Energized State Responsible for Adenosine 5′-Triphosphate Synthesis in Preilluminated Chloroplast Lamellae†

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ABSTRACT: The evidence supporting the chemiosmotic mechanism of photophosphorylation in chloroplasts can be succinctly stated. Light-induced electron transport acidifies the inside of the lamellar vesicles while at the same time the vesicles acquire an ability to phosphorylate ADP. Preincubation of the vesicles with acid also confers on them the ability to phosphorylate ADP when the pH is raised. For these reasons, the effects of preillumination have been equated to the effects of acid preincubation, and both have been equated to an acidification of the inner aqueous phase of the vesicles. However, the energized state of the lamellae following illumination cannot be the same as the state obtained through acid–base transitions in the external medium. Apparently, acid–base transitions create a condition which is delocalized and thermodynamically limited whereas the phosphorylation phenomenon associated with preillumination is easier to explain in terms of local intramembrane effects, stoichiometrically but not thermodynamically limited. The reasons for these conclusions are the following: (1) The ability of preilluminated lamellae to phosphorylate ADP (X_E) decays exponentially in a subsequent dark period as does the light-induced ionic disequilibrium responsible for the rise in the pH of the external medium and for the fall in the pH of the internal aqueous phase of the vesicles. However, the rate constants of the two exponential processes are rarely identical. Moreover, the fact that there is a known requirement for a large transmembrane hydrogen ion activity difference necessary to sustain phosphorylation should preclude an exponential decay of X_E if X_E is simply a consequence of the exponentially decaying hydrogen ion activity difference. (2) Additional transmembrane protonmotive forces induced by postillumination K^+ increases do not add at all to the yield of X_E if X_E is measured promptly after the light is turned off. In contrast, K^+ diffusion potentials and pH increases are strictly additive in acid–base transition phosphorylation. However, additional protonmotive forces added after a period of partial decay do increase ATP synthesis and result in what appears to be a change in the rate constant of the exponential decay of X_E. Sixty millivolts of protonmotive force, applied in the form of either an increase of 1 pH unit or a 10-fold increase in the concentration of an alkali ion, doubles the X_E half-life. (3) These effects of K^+ increases on the amount of ATP made after partial decay of X_E do not require the presence of valinomycin, whereas the qualitatively different effects of K^+ increases on acid–base-induced phosphorylation do require valinomycin.

The chemiosmotic theory of photophosphorylation attributes the driving force for ATP synthesis to a transmembrane hydrogen ion activity difference (Mitchell, 1968). The evidence supporting this general concept has seemed overwhelming. Light-dependent electron transport acidifies the inside of the lamellar vesicles while at the same time the vesicles acquire an ability to make ATP (X_E)[1] in a subsequent dark period (Hind & Jagendorf, 1963; Jagendorf, 1977). Acidification of the vesicles by incubation in an acid medium also confers on them the ability to make ATP when the pH is raised (Jagendorf & Uribe, 1966). Finally, electron transport does not lead to ATP synthesis unless the electron donor produces H^+ on oxidation (Trebst & Pistorious, 1965; Izawa et al., 1966; Izawa & Ort, 1974). Thus, the effects of acid incubation and the effects of preillumination have been equated to each other, and both have been equated to the acidification of the inner aqueous phase of the vesicles. While this view has been valuable as a first approximation, it now seems somewhat oversimplified. The analogy between acid incubation and preillumination does not stand up to close inspection.

The involvement of ion gradients in ATP synthesis can be considered established, but other essential features of the mechanism remain obscure. There is little known for certain about the relationship between pH changes and the fluxes of ions other than H^+, even though such fluxes are obligatorily coupled to pH changes. Under many conditions, it seems to be Mg^{2+} which moves (Hind et al., 1974). Nothing is known of the reversible chemical changes which must accompany protonation of the inner regions of the membranes. There is even a good deal of uncertainty about the locale of the events involved, whether they are in the membrane, on the membrane surface, or across the entire membrane separating the inner and outer aqueous phases. For instance, there is evidence that events within the membrane or on the membrane surfaces can couple electron transport to ATP synthesis without direct involvement of the inner aqueous phase and without any transmembrane charge (Ort et al., 1976; Graan et al., 1981; Ventaroli & Melandri, 1982). Yet there are other lines of evidence, such as the involvement of hydrogen ions stored via accumulation of permeant amines in the lumen of the vesicle during postillumination phosphorylation, which certainly show that delocalized membrane phenomena can be used for phosphorylation (Nelson et al., 1971; Avron, 1972). These

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[1] Abbreviations: X_E, energized state of preilluminated chloroplast lamellae responsible for ATP synthesis in the subsequent dark period; ΔpH, pH difference across the lamellar membranes whether caused by externally imposed pH changes or by light-induced acidification of the inside of the vesicles; $\Delta\psi$, electric charge across the lamellar membrane attributable to a change in the concentration of alkali ions in the external medium, i.e., a transmembrane diffusion potential; pmf, protonmotive force, i.e., the total work capacity of the hydrogen ions traversing the lamellar membrane; Mes, 2-(*N*-morpholino)ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; Mops, 3-(*N*-morpholino)propanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine; Bistrispropane, 1,3-bis[[tris(hydroxymethyl)methyl]amino]propane.

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problems are unlikely to be resolved until the coupling of ion movements to ATP metabolism is better understood.

In the study reported here, we have analyzed the dark decay of X_E in the absence of permeant amines (which accumulate during the light period and greatly complicate the decay kinetics). We made the study because we felt that the energized state caused by preillumination, especially the energized state formed in the absence of permanent amines, would be more representative of normal steady-state phosphorylation than the energized state caused by acid preincubation of the lamellar vesicles if, indeed, there were any differences. The results of the kinetic experiments to be described are not easily interpreted and may pose more problems than they answer, albeit problems which now must be addressed. Our observations show that the energized state of the membranes caused by illumination is definitely not the same as the energized state developed when the membranes are incubated in acid and then exposed to more alkaline media. Nevertheless, it does seem that hydrogen ion activity changes are somehow involved in both cases.

Materials and Methods

Chloroplasts were isolated from market spinach (*Spinacia oleraceae* L.) or occasionally from peas (*Pisum sativum* L.) as described by Graan & Ort (1981) except that the resuspension medium was buffered at pH 6.7 with 5 mM Mes–Bistrispropane. Preillumination, dark decays, phosphorylation reactions, and measurements of all pHs were conducted at 3 °C. Phosphorylation reactions were carried out by employing the following two-stage protocol.

(*A*) *Stage I.* (a) For acid–base ATP synthesis, the chloroplast lamellar vesicles were incubated for 3 min in 2 mL of reaction medium at the indicated pH with the indicated concentrations of KCl and valinomycin. (b) For postillumination ATP synthesis (X_E), the lamellar suspensions were illuminated at saturating light intensities with pyocyanin or methylviologen at the indicated pH for 30 s and then incubated in the dark at the same pH for the indicated dark decay period.

(*B*) *Stage II.* Rapid addition of 1 mL of phosphorylation mixture to the stage I reaction medium brought the external pH to the stated levels (usually pH 8.5) and the KCl concentration to the stated levels. ADP and P_i were usually supplied in this medium although in some experiments ADP was present also in stage I. After 10 s, the phosphorylation reaction was terminated with the addition of 1 mL of 2 M perchloric acid containing 10 mM EDTA. Control experiments showed that ATP synthesis was completed within 1 or 2 s, regardless of whether the energy was supplied by preillumination or by an imposed ΔpH or an imposed $\Delta\psi$.

