated by the following graphs (Fig. 2). In each of the three graphs two scales are depicted: the left one indicates the actual proton motive force across the thylakoid membrane, and the right one indicates an estimated proton motive force, which would correspond to the actual ATP/ADP-ratio, if there was thermodynamic equilibrium. The value of the latter is defined by Equation (1). A high (low) ATP/ADP-ratio corresponds to a high (low) value of the equilibrium proton motive force.

Two different experimental situations have to be distinguished: either the time span between the insertion of chloroplasts into the reaction cuvette and the firing of the first flash group was sufficient to establish the dark equilibrium as defined by Eqn. (1) or it was not. The first case is depicted in Fig. 2A. In equilibrium, the actual proton motive force equals the equilibrium value. Clearly, any

increase of the actual proton motive force induced by illumination should shift the ATP/ADP-couple towards ATP, no matter how small this increase was. Therefore, a critical proton motive force or a critical electrical potential difference, which forms part of it, cannot be understood in these terms.

However, the situation is different in the second case, where the actual ATP/ADP-ratio, for some reason, had not equilibrated with the actual proton-motive force across the membrane. This is depicted in Fig. 2B. Then an ATP synthesis can be expected only if the light induced proton-motive force had exceeded the value which would correspond to the ATP/ADP-ratio in the reaction cuvette, if the equilibrium had been established. Thus from the chemiosmotic hypothesis the existence of a critical electrochemical potential difference can be predicted, if the ADP/ATP couple had not equilibrated with the electrochemical potential difference, when illumination is started.

Energetic questions arising from this interpretation will be discussed elsewhere.

The basic assumption for this equilibrium thermodynamic interpretation was the reversibility of the ATPase system. The validity of this assumption deserves detailed discussion.

#### *Open Questions as the Reversibility of the ATPase System*

The term "reversibility of the ATPase system" shall indicate simply, that there is an enzyme system, located at the thylakoid membrane, which is able to synthesize as well as to hydrolyse ATP, depending on the actual ATP/ADP-ratio and the actual proton motive force. Mechanistic questions of the enzyme system will not be regarded.

While there is no question as to the reversibility of the  $Mg^{++}$ -dependent ATPase system on class I-chloroplasts [5], hydrolysis experiments on class II-chloroplasts have revealed a partial inability of the  $Mg^{++}$ -dependent ATPase to hydrolyse ATP. The most important results are as follows.

On class II-chloroplasts there is a  $Mg^{++}$ -dependent ATPase which can hydrolyse ATP under appropriate conditions (see below) [6]. That this ATPase is sensitive to the same poisons as the phosphorylating ATPase [7, 8] makes it probable that both are identical, except their working in reverse. However, ATP hydrolysis has been observed under two conditions only: if the ATPase is triggered, either by light [6] or by an artificially induced pH-difference [9] and if SH-agents are present [6, 9].

Class II-chloroplasts without addition of SH-compounds have been used in the experiments which are discussed in this paper. Thus these chloroplasts have not been able to hydrolyse ATP. An operational mechanism giving rise to this inability

will be searched for. The argument will be based on three assumptions:

- a) The ATPase system is fully reversible.
- b) The activity of the ATPase system is high, if the light induced potential difference exceeds a certain triggering level, and it is low, if it does not.
- c) The triggering level is higher in the absence of SH-compounds than in the presence of them.

This is best illustrated by discussing an extreme example. It will be assumed that the level of the proton motive force, which is required for triggering, exceeds the value which in equilibrium would correspond to the actual ATP/ADP-ratio in the hydrolysis experiments (see Fig. 2C): Obviously, under this condition no ATP hydrolysis should be observed, since the ATPase system starts its activity at a proton motive force which will lead to the synthesis of ATP.

If a synthesis experiment is carried out, which starts from a rather low ATP/ADP-ratio, a rapid ATP synthesis should be expected, if the light induced proton motive force does exceed the triggering level. (The situation in the presence of SH-compounds can be understood from the third assumption in an analogous way.) This interpretation of the observed irreversibility of the ATPase system can easily be extended to more general examples. In the context of this paper it is sufficient to state that the apparent inability of the ATPase to hydrolyse ATP may reflect the triggering requirements of an intrinsically reversible enzyme system. This has consequences for the interpretation of the observed critical electric potential difference.

#### *The Critical Electric Potential Difference and the Triggering Requirements of the ATPase System*

One may argue that the critical electric potential difference reflects the triggering level of the ATPase system. While quantitative aspects of this suggestion will be evaluated in the appendices, the qualitative aspects will be discussed here. The main question is, in which way the activity of the enzyme system may be modulated by the electric potential difference.