Figure 1 illustrates the sequence of reactions involved in the measurement of X_E dark decay. Note that in almost all X_E experiments, those described here and those reported elsewhere [cf. Hind & Jagendorf (1963) and Izawa (1970)], a part of the hydrogen ion activity difference is actually contributed by a pH increase in the medium and sometimes by an imposed diffusion potential, both applied after the illumination period and after any decay period. The magnitude of these additional energy sources must be kept in mind when we are analyzing X_E experiments. Note particularly that any externally imposed pH changes and diffusion potentials were applied *after* the period of decay being assessed and that these additional pH gradients and cation gradients were not in operation during the decay. Hence, the coonsequences of the additional energy contributions from these sources were consequences of their effects on the already decayed state and *not* consequences of their effects on the decay (which occurred in their absence).

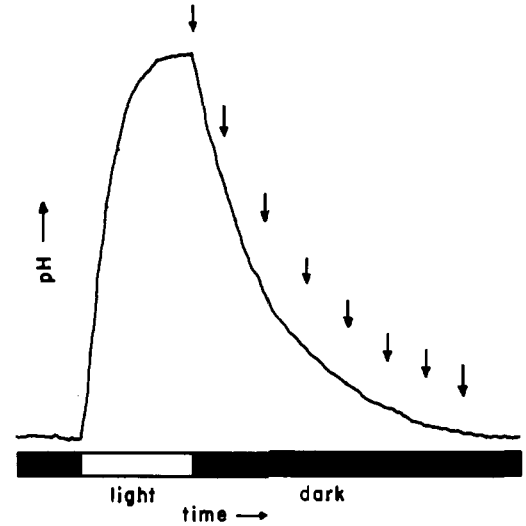

FIGURE 1: Protocol for studying the decay of X_E and the time course of the pH changes in the external medium. When the chloroplast suspensions were illuminated, the pH of the external medium increased, and the lamellae developed the capacity to make ATP (X_E). After the light was turned off, the chloroplast lamellae were incubated in the dark for the indicated time intervals while the small pH drop in the external medium, presumably representing ion reequilibration, was monitored with a glass electrode. Then a mixture of ADP, P_i, KCl, and buffer (to establish the final pH) was added at the times indicated by the arrows and the residual X_E then measured as the amount of ATP synthesized. It must be emphasized that in any one experiment the conditions of dark decay prior to phosphorylation were identical, regardless of the final pH of the medium or the magnitude of any imposed diffusion potential, these being established *after the period of decay indicated by the arrows*, when the residual X_E was measured. The figure shows the measured rise in the pH of the external medium and the subsequent fall of the pH in the following dark period.

Table I: Valinomycin Requirement for Establishing a $\Delta[K^+]$ Effect in Acid Incubation and Postillumination (X_E) Experiments[a]

	$\Delta[K^+]$ enhancement of ATP synthesis [nmol of ATP (mg of chlorophyll)$^{-1}$]	
valinomycin	acid incubation	residual X_E after 2.5 min in the dark
+	16.8	10.2
−	3.9	10.2

[a] Acid incubation experiments involved raising the pH from 6.5 to 8.5 while increasing $[K^+]$ from 1 to 100 mM ($\Delta\psi$ = 120 mV). ATP synthesis was less than 1 nmol (mg of chlorophyll)$^{-1}$ in the presence or absence of valinomycin when there was no $\Delta[K^+]$ imposed. X_E experiments involved illumination of lamellae at saturating light intensities in the presence of 10 μM pyocyanin and 10 mM $[K^+]$ for 30 s at pH 6.5. After a 2.5-min dark period, the pH was raised to 8.5 while increasing $[K^+]$ to 100 mM ($\Delta\psi$ = 60 mV). Preillumination-induced ATP synthesis was 14 nmol (mg of chlorophyll)$^{-1}$ in the presence of valinomycin and 21.4 nmol (mg of chlorophyll)$^{-1}$ in the absence of valinomycin when there was no $\Delta[K^+]$ imposed but 24.2 and 31.6 nmol (mg of chlorophyll)$^{-1}$, respectively, when $[K^+]$ was raised from 10 to 100 mM.

It should be noted that the differences between preillumination-dependent phosphorylation and acid preincubation dependent phosphorylation cannot be attributed in any way to the acid treatment. It is a characteristic of almost all X_E experiments that the initial pH is low and that the pH is raised when phosphorylation is initiated. For instance, in Table I the "acid preincubation" and the preillumination experiments involved exactly the same pH changes, the only difference being that in the former the requisite extra energy was contributed by a diffusion potential and in the latter the requisite extra energy was contributed by a period of electron transport.

Further details of reaction conditions are given in the legends of figures and tables.

ATP synthesis was determined by measuring the incorporation of [^{32}P]P_i into ATP, after adsorption of the nucleotides on charcoal, deadsorption, and separation of the ATP on a Dowex AG 1X4 amine-exchange column as described by Graan & Ort (1981). ATP hydrolysis was measured as the release of [^{32}P]P_i from [γ-^{32}P]ATP. The [^{32}P]P_i was extracted as the phosphomolybdate complex into isobutyl alcohol–xylene (1:1) essentially as described elsewhere (Saha & Good, 1970).

Results

Kinetics of X_E Decay. As several other studies have already shown (Jagendorf & Uribe, 1966; Hangarter & Good, 1982), no ATP is made by acid-incubated lamellae until the combined ΔpH and $\Delta\psi$ approach 180 mV. Figure 2 documents this fact yet again and shows the amount of ATP formed as a function of the increase of the pmf beyond the threshold energy requirement.[2] In the experiments illustrated by the figure, the various pmf's were achieved by widely different combinations of pH changes and potassium ion concentration changes. It should be noted that the amount of ATP formed always depends on the combined pmf's, in spite of the relative contributions of ΔpH and $\Delta\psi$ [see also Hangarter & Good (1982)]. Thus, it is clear that ΔpH and $\Delta\psi$ are strictly additive, as one would expect if the limitations were thermodynamic rather than stoichiometric.[3] It should also be noted that, up to levels of about 50 nmol of ATP per mg of chlorophyll, there is no evidence of a saturation effect.

The situation is strikingly different when we substitute a preillumination period for any part of the pH change. Thus, if we incubate the lamellae in the dark at pH 6.5 and then raise the pH to 8.5, while at the same time imposing a 10-fold increase in the K^+ concentration, we can assume that the threshold pmf has just been reached (ΔpH + $\Delta\psi$ = 180 mV). Then little ATP synthesis is to be expected, and little is found (see Figure 2 for a pmf of 180 mV). If we preilluminate in the presence of an electron acceptor before we add the ADP and P_i, raise the pH, and add more K^+, a good deal of ATP is made (Figures 3–5). Since the preillumination itself raises the pH of the external medium slightly (Hind & Jagendorf, 1963), it has seemed reasonable to assume that the pH inside the lamellar vesicles has been lowered considerably and that the resulting ATP synthesis is a consequence of the increased internal hydrogen ion concentration pushing the total pmf well above the thermodynamic threshold. Furthermore, since the supplementary pmf imposed after the decay period is by itself

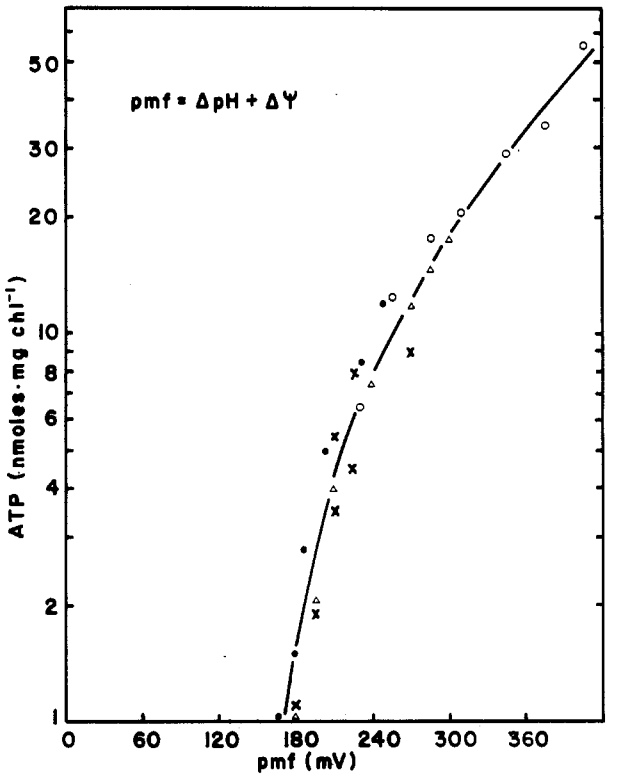

FIGURE 2: Dependence of ATP yield on the magnitude of the protonmotive force. The various protonmotive forces were generated by imposing different combinations of external pH changes and external K^+ concentration changes as described previously (Hangarter & Good, 1982). The data are taken from the results of an extensive study involving a wide range of conditions and many widely different values of ΔpH and $\Delta\psi$ [(○) $\Delta\psi$ = 210 mV; (△) $\Delta\psi$ = 180 mV; (●) $\Delta\psi$ = 150 mV; (×) $\Delta\psi$ = 120 mV; the remainder of the pmf was contributed by varying the imposed ΔpH]. The relationship between ATP yield and externally imposed protonmotive force is clearly neither exponential nor linear near the thermodynamic threshold, and therefore, an exponential decay of the protonmotive force should not lead to an exponential decay of the ability to make ATP.

overcoming the thermodynamic threshold, one might expect the decay of the ability of the preparation to phosphorylate ADP to parallel the decay of that part of the driving force contributed by preillumination, namely, that part associated with the light-induced pH change in the external medium. Thus, the expected effects of thresholds on the decay kinetics should be circumvented. Figure 3, upper curve, and Figure 4, upper curve, do indeed show a close parallel between the relaxation of the pH change in the medium and the decay of the phosphorylation capacity when no part of the illumination has to be used to overcome part of the thermodynamic threshold. This is also evidence that the pH change in the medium is quantitatively related to the phosphorylation driving force added by preillumination.

If we ask the preillumination to overcome a part of the thermodynamically dictated threshold pmf, that is to say, if we add less than the threshold pmf externally after the illumination period, the chemiosmotic model predicts two consequences: (1) less ATP should be made because the excess of the total pmf (light-induced and externally applied) above the threshold is smaller, and (2) the decay of the ability of the vesicles to make ATP after the light is turned off should deviate from the exponential if the driving force does decay exponentially, as the external pH changes indicate; in other words, the two processes should not both be exponential if one condition drives the other in a thoroughly nonlinear fashion as is the case when a threshold energy requirement is introduced. Neither of these predicted consequences occurs. The

[2] In equilibrium thermodynamics, the expression "threshold energy requirement" is without meaning. A threshold becomes meaningful when a disequilibrium is defined and a direction of reaction specified. Thus, when we speak of threshold energies, we do so in the context of *net* ATP synthesis with known or plausible concentrations of ADP, ATP, and P_i. Under these conditions, not only is the threshold meaningful but also its magnitude is well-known. Furthermore, since the catalysis by the coupling factor is effectively unidirectional for reasons not fully understood, the threshold concept has special force. The thermodynamic threshold for chloroplast phosphorylation may have the same meaning as the thermodynamic (voltage) threshold for diode conduction. Note that in our computations of energies we have used the more familiar values for 27 °C rather than the values for 3 °C. Since the energies available and the energies required change in the same way with temperature, our arguments are in no way affected.

[3] By thermodynamically limited, we mean that some substance such as a donor of hydrogen ions is abundant but that little can be used without lowering the activity of the substance in the pool below the energetic requirement for net phosphorylation of ADP. By stoichiometrically limited, we mean that the substance yielding H^+ (or Mg^{2+} etc.) to drive phosphorylation is limited by its abundance but that it can deliver almost all H^+ ions before energetic limitations intrude.

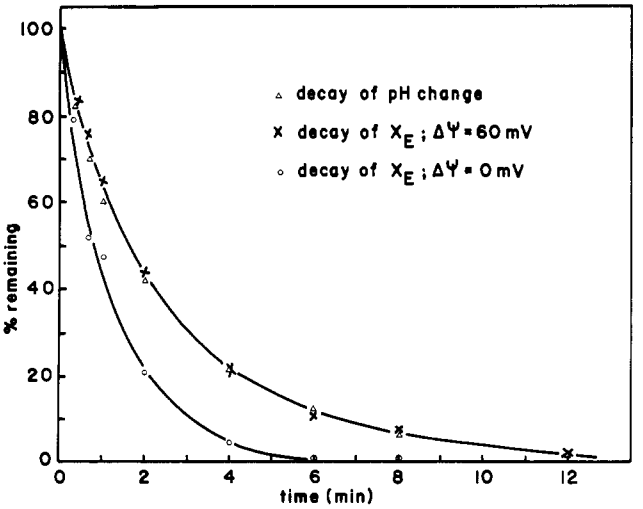

FIGURE 3: Dark disappearance of the light-induced pH rise in the external medium and the concurrent decay of the capacity of the lamellae to make ATP. Two milliliters of a chloroplast suspension containing 50 μg of chlorophyll in 4 mM Mes–Bistrispropane (pH 6.5) with 2 mM $MgCl_2$, 50 mM sorbitol, 1 μM valinomycin, 10 μM pyocyanin, and 10 mM KCl was illuminated for 30 s. At the indicated times after the light was turned off, 1 mL of 200 mM Tricine–Bistrispropane (pH 8.5), 2 mM $MgCl_2$, 3 mM $Na_2H^{32}PO_4$, 150 μM ADP, and 10 or 300 mM KCl was rapidly added ($\Delta\psi$ = 0 was obtained by having [KCl] at 10 mM before and after the addition of the base stage, whereas $\Delta\psi$ = 60 mV was obtained by raising [KCl] from 10 to 100 mM). The relaxation of the light-induced pH rise was measured in parallel experiments but with lower buffer concentration, 0.4 mM Mes–Bistrispropane. The 100% level of ATP (before decay) was the same, 65 mmol (mg of chlorophyll)$^{-1}$, regardless of the $\Delta\psi$ imposed. Note that with the two curves the conditions during the light and during the subsequent dark decay periods were identical, the different diffusion potentials ($\Delta\psi$) having been imposed after the partial decay, not during it. Note also that when the externally imposed part of the pmf was near the threshold required for ATP synthesis to begin, the relaxation of the pH rise and the decay of the phosphorylation capacity were parallel. When part of the threshold pmf had to be overcome by preillumination, the decay was much faster but still exponential.