The influence of SH-compounds on the activity of the ATPase system suggests that the activity may be modulated *via* the conformation of the enzyme. And so, one may argue, the electric field strength across the membrane acts on the enzyme conformation. To give an operational example for this: if the membrane bound enzyme could exist in two conformations A (inactive) and A\* (active) with different static dipole moments ( $\vec{\mu} \neq \vec{\mu}^*$ ), then the equilibrium between A and A\* would depend on the electric field strength across the membrane. And so would the average ATPase activity. A quantitative treatment of this model is given in Appendix II.

This model can be classified as an allosteric effect of the electric field on an enzyme system. The model is able to account, in principle, for the hydrolysis experiments as well as for the observed critical electric potential difference necessary for the synthesis of ATP.

In contrast to the first model, which describes a real triggering process, another operational model can be derived, where the modulation of the phosphorylating activity is interpreted as an intrinsic kinetic property of the ATPase coupled proton conductivity. This model is based on the assumption that the translocation of three protons per ATP is the rate determining step in phosphorylation. This model is treated quantitatively in the Appendix I. An absolute rate theory approach based on very simple assumptions as to the mechanism of the ATPase coupled proton conductivity yields an approximately exponential dependence of the phosphorylation rate on the electric potential difference. This model requires the fit of only two free parameters to yield a quantitative description of the experiments [1] on the critical electric potential difference. However, this model does not account by itself for the seeming irreversibility in the hydrolysis experiments. To do so, further assumptions are required. These will be specified elsewhere.

#### CONCLUSIONS

In the preceding paper it has been demonstrated, that the electric potential difference across the thylakoid membrane has to exceed a certain "critical value" before ATP can be synthesized [1].

This phenomenon is related to lag phenomena which have been reported in the literature, previously. A time lag [10] and an intensity lag [11–14] as well as a critical pH-difference [15] have been observed for photophosphorylation. While Sakurai *et al.* have interpreted lag phenomena in terms of a detailed reaction mechanism of a chemical high energy intermediate [14], Schwartz has inclined to an interpretation of the pH-lag on the basis of the equilibrium thermodynamic formulation of the chemiosmotic hypothesis [15].

In contrast to the first, the latter interpretation has not been formulated thoroughly. Since the experimental results presented in the preceding paper favour the chemiosmotic hypothesis, this hypothesis has been chosen as a starting point for the present discussion, too.

As shown above, the interpretation of the critical electric potential difference for phosphorylation from the equilibrium thermodynamic formulation of the chemiosmotic hypothesis has led to grave difficulties. These difficulties have arisen from the seeming irreversibility of the ATPase system on class II-chloroplasts.

The seeming irreversibility does not exclude the chemiosmotic postulate that the electrochemical potential difference of the proton is the energy source for the ADP ~ P bond. But, it excludes the identification of a critical potential difference with that value of the proton motive force, where, under the actual ADP/ATP ratio in the solution, the rates of the ATP synthesis and the ATP hydrolysis balance each other. In looking for a mechanism which may give rise to the apparent inability of the ATPase to hydrolyse ATP, an interpretation involving the triggering requirements has been presented.

This has finally led to the identification of the critical electric potential difference with the triggering level of the ATPase system. This level represents the transition point from a low to a high activity of the enzyme system.

Although the concept of an ATPase system whose activity is modulated by the electric potential difference across the thylakoid membrane is, in principle, compatible with a chemiosmotic mechanism, it is not necessarily linked to it. Thus, in this paper no conclusion can be derived as to the question, if the chemiosmotic hypothesis is valid or not.

It is hoped that the emphasis which has been given to the triggering role of the electric potential difference in phosphorylation may stimulate further studies on this subject. Possibly these may reveal new aspects of a long range interaction of enzyme molecules in biological membranes.