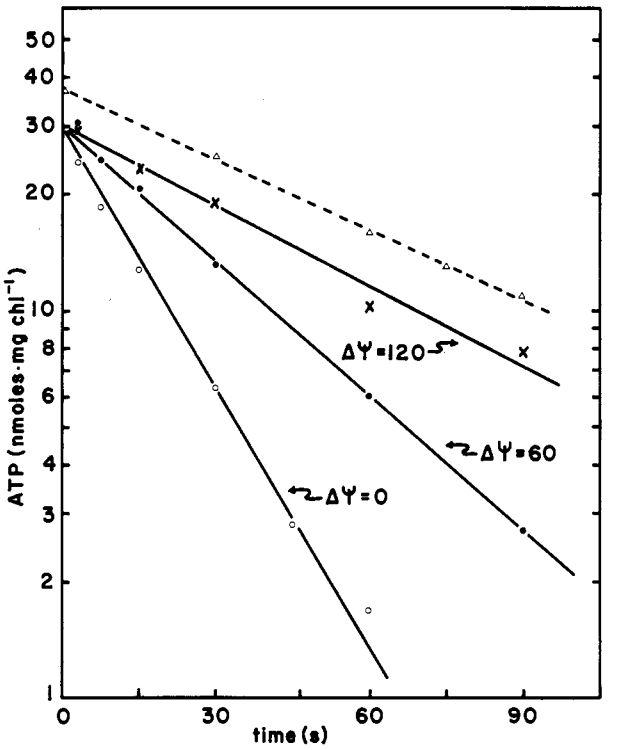

FIGURE 4: Influence of postdecay membrane potentials on the apparent decay of X_E at pH 7.5. Conditions were the same as in Figure 3 except that the buffer was 4 mM Mops–NaOH (pH 7.5) and the [KCl] was changed from 1 to 100 mM for $\Delta\psi$ = 120 mM and from 10 to 100 mM for $\Delta\psi$ = 60 mV or kept at 10 mM for $\Delta\psi$ = 0 mV. Again, the conditions during the light period and subsequent dark period were nearly identical, the different membrane potentials having been added after the decay had taken place. Immediately after the light was turned off, the membrane potentials had no effect on X_E, but addition of membrane potentials after the decay period resulted in a stimulation of ATP synthesis which gave the appearance of the decay being slowed. In all cases, the apparent decay remained exponential. The dashed line shows the dark decay of the light-induced pH change in the external medium in arbitrary units.

yield of ATP (when measured at the time the light is turned off) is unaffected by the magnitude of the dark-imposed part of the pmf and therefore by the magnitude of that part of the threshold pmf which must be overcome by illumination. Furthermore, the decay of the ability to make ATP remains inexplicably exponential, even though some of the driving force should be ineffectual being below threshold. However, "the apparent rate constant" of the decay is increased, and the half-life of the ability of the preparations to make ATP is correspondingly decreased (see Figures 3–5) whenever light is being used to bring the state of the membranes up to the energy threshold for ATP synthesis. The larger the proportion of the energy which must be contributed by illumination, the larger the apparent rate constant of the decay of X_E and the slower the half-life. We use the term apparent rate constant advisedly since this factor, which seems a property of the decay system, is in fact no such thing. It is determined by the dark-imposed ΔpH and $\Delta\psi$, conditions introduced after the decay period is over, and presumably reflects the extent of decay required before the capacity for phosphorylation disappears.

These observations span a wide range of ATP yields, a wide range of electron transport rates, a wide range of imposed pH differences, and a wide range of imposed K^+ "diffusion potentials". In no case did we find the decay of X_E other than exponential, and rarely did dark-imposed $\Delta\psi$ increase the yields when the yields were measured promptly after the light was turned off. This is illustrated most clearly in Figure 5. Electron transport rates were varied by varying the light intensity (Figure 5A) with the result that the alkalinization of the external medium and the yield of ATP also varied. ATP production was about 60 nmol/mg of chlorophyll at high light intensity and about 14 nmol/mg of chlorophyll at low light intensity. In neither case did the imposition of a $\Delta\psi$ of 60 mV have any effect on the promptly measured ATP yield. However, when the preparation exposed to high light intensity was held in the dark until the potential ATP yield had dropped from 60 to 14 nmol, the addition of 60 mV doubled the yield of ATP. Both preparations, the one exposed to low light intensity and the one exposed to high light intensity and then held in the dark for 2 min, had the same capacity to make ATP (14 nmol/mg of chlorophyll), but the two states of the membranes must have been fundamentally different.

Exactly the same phenomenon was observed in the experiments described in Figure 5B. In that case, partial inhibition of electron transport by DCMU replaced inhibition of electron transport by low light intensity. Again, the low level of ATP-generating capacity associated with low rates of electron transport represented a condition which was different from the condition achieved by high rates of electron transport followed by partial decay of the phosphorylation capacity.

It should be pointed out, however, that a supplementary postillumination pmf in the form of an increase in the concentration of K^+ does increase the yield of ATP somewhat, even when that yield is promptly measured, if the pH of the phosphorylation stage is unfavorably low (Figure 6).

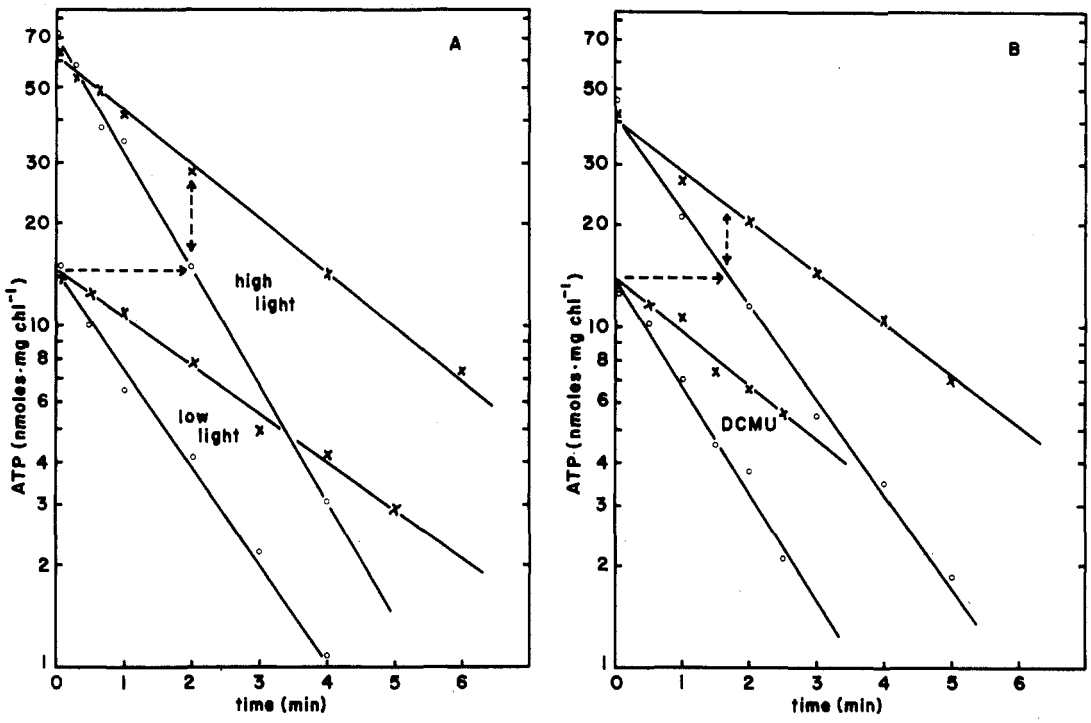

FIGURE 5: Influence of postdecay membrane potentials on the apparent decay of high and low levels of X_E. Conditions were the same as in Figure 3 except that in panel B the electron acceptor was methylviologen instead of pyocyanin. Limiting levels of X_E were obtained by using low light intensity in panel A and DCMU (5 μM) in panel B: (×) $\Delta\psi$ = 60 mV; (○) $\Delta\psi$ = 0 mV. Even when X_E was small, the addition of a membrane potential immediately after the light was turned off failed to increase the amount of ATP formed. However, when X_E decayed from a high level to the level obtained by low light intensity or DCMU treatment (see arrows), addition of a membrane potential did increase the yield of ATP. Thus, the lack of additivity at zero time cannot be due to saturation. Furthermore, the same level of X_E must represent different states of the membrane, depending on whether or not there has been a decay.

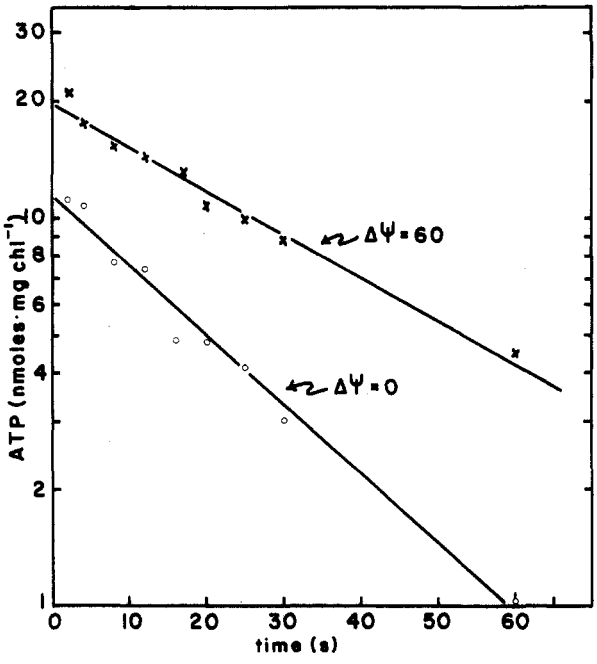

FIGURE 6: Influence of postdecay membrane potentials on the apparent decay of X_E in the absence of a postdecay pH change in the external media. Conditions were the same as those in Figure 4 except that the electron acceptor was 50 μM methylviologen and the pH of the base stage was only 7.5 (200 mM Mops–NaOH) instead of 8.5. Note that when the pH of the phosphorylation stage was unfavorably low, a postdecay K^+ concentration increase did increase the yield of X_E immediately after the light was turned off. Again, the apparent decay remained exponential, and the rate of the decay appeared to be slowed ($t_{1/2}$ = 17 s at $\Delta\psi$ = 0 mV, $t_{1/2}$ = 27 s at $\Delta\psi$ = 60 mV).

Yet another difference between X_E and acid incubation is to be found in the nature of the K^+ changes necessary to elicit the two different "membrane potential" effects. Those K^+

Table II: Cation Enhancement of ATP Synthesis in Preilluminated Chloroplasts after Partial Dark Decay of X_E[a]

	enhancement [nmol of ATP (mg of chlorophyll)$^{-1}$]			
	Li^+	Na^+	K^+	Rb^+
chloride salt kept at 10 mM	19.1	18.8	13.4	16.6
chloride salt raised from 10 to 100 mM	24.5	29.4	23.2	24.4
	5.4[b]	10.6[b]	9.8[b]	7.8[b]

[a] Chloroplasts were illuminated for 30 s at pH 6.5 in the presence of 10 μM pyocyanin and 10 mM each of the various chloride salts. After a 3-min dark period, the pH was raised to 8.5 either while keeping the same salt at 10 mM (no $\Delta\psi$) or while raising the concentration to 100 mM ($\Delta\psi$ = 60 mV). [b] Increased yield.

diffusion potentials which are additive with ΔpHs induced by acid preincubation are almost entirely dependent on the presence of valinomycin to facilitate K^+ movement through the membranes, whereas the K^+ concentration changes which influence the half-life of X_E are quite independent of valinomycin (Table I). Presumably more K^+ ions move in the acid preincubation system then in the X_E system in order to provide an effective $\Delta\psi$, and they move to a different place. This suggests that the "potentials" developed are sometimes local (see discussion). In any event, neither of the two different effects of changing K^+ concentrations can be attributed to K^+ requirements per se since the effects in both cases are functions of the logarithms of the ratios of the before and after concentrations and quite independent of the absolute concentrations (data not shown). In fact, the apparent slowing of X_E decay can be observed with increases in the concentrations of any of the alkali cations (Table II) in the absence of ionophores.

Table III: Influence of DTT on Light-Activated ATPase Activity[a]

initial pH	DTT	μmol of $[^{32}P]P_i$ released (mg of chlorophyll)$^{-1}$ h^{-1}
7.5	−	12.2 ± 0.5
7.5	+	26.9 ± 1.0
6.5	−	9.0 ± 0.7
6.5	+	23.1 ± 1.1

[a] Chloroplasts were illuminated for 2 min at the indicated pH in the presence of 50 μM methylviologen and 10 mM dithiothreitol (DTT). Five seconds after the light was turned off, the pH was raised to 8.5, and 200 μmol of $[^{32}P]$ATP was added. After 5 min, the reaction was terminated by the addition of perchloric acid. The data are the averages of three samples and have been corrected for the dark ATPase activity [3.4 μmol (mg of chlorophyll)$^{-1}$ h^{-1}].

Are the Observed Decays of X_E Functions of Coupling Factor Deactivation? There is a good deal of evidence that the enzyme system in chloroplasts catalyzing ATP synthesis is activated by the protonmotive force, which provides the requisite energy for phosphorylation, and is deactivated when the protonmotive force is depleted. This evidence is of two kinds. The coupling factor performs a unidirectional catalysis by synthesizing ATP without promoting any ATP hydrolysis or ATP–P_i exchange (Jagendorf, 1977). This implies activation only under conditions in which the forward reaction is feasible. Furthermore, energization of the membrane system, either by light-driven electron transport or by acid–base transitions, induces a persistent ATPase and ATP–P_i exchange activity if strongly reducing sulfhydryl reagents are present (Bakker-Grunwald & Van Dam, 1973; Kaplan et al., 1967). If activation and deactivation of the coupling factor are integral parts of the synthesis reaction, that is, if the formation of ATP or the release of ATP from the coupling factor in themselves inactivate the catalytic function, the process need not concern us in our analysis of X_E decay. But if the activation of the enzyme has even a brief separate existence from the energization of the system (as is certainly the case in the presence of sulfhydryls such as dithiothreitol) or if the activation of the coupling factor has requirements in any way different from the energy requirements for ATP synthesis, a serious complication arises in the interpretation of X_E decays. Then we must ask ourselves if the decay we have observed is an expression of a diminishing energy source or an expression of diminishing activation of the coupling factor.

In an attempt to answer this question, we investigated the effects of dithiothreitol and ADP on X_E and its decay (Figure 7). Dithiothreitol, under conditions which markedly stimulate ATPase activity (Table III), has no effect whatsoever on either the magnitude or the lifetime of X_E. On the other hand, the presence of ADP during illumination and during the subsequent dark decay period actually slows the decay of X_E somewhat, in spite of the fact that ADP is known to hasten the inactivation of already activated coupling factor (Carmeli & Lifshotz, 1972; Dunham & Selman, 1981). Thus, inactivation of the coupling factor, at least as measured by changes in ATPase activity, has nothing to do with the diminishing X_E we have been observing (see the Discussion for other reasons for discounting the decay of the activity of the coupling factor as an explanation for the decay of X_E).