#### APPENDIX I

##### *The Single Potential-Barrier Model for an Activity Modulation of the ATPase System*

The biphasic decay of the electric potential difference in the flash group experiments (see Fig.1) has been interpreted in the preceding paper as due to

two different types of electric currents across the thylakoid membrane: an ATPase coupled proton current ( $J_{H^+}^*$ ) which is only present if the electric potential difference exceeds the "critical value", and a basic current ( $J_B$ ). Denoting by  $J$  the current density and by  $C$  the capacity per unit area of the membrane, the following differential equation describes the decay of the electric potential difference:

$$\frac{d}{dt} \Delta\psi = + \frac{1}{C} (J_{H^+}^* + J_B). \quad (2)$$

The experiments under non-phosphorylating conditions have revealed that the decay of the electric potential difference which is due to the basic current is of first order, approximately. Consequently the basic current fulfils Ohm's law:

$$J_B = -G_B \times \Delta\psi \quad (3)$$

where  $G_B$  is the basic conductivity of a unit area of the membrane. Thus, the rate constant of the observed first order decay equals  $G_B/C$ . The validity of Ohm's law is not at all trivial on very thin membranes. The rather restrictive properties which make a membrane behave ohmic have been specified by Ciani [17] from an absolute rate theory approach. Grossly speaking, Ohm's law is valid, only if the membranes can be represented by many potential barriers for the penetrating ion. However it fails if there are only a few barriers. (This is due to the fact that the exponential dependence of a transition state flux on the electric potential difference of an ion is small as compared with  $kT$ .)

If, as depicted in Fig.3, there are restricted "thin" areas in the membrane, where a permeating ion "sees" only one potential barrier the current density

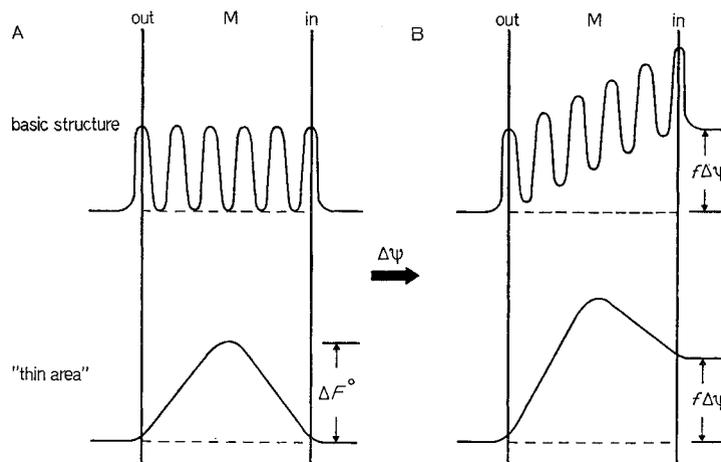


Fig.3. Schematic representation of the potential energy of the proton across the thylakoid membrane, (A) without and (B) with a superimposed electric field

has to be described by an expression different from Eqn. (3):

$$J_{\text{H}} = [\text{H}_0^+] k^+ - [\text{H}_1^+] k^- \quad (4)$$

Here,  $J_{\text{H}}$  denotes the current density of an arbitrary ionic species, say  $\text{H}^+$ ,  $[\text{H}_1^+]$  is the particle density of  $\text{H}^+$  in the inner —  $[\text{H}_0^+]$  in the outer phase.  $k^+$  and  $k^-$  denote the inward and outward rate constants, respectively. According to Eyring's theory the rate constants  $k^+$ ,  $k^-$  are given by:

$$k^\pm = \alpha \frac{kT}{h} \exp(-\Delta^\pm F^*/RT) \quad (5)$$

where  $\alpha$  is a factor which takes the density of "thin" areas into account and  $\Delta^\pm F^*$  is the free energy difference between the top of the potential barrier and the outer and the inner aqueous phase, respectively. The free energy difference can be split up into a constant term and a variable term due to a superimposed electric field:

$$\Delta^\pm F^* = \Delta^\pm F_0^* + \mathfrak{F} \Delta^\pm \psi \quad (6)$$

where  $\mathfrak{F}$  denotes the Faraday (a monovalent ion assumed). From Equations (4), (5), (6) it follows that the current density depends exponentially on the electric potential difference across the membrane. This may be tentatively used to describe the ATPase coupled proton current across the thylakoid membrane, which depends with higher than first order on the electric potential difference. Making use of the very probable assumption that the Donnan potential is zero under the 10 mM of potassium chloride which have been used in the experiments, the proton concentrations on both sides of the membrane are equal in the dark equilibrium. Thus it follows:

$$\Delta^+ F_0^* = \Delta^- F_0^* = \Delta F_0^*$$

Under this assumption and setting:

$$T_{\text{H}} = \alpha \frac{kT}{h} \exp(-\Delta F_0^*/RT)$$

the density of the proton current becomes:

$$J_{\text{H}^+}^* = -T_{\text{H}}([\text{H}_1^+] \exp(-\mathfrak{F} \Delta^- \psi) - [\text{H}_0^+] \exp(-\mathfrak{F} \Delta^+ \psi)) \quad (7)$$

$T_{\text{H}}$  is the transmission coefficient for protons. The star on  $J_{\text{H}^+}^*$  denotes, that  $J_{\text{H}^+}^*$  is identified with the current density of those protons which flow across the special ATPase coupled pathway.