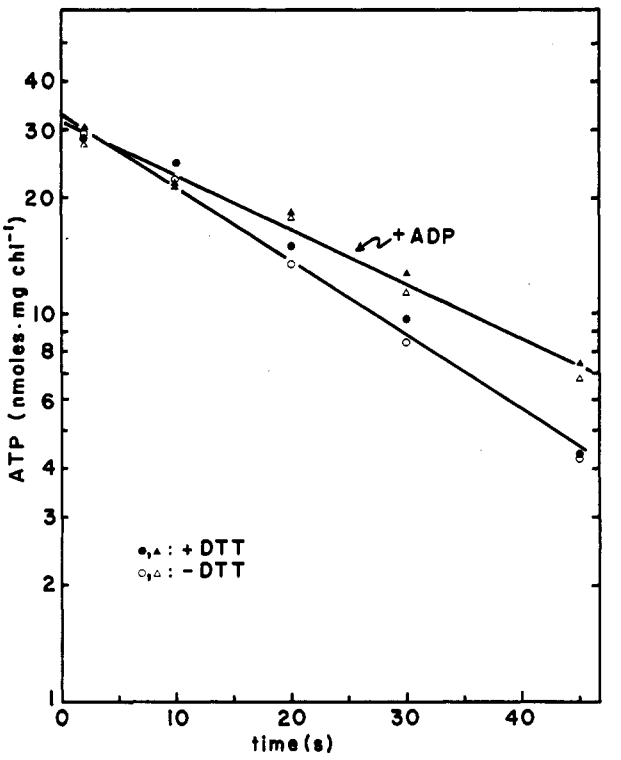

FIGURE 7: Effects of ADP and dithiothreitol on the decay of X_E. Conditions were the same as those in Figure 4 for $\Delta\psi = 0$ except that the electron acceptor was methylviologen, 20 μM ADP and 10 mM DTT were included in stage I as indicated, and the chloroplasts were preilluminated for 2 min. Note that the presence of ADP slowed the decay of X_E. However, ADP has been shown to stimulate the inactivation of the ATPase activity of "activated" coupling factor (Dunham & Selman, 1981). Furthermore, dithiothreitol had no effect on X_E, even though it stimulated ATPase activity (Table III). Thus, activation of the coupling factor, as indicated by ATPase activity, seems to have little relevance to the magnitude of X_E.

Discussion

Any model describing the light-energized state of the membranes must accommodate these observations: The capacity of preilluminated chloroplast lamellae to make ATP in a subsequent dark period almost always disappears exponentially as the dark period is extended. The rates of disappearance are strictly proportional to the remaining capacity over a wide range of conditions, encompassing a wide range of decay rates. Furthermore, when the pH of the phosphorylation stage is 8.5, the optimum for steady-state phosphorylation, an additional energy source in the form of a diffusion potential does not contribute at all to X_E, the postillumination yield of ATP. This is true no matter how large or small the yield. However, for reasons not understood, either a membrane potential or a pH rise imposed after partial decay affects the yield of ATP in such a manner that the rate constant of the decay process seems to decrease. Since the decay process occurs before the additional protonmotive force is applied, this apparent increase in the rate constant must really be an expression of the amount of decay required to eliminate the phosphorylation capacity.

First we must decide what loss is responsible for the declining ability of the preilluminated, dark-stored lamellae to phosphorylate ADP. There would seem to be three possibilities. Perhaps we have been observing a progressive deactivation of the ATP-synthesizing coupling factor, perhaps we have been observing the leaking away of accumulated ions, or perhaps we have been observing the relaxation of energy-supplying protein rearrangements, states which are only secondarily responsible for ion fluxes. Let us examine these possibilities.

Activation and Deactivation of the Coupling Factor. There has been a great deal of interest in light-induced activations

of the coupling factor, activations which turn on the ATPase activity of the system and initiate ADP–P_i exchange reactions [cf. Petrack et al. (1965) and Carmeli & Avron (1967)]. Thus, it has long been thought that the coupling factor is converted into an active form by the energized state of the membranes and then becomes inactive again when the energy is dissipated. It is therefore natural to suspect that the decays of X_E that we have investigated may be directly related to decays in the active state of the coupling factor and only indirectly related to the disappearance of the source of energy being used for ATP synthesis. Causes of the decay would then reside in structural changes in the enzyme, conformational alterations which might well relax exponentially. However, it does not seem possible that the decays of X_E we report here represent diminishing activity of the coupling factor. The reasons for discounting changes in the efficiency of the coupling factor are many and, taken together, compelling: (a) When the threshold driving force for phosphorylation is all provided by a postdecay, externally imposed protonmotive force, the loss of X_E and the disappearance of the light-induced pH rise in the medium coincide exactly (Figure 3 and 4). Since it can be confidently assumed that the ion disequilibrium represented by the light-induced pH rise then provides the energy responsible for all of the ATP synthesis, it is clear that in this case the decay of X_E is in no way related to a diminishing level of active coupling factor. A changing level of active coupling factor, if it lowered the efficiency of the use of the phosphorylation-driving ion gradients, would destroy the correspondence between the remaining X_E and the remaining ion disequilibrium. On the other hand, when the supplementary protonmotive force added in the dark after the decay is less than the thermodynamic threshold for ATP synthesis, the residual ability of the chloroplasts to phosphorylate ADP during the decay of X_E is still a function of the ion disequilibrium, albeit a more complex function. Since we know that the energy source, as measured by the ion disequilibrium, is decreasing exponentially, the imposition of a second decay (that of the coupling factor) would be most unlikely to produce a resultant which remained exponential. Yet, the decay of X_E is, we repeat, strictly exponential over a very wide range of conditions. (b) The persistent activations of the coupling factor illustrated by the sulfhydryl-dependent induction of ATPase activity and by the ATP–P_i exchange reactions may have little relevance to those factors limiting steady-state phosphorylation and as shown here obviously have no relevance at all to those factors limiting postillumination phosphorylation. Such activations of the coupling factor are strongly inhibited by excess ADP, and the decay of already existing activation is hastened by ADP (Carmeli & Lifshitz, 1972; Dunham & Selman, 1981). In contrast, the dark decay of the system's ability to make ATP is actually somewhat *slowed* by the presence of ADP (Figure 7). Furthermore, the activation of the ATPase is enhanced and stabilized by reducing sulfhydryls such as dithiothreitol (Table III), but as we have already pointed out, dithiothreitol has no effect whatsoever either on the yield of ATP or on the persistence of X_E in the dark (Figure 7).

In any event, many of our observations do not seem at all susceptible to interpretation on the basis of coupling factor activation. This is especially true of the lack of additivity of ΔpH and $\Delta\psi$ in our X_E experiments, even when X_E is limited by limited electron transport (Figure 5).

For these reasons, it seems exceedingly improbable that the kinetics of X_E decay described here are related to the activation or inactivation of the ATP-synthesizing enzymes, except to the extent that activation and energization may be considered parts of a single process. We must look for explanations of the decay in the nature of the energy conservation itself, in the nature of the ion activity differences, and in the nature of the reservoirs maintaining these differences.