The expression for the ATPase coupled proton current can be somewhat simplified. A first simplification arises from the difference in the buffer capacities of the outer and the inner phase of the thylakoids. Under the experimental conditions specified in the preceding paper the proton concentration in the outer phase is practically constant ( $[\text{H}_0^+] = \text{const.}$ ). A second simplification results

from the relative ignorance as to the detailed topology of the membrane. Since there is no separate structural information as to the location of an activation barrier of the ATPase with respect to the outer and the inner phase respectively, one may assume, that the activation barrier lies close to the outer phase. Then the superimposed electric potential between the barrier and the outer phase comes close to zero:

$$\Delta^+ \psi = 0$$

while the full superimposed potential difference can be measured between the barrier and the inner phase:

$$\Delta^- \psi = -\Delta \psi.$$

Thus, the current density mediated by the ATPase becomes:

$$J_{\text{H}^+}^* \cong -T_{\text{H}}([\text{H}_1^+] \exp(\mathfrak{F} \Delta \psi/RT) - [\text{H}_0^+]) \quad (8)$$

Both the ATPase coupled proton current [Eqn. (8)] and the basic current [Eqn. (1)] contribute to the decay of the light induced electric potential, which can be described by the following differential equation:

$$\frac{d}{dt} \Delta \psi = -\frac{1}{C} (G_{\text{B}} \Delta \psi + T_{\text{H}}([\text{H}_1^+] \exp(\mathfrak{F} \Delta \psi/RT) - [\text{H}_0^+])) \quad (9)$$

This non-linear, first order differential equation contains two time dependent variables:  $\Delta \psi$  and the inner proton concentration  $[\text{H}_1^+]$ . For a complete solution, therefore, a second differential equation for  $[\text{H}_1^+]$  is required. The decrease of the inner proton concentration is due to the ATPase coupled proton current and to the proton component of the basic current:

$$\frac{d[\text{H}_1^+]}{dt} = \beta (J_{\text{H}^+}^* + J_{\text{H}^+}^{\text{B}})$$

( $\beta$  is related to the buffer capacity of the inner phase of the thylakoid. Due to the small variation of  $[\text{H}_1^+]$  in the experiments,  $\beta$  is approximately constant.)

Studies on the basic proton current under non-phosphorylating conditions in flash illumination have revealed that it is much less than the basic currents of the other ions under short flash excitations [18]. The contribution of  $J_{\text{H}^+}^{\text{B}}$  therefore, may be neglected for a description of the events in the first 200 msec. The approximated differential equation for the inner proton concentration becomes:

$$\frac{d}{dt} [\text{H}_1^+] = -\beta \frac{T_{\text{H}}}{C} ([\text{H}_1^+] \exp(\mathfrak{F} \Delta \psi/RT) - [\text{H}_0^+]) \quad (10)$$

Equations (9) and (10) can be solved to describe the ion transport phenomena on the basis of the rather rough model given above. This highly simpli-

fyng model distinguishes between two types of electric currents only: the basic one, due to other ions than  $H^+$  and the ATPase coupled  $H^+$ -current. The model neglects basic ion fluxes driven by chemical forces.

#### The Time Course of the Field Decay

Firstly, the parameters in Equation (9) and (10) have been adjusted so as to yield the best fit with the measured time course of the electric potential difference under phosphorylating conditions.

The best fit analog computer solution for the time course of the electric potential is depicted in Fig. 4. As in the corresponding experiments from the preceding paper (Fig. 2 [1]) four solutions are depicted which belong to four different initial values of the

tration in the inner phase of the thylakoid after a single short flash of light; this has been estimated for the experimental conditions (pH 8,  $[P_i] = 5$  mM) by Dr. Rumberg (private communication) as 13 nM. The other initial values of the inner proton concentration after a group of  $n$  flashes have been assumed to be proportional to the initial values of the electric potential difference.

$$[H_1^{+(n)}] = [H_1^{+(1)}] \times \frac{\Delta\psi(n)}{\Delta\psi(1)}.$$

$G_B/C$ , the velocity constant of the basic decay of the electric potential, has been extrapolated from the slow decay phase. It is practically equal to the rate constant of the decay under non-phosphorylating conditions, i.e.  $3 \text{ sec}^{-1}$ . The parameter  $\beta$  is deter-

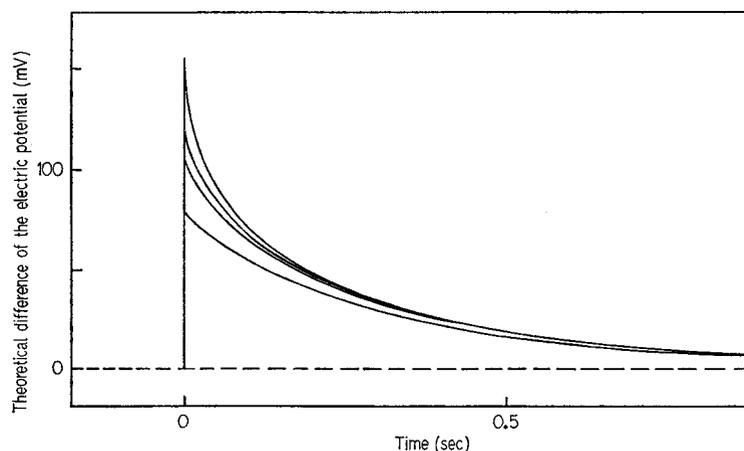


Fig. 4. Theoretical time course of the electric potential difference from the "single-potential-barrier-model". Four different initial values of the electric potential difference have been chosen corresponding to the experiment depicted in Fig. 2 from the preceding paper

electric potential difference. Although the fit with the corresponding experimental results is not perfect, the calculated time course of the electric potential represents the following features of the experiments:

a) The decay under phosphorylating conditions is biphasic.

b) The fast phase can be observed only above a certain "critical" potential difference.

c) The decay constant of the slow phase is independent of the initial value of the potential difference.

It is rather satisfying that this fit has been achieved by variation of only two free parameters.

The numerical values of all the other parameters have been determined in independent experiments. These are:  $[H_o^+]$ , the proton concentration in the outer phase; this was measured with a glass electrode and adjusted to 10 nM.  $[H_1^{+(1)}]$ , the proton concen-

tration in the inner phase after a single flash, was determined by the choices for  $[H_1^{+(1)}]$  and  $\Delta\psi^{(1)}$  and found to be  $0.89 \times 10^{-6} \text{ V}^{-1} \times \text{M}$ .

The two parameters which have been varied until the best fit was obtained are:  $T_H/C$ , the ATPase coupled proton permeability of the membrane which was chosen as  $9.3 \times 10^5 \text{ V}^{-1} \text{ M}^{-1}$ .

$\Delta\psi^{(1)}$  the electric potential difference in a single short flash of light, was chosen as 78 mV.

The choice of the latter parameter was most critical, since it contributes to the decay rate of the electric potential exponentially. A variation of  $\Delta\psi^{(1)}$  by more than  $\pm 30\%$  yielded a different time course which could not be corrected for by a larger variation of  $T_H/C$ . As the value  $\Delta\psi^{(1)} = 78 \text{ mV}$  is rather critical, the physical realm of the model can be tested by comparing this value to a previous estimation for the electric potential difference after a single short flash. It is rather satisfactory that it comes close to a value of 50 mV which has been

estimated on the basis of completely different arguments [3].

### *The Critical Potential Difference for Phosphorylation*

In the preceding paper it has been demonstrated that about 3 protons passing across the ATPase coupled pathway lead to the production of one molecule of ATP. This can be used to calculate an expectation for the ATP yield from the theoretical time course of the electric potential difference. It should be questioned whether this theoretical ATP yield does account for the fact, that below a certain critical potential difference practically no ATP is synthesized in flash group experiments.

that only about 1.7 protons can flow back across the ATPase coupled pathway. The remaining electric potential difference decays due to the basic current.

The unrealistic fate of the rest of 2.3 protons which stay in the thylakoid forever, is due to the fact, that the basic ion diffusion and the basic proton permeability have been neglected.

Analogous to Fig.5 the number of protons, which have flowed across the ATPase coupled pathway has been calculated for different initial values of the electric potential difference.