Decay of X_E. If we assume that the light-induced driving force for ATP synthesis does decay exponentially in the dark (and the light-induced pH change in the external medium certainly does), it is by no means clear why the ability of the membranes to make ATP should also decay exponentially. As we have already stressed, thermodynamic considerations and acid–base transition studies with lamellae both indicate that there must be a threshold hydrogen ion activity difference before there can be any net ATP synthesis at plausible phosphate potentials (Jagendorf, 1977; Hangarter & Good, 1982). As a consequence, the ability of the membranes to make ATP should be lost with decaying protonmotive force long before that force has disappeared and long before the light-induced pH change in the medium has completed its relaxation. In the presence of such a threshold, it would seem impossible for both the driving force and the phosphorylation capacity to decay exponentially. Yet the decay of the light-induced ion disequilibrium and the decay of X_E are almost always both exponential [see also Izawa (1970)].

Alternatively, we might postulate that the protons relevant to the phosphorylation process are all stored at an activity which confers on them a driving force above the thermodynamic threshold. In other words, the buffering group responsible for storing protons for phosphorylation might be a select group with a quite low pK_a, in which case its proton could not be in equilibrium with the protons of the bulk proteins which buffer over a very wide pH range. Thus, kinetic evidence from the decay rate of X_E and kinetic evidence from the decay rate of the light-induced pH change in the medium both point in the same direction, that special, isolated, low-pK groups provide the driving force for phosphorylation. This picture of local energizations of the membrane would also help to explain the fact that transmembrane diffusion potentials add nothing to the phosphorylation capacity if the ATP synthesis is measured as soon as the light is turned off.[4] Thus, if the energizing reactions all stored protons at a potential sufficient to drive phosphorylation, the capacity for phosphorylation would simply reflect the number of such protonations. Then ATP synthesis would be stoichiometrically limited and not thermodynamically limited, as is clearly the case.

This condition is in striking contrast to the condition arising from acid–base transitions. Externally imposed pH changes and membrane potentials are precisely additive, both having

[4] It is possible that light or light-induced electron transport greatly increases the permeability of the membranes to anions such as chloride. The failure of added KCl to contribute to X_E yield might then reside in the inability of KCl to generate a diffusion potential across the light-exposed membranes. Similarly, the gradual restoration of KCl effects during a subsequent dark period might represent recovery of the dark state with decreasing permeability to chloride and a consequent gradual restoration of the ability of KCl to generate a diffusion potential. However, this hypothesis is untenable as an explanation of the observations. Potassium chloride and potassium glutamate give almost identical results: the same initial absence of enhancement of X_E and the same effects of added K^+ on the apparent rate constant of X_E decay. The anion permeability hypothesis would only fit the data if the permeabilities to chloride and to glutamate were identical in the dark and were affected in identical ways by illumination of the membranes. This is extremely improbable. In any event, no explanation involving changes in the relative permeability of the membranes to potassium ions and anions can be valid since the presence and absence of valinomycin make no difference in the observations.

identical effects on dark ATP synthesis (Hangarter & Good, 1982). Furthermore, the magnitude of these necessarily delocalized driving forces, derived from external acid–base transitions, determined the yield of ATP. Indeed, the very delocalization virtually guarantees that the yield will be thermodynamically and not stoichiometrically limited since there can be no dearth of available hydrogen ions in the bulk aqueous phase.

Yet another observation points in the direction of light energization by protonation of local, low-pK_a groups. The K^+ diffusion potentials which add to the yield of ATP in acid–base transition studies depend on valinomycin, whereas the "diffusion potentials" which appear to alter the rate constant of X_E decay are completely independent of the presence of valinomycin (Tables I and II). Again, the simplest explanation is that the two phenomena, acid–base transition and preillumination, involve different regions so that the diffusion pathways are different (unless, of course, the light treatment is opening new cation transport channels[4]). This means that we may have to postulate again local events in the membrane to account for the effects of K^+ concentration increases on decaying X_E.

At first, arguments about "local diffusion potentials" seem nonsensical. A diffusion potential, being an electric field, cannot possibly be local. However, we may have been deluding ourselves about what happens during phosphorylation by using terms such as membrane potential. It is true that an increase in K^+ concentration can create a transmembrane diffusion potential which can help to push the positively charged hydrogen ions across a membrane, thereby increasing their work potential. However, we should not forget that this particular mechanism constituted but one of many possible K^+–H^+ exchange mechanisms, all of which would have the same thermodynamic consequences. Many kinds of specific cation–H^+ exchange reactions could be confined to specific regions of the membrane and involve special protonatable groups. Thus, no delocalized electric field need be postulated for some plausible explanations of the effect of increasing cation concentration.

We would embrace the idea of intramembrane events, specific proton-storage intermediates, and specific cation–H^+ exchanges with fewer reservations if we better understood the effects of these pseudodiffusion potentials on the apparent rate constants of X_E decay. The effects are most easily interpreted in terms of the very energy thresholds we have tried to explain away. If energy thresholds are involved, one can readily imagine that more decay of the light-contributed driving force would be required before ATP synthesis need stop if a supplementary energy source is contributed by a cation concentration increase. Then an increase in K^+ concentration would be expected to increase the half-life of the capacity to make ATP, as it does. Clearly, ATP synthesis is not thermodynamically limited immediately after the light is turned off, but it is equally clear that thermodynamic limitations begin to intrude as soon as X_E decay sets in, if only because added energy begins to be effective. This would be easy to accept were it not for the fact that there is no evidence whatsoever for a transition between stoichiometrically limited and thermodynamically limited states; the decay of X_E is almost always monotonically exponential. Yet there can be little doubt that thermodynamic limitations hover in the background, even without X_E decay. When the pH of the phosphorylation stage is 7.5 or lower (and the available transmembrane H^+ activity difference following illumination is presumably lower than when we use our standard base stage at pH 8.5), supplementary energy from a rise in K^+ concentration does increase the yield of X_E somewhat, whether or not any X_E decay has occurred (Figure 6).

In summary, the energized state of the membranes which is associated with an externally imposed pH rise (acid–base phosphorylation) and the energized state of the membrane associated with preillumination (X_E) are quite different. Both the exponentiality of the decay of X_E without any kinetic evidence of a thermodynamic threshold and the absence of any increase in X_E when there is supplementary energy diffusion potentials point to a process limited by the supply of something and not limited by a driving force acting on the ubiquitous hydrogen ions of a bulk water phase. This argument is the more convincing in that phosphorylation associated with acid–base transitions *is* limited by the driving force, even though the amounts of ATP synthesized in the two systems are comparable (see Figure 2 vs. Figures 4 and 5). Thus, it is very hard to avoid the conclusion that energization of the membrane by light-driven electron transport involves local, intramembrane phenomena, whatever these phenomena may be. This conclusion is consistent with the previously mentioned observations (Ort et al., 1976; Graan et al., 1981) that ATP synthesis can begin long before the inner aqueous phase of the lamellar vesicles has been acidified and before any appreciable transmembrane electric potential can have developed [see also Venturoli & Melandri (1982)]. Such a conclusion also has wider implications regarding the mechanism of normal, steady-state ATP synthesis.

Registry No. ATP, 56-65-5; hydrogen ion, 12408-02-5.

References

Avron, M. (1960) *Biochim. Biophys. Acta 40*, 257–272.

Avron, M. (1972) *Proc. Int. Congr. Photosyn., 2nd*, 861–871.

Bakker-Grunwall, T., & Van Dam, K. (1973) *Biochim. Biophys. Acta 292*, 808–814.

Carmeli, C., & Avron, M. (1967) *Eur. J. Biochem. 2*, 318–326.

Carmeli, C., & Lifshitz, Y. (1972) *Biochim. Biophys. Acta 267*, 86–95.

Dunham, K. R., & Selman, B. R. (1981) *J. Biol. Chem. 256*, 212–218.