The result is represented as a fat curve in Fig.6. The scale for the number of protons is drawn on the right side of the diagram, the scale for the electric potential difference is marked above. Under the

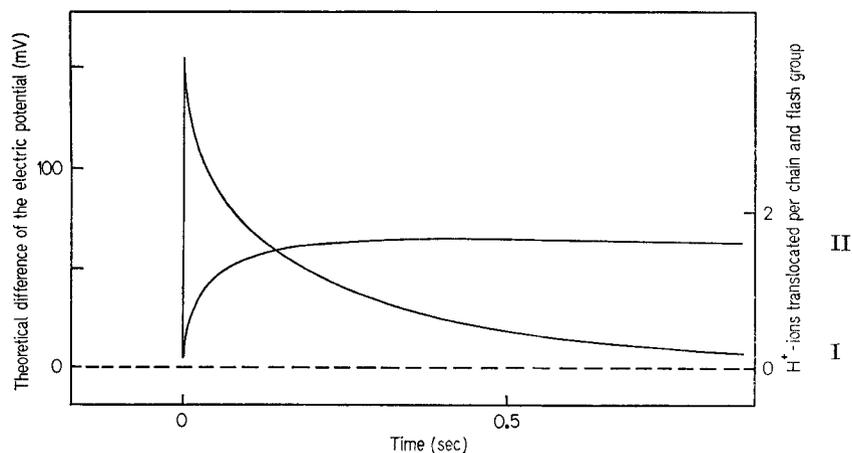


Fig.5. Theoretical time course of the electric potential difference (I) and of the number of protons translocated by the ATPase (II)

In Fig. 5 the already known theoretical time course of the electric potential difference is depicted. A second curve represents the number of protons which have flowed for time  $t$  across the ATPase coupled pathway.

The scale factors of the electric potential difference and of the number of protons translocated per electron transported have been normalized so that the electric potential difference induced in a short flash of light corresponds to two protons per electron. This correlation has been confirmed for the excitation process in a previous paper [3]. The two theoretical curves depicted in Fig.5 may be interpreted as follows. As an initial condition an electric potential difference of about 156 mV has been set on across the thylakoid membrane and correspondingly about four protons have been pumped into the inner phase per electron transported. The decay of the electric potential in the first 50 msec is dominated by the ATPase coupled proton flux. But with decreasing  $\Delta\psi$  this flux is slowed down practically to zero, so

assumption that about 3  $H^+$  produce one molecule of ATP this curve shall be compared with the measured dependence of the number of ATP molecules on the initial value of the absorption change at 523 nm (refer to Fig.3 [1]). This dependence is indicated by open circles in Fig.6. Again the scale for the absorption change, as indicated on the bottom of the diagram, has been normalized so that the amplitude on excitation with a single short flash of light ( $\Delta I/I = 3 \times 10^{-3}$ ) corresponds to a voltage of 78 mV. The excellent agreement between calculated and the measured ATP yields in Fig.6 demonstrates that the model of an "ATPase-coupled-single-potential-barrier-proton-conductivity" is able to explain that phosphorylation occurs only above a critical electric potential difference. The critical value of the electric potential difference is determined by the competition between the ATPase coupled and the basis conductivity. Below the critical potential difference the basis conductivity is dominating, and above it, the ATPase coupled one is.

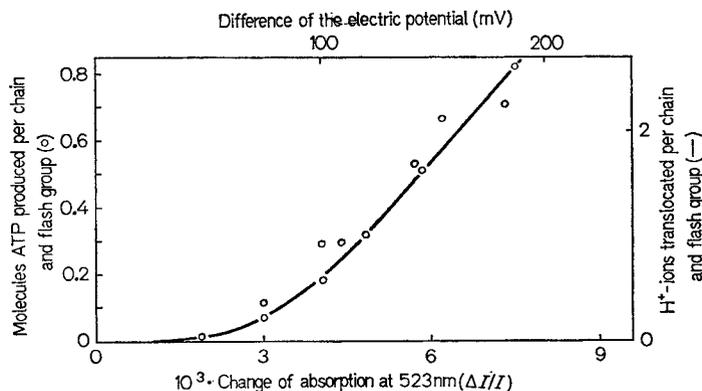


Fig. 6. Theoretical ATP yield in flash group experiments (curve) as compared with the experimental ATP yield (open circles) from the preceding paper [1]

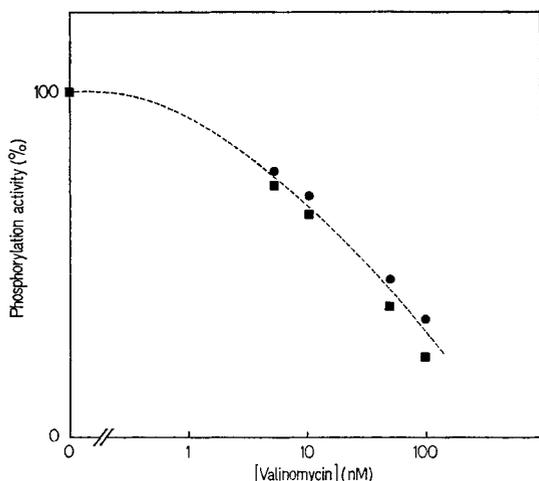


Fig. 7. Comparison between the relative ATP yield measured in flash group experiments [1] (■) and calculated from the "single-potential-barrier-model" (●), dependent on the valinomycin concentration

#### The Valinomycin-Sensitivity of Photophosphorylation in Short Flash Groups

Since the observed ATP yield can be explained from the competition between two conductivities for the electric potential difference, can the valinomycin sensitivity of phosphorylation observed in short flash groups be explained in these terms, too? From the measured time course of the electric potential difference, it can be concluded (see Fig. 4 from the preceding paper) that the basic decay rate is increased by valinomycin. The numerical value of  $G_B/C$  which corresponds to a given valinomycin concentration has been read out from measured decay curves (similar to Fig. 4 from the preceding paper). This value has been inserted into the two differential equations [Eqn. (9), (10)] to calculate the number of

protons, which cross the ATPase coupled pathway for the corresponding valinomycin concentration.

Taking into account the constant  $H^+/ATP$  stoichiometry, the dependence of the ATP production on the valinomycin concentration has been calculated and plotted by full circles into Fig. 7. A comparison with the measured values, which are indicated by full squares (as in Fig. 5 [1]), reveals a rather good agreement.

#### Discussion of the Single-Potential-Barrier-Model

Thus the very simple model for the ion transport phenomena under phosphorylating conditions describes the experiments quantitatively, which have been carried out under short flash group excitation. This model has been based on three types of assumptions:

a) The thylakoid membrane is basically permeable to ions. The basic conductivity is lower for protons than for other ions [18]. The basic conductivity is ohmic and diffusional ion fluxes can be neglected over a certain time range ( $\leq 250$  msec) after excitation.

b) Under phosphorylating conditions (on addition of  $Mg^{++}$ ,  $P_i$  and ADP) there exist an additional ATPase coupled proton current which behaves as if the protons had to jump across a single potential barrier. This gives rise to an approximately exponential dependence of the proton current on the electric potential difference.

So far, the model is a gross simplification of the electric phenomena at the thylakoid membrane. The third type of assumption links the ATP production to it:

c) If protons pass across the special ATPase coupled permeability, one molecule of ATP is formed for any three protons, which have passed.

Thus the ATP production is determined by the competition for the electric potential difference be-

tween the basic permeability and the ATPase coupled permeability.

The model takes no account of the molecular features of the ATPase coupled proton permeability. When it claims that this permeability behaves as if the protons had to cross only a single potential barrier, nothing can be concluded as to the kind of barrier. However, it is probable that the rate determining single potential barrier lies on the reaction coordinate of a protolytic reaction. It is an open question, whether the ATPase coupled proton current is merely a proton current. Mitchell has pointed out several mechanisms which are equivalent to a net proton flux across the membrane: for instance a flux of OH<sup>-</sup> in the inverse direction or a transport of H<sup>+</sup> on a carrier I<sup>-</sup>, coupled to the back flux of the carrier in the inverse direction [2].

The basic feature of the model is that it does not require any complicated mechanism acting on the enzyme, to describe the observed dependence of the ATPase activity on the electrochemical potential difference. Instead, this dependence is interpreted as an intrinsic property of the rate determining permeation of protons across a single potential barrier.

Clearly, one may think of alternative mechanisms for an interpretation of the approximately exponential dependence of the ATPase coupled proton flux on the electric potential difference. For instance, this flux may intrinsically obey the Nernst-Planck equation, which is linear in  $\Delta\psi$ , but, the number of ATPase channels (or translocators) may depend strongly on the electric potential difference.

This will be discussed briefly in the following.

## APPENDIX II

### *A Model for Electrically Triggered Conformation Changes of an ATPase System*

The model shall be based on the assumption that the number of ATPase molecules, which are in an active conformation, depends on the average electric field strength at the enzymes. Such a "field triggered ATPase" may be described by the following reaction scheme:



where A and A\* stand for the enzyme and  $\vec{\mu}$  and  $\vec{\mu}^*$  denote the static dipole moments of the ATPase in the inactive and the active state, respectively.

The static dipole moments are introduced to yield an operational scheme for the triggering mechanism.