Graan, T., & Ort, D. R. (1981) *Biochim. Biophys. Acta 637*, 447–456.

Graan, T., Flores, S., & Ort, D. R. (1981) in *Energy Coupling in Photosynthesis* (Selman, B. R., & Selman-Reimer, S., Eds.) Elsevier/North-Holland, Amsterdam.

Hangarter, R. P., & Good, N. E. (1982) *Biochim. Biophys. Acta 681*, 397–404.

Hind, G., & Jagendorf, A. T. (1963) *Proc. Natl. Acad. Sci. U.S.A. 49*, 715–722.

Hind, G., Nakatani, H. Y., & Izawa, S. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1484–1488.

Izawa, S. (1970) *Biochim. Biophys. Acta 223*, 165–173.

Izawa, S., & Ort, D. R. (1974) *Biochim. Biophys. Acta 357*, 127–143.

Izawa, S., Connolly, T. N., Winget, G. D., & Good, N. E. (1966) *Brookhaven Symp. Biol. No. 19*, 169–187.

Jagendorf, A. T. (1977) *Encycl. Plant Physiol., New Ser. 5*, 307–337.

Jagendorf, A. T., & Uribe, E. (1966) *Proc. Natl. Acad. Sci. U.S.A. 55*, 170–177.

Kaplan, J. H., Uribe, E., & Jagendorf, A. T. (1967) *Arch. Biochem. Biophys. 120*, 365–375.

McCarty, R. E., Fuhrman, J. S., & Tsuchiya, Y. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 2522–2526.

Mitchell, P. (1968) *Chemiosmotic Coupling and Energy Transduction*, Glynn Research Ltd., Bodmin, U.K.

Nelson, N., Nelson, H., Naim, V., & Neumann, J. (1971) *Arch. Biochem. Biophys. 145*, 263–267.
Ort, D. R., Dilley, R. A., & Good, N. E. (1976) *Biochim. Biophys. Acta 449*, 108–124.
Petrack, B., Cranston, A., Sheppy, F., & Farron, F. (1965) *J. Biol. Chem. 250*, 906–914.
Saha, S., & Good, N. E. (1970) *J. Biol. Chem. 245*, 5017–5021.
Trebst, A., & Pistorious, E. (1965) *Z. Naturforsch. C: Biochem., Biophys., Biol., Virol. 20C*, 143–147.
Venturoli, G., & Melandri, B. A. (1982) *Biochim. Biophys. Acta 680*, 8–16.

Effect of Lipid Composition on the Calcium/Adenosine 5′-Triphosphate Coupling Ratio of the Ca^{2+}-ATPase of Sarcoplasmic Reticulum†

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ABSTRACT: The Ca^{2+}-ATPase of sarcoplasmic reticulum was purified and depleted of proteolipids by solubilization in Triton X-100 and by fractionation on a DE-52 column. The protein reconstituted by deoxycholate–cholate dialysis at low lipid to protein ratios (2–5 mg of lipid/mg of protein), with either dioleoylphosphatidylethanolamine or monogalactosyldiglyceride, exhibited high initial rates of ATP-dependent Ca^{2+} uptake [300–900 nmol min^{-1} (mg of protein)$^{-1}$] and coupling ratios (Ca^{2+} transported/ATP hydrolyzed) up to 1.2. Ca^{2+}-ATPase reconstituted with lipids of increasing degrees of methylation (dioleoylphosphatidylethanolamine, dioleoylmonomethylphosphatidylethanolamine, dioleoyldimethylphosphatidylethanolamine and dioleoylphosphatidylcholine) or increasing degrees of glycosylation (monogalactosyldiglyceride and digalactosyldiglyceride) revealed a progressive decrease in both ATP-dependent Ca^{2+}-uptake and coupling ratios. The rate and extent of Ca^{2+} uptake decreased as the dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine or monogalactosyldiglyceride/dioleoylphosphatidylcholine molar ratios in the reconstituted vesicles were reduced. Vesicles reconstituted with high molar ratios of dioleoylphosphatidylethanolamine/dioleoylphosphatidylcholine or monogalactosyldiglyceride/dioleoylphosphatidylcholine and at a high lipid to protein ratio became leaky and released the Ca^{2+} accumulated inside the vesicles when the temperature of the incubation mixture was increased (e.g., from 20 to 37 °C). Freeze–fracture electron microscopy of reconstituted vesicles incubated at 37 °C demonstrated fusion of vesicles and formation of hexagonal II structures. Reconstitution of the Ca^{2+}-ATPase with other phospholipids such as dioleoylphosphatidylcholine, dioleoylphosphatidylglycerol, cardiolipin, bovine brain phosphatidylserine, phosphatidylinositol, and mixtures of dioleoylphosphatidylcholine and cholesterol catalyzed Ca^{2+}-dependent ATP hydrolysis [0.5–2 μmol of P_i min^{-1} (mg of protein)$^{-1}$] but low rates of Ca^{2+} uptake [5–10 nmol min^{-1} (mg of protein)$^{-1}$]. Our results suggest that the "coupling state" of the Ca^{2+}-ATPase as numerically expressed as the Ca^{2+}/ATP ratio is stabilized by cone-shaped lipid molecules (e.g., dioleoylphosphatidylethanolamine and monogalactosyldiglyceride).

Ca^{2+}-ATPase[1] of sarcoplasmic reticulum membranes couples the hydrolysis of ATP to Ca^{2+} transport. This protein has been purified and reconstituted into phospholipid vesicles by several methods (Racker, 1979). Reconstitution experiments with the Ca^{2+}-ATPase by deoxycholate–cholate dialysis demonstrated that phosphatidylethanolamine is required for Ca^{2+} uptake. Reconstitution of the enzyme with acetyl-PE yielded vesicles lacking both ATP-hydrolysis and Ca^{2+}-transport activities, which were restored by addition of suitable amounts of stearoylamine or oleoylamine (Knowles et al., 1975). Recently, Hidalgo et al. (1982) demonstrated that blockage of the amino group of PE in sarcoplasmic reticulum vesicles with fluorescamine resulted in low coupling of the Ca^{2+}-ATPase. Ca^{2+} transport was inhibited, but Ca^{2+}-dependent ATP hydrolysis was unaffected. These observations suggest that the amino group of PE is essential for coupling in the Ca^{2+}-ATPase of sarcoplasmic reticulum. However, reconstitution by freeze–thaw sonication yielded different results (Zimniak & Racker, 1979; Caffrey & Feigenson, 1981). Phosphatidylcholine vesicles transported Ca^{2+} though not nearly as well as phosphatidylcholine–phosphatidylethanolamine vesicles. In order to explore the role of phosphatidylethanolamine, we considered the possibility that coupling of the Ca^{2+}-ATPase depends on the presence of lipids capable of adopting nonbilayer structures (e.g., PE's tend to assemble into hexagonal II structures) as described by Cullis et al. (1982). We have therefore reconstituted the Ca^{2+}-ATPase with several lipids and examined Ca^{2+} transport and their tendency to form hexagonal structures. Our results support

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[1] Abbreviations: ATPase, adenosinetriphosphatase; PE, phosphatidylethanolamine from natural sources; DOPE, dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; MGDG, monogalactosyldiglyceride; PI, phosphatidylinositol; PS, phosphatidylserine; CL, cardiolipin; DOPG, dioleoylphosphatidylglycerol; DGDG, digalactosyldiglyceride; DO(1M)PE, dioleoylmonomethylphosphatidylethanolamine; DO(2M)PE, dioleoyldimethylphosphatidylethanolamine; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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