If the components of the dipole moments in the direction of the field strength ( $\vec{E}$ ) were different:

$$\vec{\mu} \cdot \vec{E} \neq \vec{\mu}^* \cdot \vec{E} \quad (12)$$

then the equilibrium between A and A\* would be shifted, if the electric field strength changes. Since conformational changes occur rather rapidly, one may assume that they follow the field changes without a measurable delay and then the ratio between active and inactive ATPase molecules would be given by the Boltzmann expression:

$$\frac{[A^*]}{[A]} = \exp(-\Delta F^\circ/RT \cdot \Delta\vec{\mu} \cdot \vec{E}/kT). \quad (13)$$

In this expression  $\Delta F^\circ$  indicates the standard free energy of the ATPase activation reaction in the absence of a superimposed electric field and  $\Delta\vec{\mu} = \vec{\mu}^* - \vec{\mu}$ .

The overall surface density of ATPase molecules on the thylakoid membrane may be denoted by  $\sigma_0$ . Then from the Boltzmann expression the surface density of the active enzyme can be calculated as a function of the electric field strength:

$$\sigma^* = \sigma_0 / (1 + \exp(\Delta F^\circ/RT - \Delta\vec{\mu} \cdot \vec{E}/kT)). \quad (14)$$

Assuming that the product ( $\Delta\vec{\mu} \cdot \vec{E}$ ) is positive in sign, an increase of the electric field strength would shift the A\*/A ratio *versus* the active species A\*.

Now, it has to be demonstrated that the model above sketched is able to describe the observed bi-phasic decay of the electric potential difference under phosphorylating conditions. Again, it will be assumed that the rapid phase of the electric potential decay is due to a special ATPase coupled proton current. And it shall be furthermore assumed that the current mediated by an ensemble of active ATPase obeys a linear thermodynamic flux equation. Moreover, the observed current density depends on the surface density of active ATPase molecules. This gives rise to the following expression for the current density  $J_{H^+}^*$ :

$$J_{H^+}^* = \frac{\sigma^*}{\sigma_0} (L_1 \Delta\psi + L_2 \Delta[H^+]). \quad (15)$$

In this expression  $\Delta[H^+]$  is the concentration difference of the proton across the membrane. That a linear Nernst-Planck-type equation can be used for the description of ion fluxes across very thin membranes is not trivial. However it has been confirmed experimentally for several membrane systems by Eisenmann and coworkers [19].

Neglecting space charge effects in the membrane phase, the electric field strength at the ATPase is proportional to the electric potential difference  $|\vec{E}| \sim \Delta\psi$ . Introducing this proportionality into Equation (14) it follows a functional relationship  $\sigma^* = \sigma^*(\Delta\psi)$ .

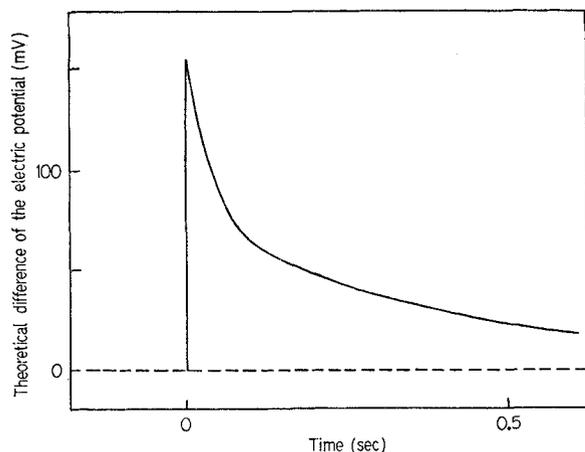


Fig. 8. Theoretical time course of the electric potential difference calculated from the model of a "field triggered ATPase"

This gives rise to a non-linear differential equation for the decay of the electric potential difference:

$$\frac{d}{dt} \Delta\psi = -\frac{1}{C} \left[ \frac{\sigma^* (\Delta\psi)}{\sigma_o} (L_1 \Delta\psi + L_2 \Delta[H^+]) + G_B \Delta\psi \right]. \quad (16)$$

This equation is analogous to Equation (9). Again  $G_B$  is the basic conductivity of the membrane, and  $C$  its capacity per unit area. Analogous to Eqn. (10) the corresponding equation for the decay of the pH difference across the membrane can be formulated. However, this will not be done explicitly. Instead, a solution of both equations will be presented, which is based on an appropriate set of parameters. This solution for the time course of the electric potential difference across the thylakoid membrane (Fig. 8) reveals a fairly good agreement with the experimentally observed time course (see Fig. 1). The set of parameters used in the calculation is not very important here, since there are rather a lot of parameters which can be chosen at will.

Thus instead of insisting on this special fit, it should be pointed out that this model of a field

triggered ATPase is able, in principle, to describe the observed critical potential difference right.

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