THE RELATIONSHIP BETWEEN PROTON FLUXES AND THE REGULATION OF ELECTRON TRANSPORT IN CHLOROPLASTS

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1. Introduction

In the light, chloroplast thylakoids accumulate hydrogen ions in a reaction which is stoichiometrically coupled to the light-driven transfer of electrons from water to photosystem I [1–3]. At the steady-state, the rate at which protons move into the thylakoid will equal the rate at which protons move out, and, as a result, the rate of electron transport coupled to inward proton translocation will depend upon the rate of proton efflux [4]. There is already evidence that at least two pathways for proton efflux exist in chloroplasts [5,6]. The first pathway consists of a non-specific leakage of hydrogen ions through the membrane, resulting from certain intrinsic properties of the membrane components. The second proton leak is via a specific pathway capable of coupling an outward directed proton flux to the phosphorylation of ADP [7]. This second pathway is thought to involve at least two components; a transmembrane, proton-specific channel [6], and the chloroplast coupling factor enzyme (CF_1) [8,9].

It has been widely observed that when the non-specific permeability of the thylakoid membrane is drastically increased (for example, with an uncoupler), no pH gradient can be maintained, and the rate of electron transport is also very much increased [4]. Similarly, if the flux of protons through the specific channel is increased by allowing ATP formation to proceed, the transmembrane pH gradient is decreased [10,11] with a concomitant stimulation of the rate of electron transport [12].

Conversely, one would expect that if the proton permeability of the membrane were decreased, the magnitude of the steady-state pH gradient would be larger and the rate of electron transport less. In fact, McCarty et al. [9] have presented evidence that the adenine nucleotides, ADP and ATP, at low concentrations, will partially inhibit the rate of electron transport occurring in the absence of phosphate (so called 'basal' electron transport) while greatly stimulating the extent of proton uptake. This adenine nucleotide effect has been shown to be a direct effect on the coupling factor enzyme, indicating that, in the presence of ATP or ADP, the CF_1 molecule somehow becomes more effective in preventing proton movement through a transmembrane channel. That is, CF_1 behaves as a regulated gate for that portion of the proton efflux utilizing the proton-specific membrane channel of the ATP-forming complex [8].

The energy transfer inhibitor triphenyltin chloride has also been found to block the movement of protons through the channel gated by CF_1 [6], although not by a direct interaction with the CF_1 molecule. Rather, triphenyltin binds with some component of the membrane involved in the passage of protons through the membrane during both ATP formation and membrane-bound ATPase reactions; i.e. some component of the channel itself.

In this paper it is shown that, like the adenine nucleotides, triphenyltin increases the extent of proton uptake while decreasing the rate of basal electron transport. However, it is also shown that the effects of ATP and triphenyltin are different in several important respects, and that, in the presence of a saturating concentration of ATP, triphenyltin will inhibit basal electron transport an additional 30–40% without further increasing the extent of proton uptake.

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2. Experimental

Chloroplasts were isolated from leaves of fresh market spinach by techniques described elsewhere [6]. Electron transport was measured spectrophotometrically as the reduction of $Fe(CN)_6^{3-}$ by continuously recording the absorbance decrease of the sample at 420 nm. Light-induced pH changes were detected with a Sargent miniature combination electrode connected to a Corning pH meter and a strip chart recorder with a full scale deflection of 0.1 pH unit. The chart was calibrated in H^+ equivalents by titrating the sample in the light [13] with 0.001 M HCl. The overall response time for the pH measuring system was $t½ \leqslant 1$ sec. Actinic illumination (> 500 kergs. $cm^{-2} \cdot sec^{-1}$) was supplied by a high intensity projector lamp. Details of the reaction mixtures are given in the figure legends.

Triphenyltin was obtained from Alpha Inorganics and recrystallized twice from ethanol before use.

3. Results

The data presented in fig.1 show clearly that ATP stimulates the steady-state extent of the light-driven proton uptake reaction while simultaneously inhibiting partially the rate of basal electron transport. These results confirm data already published [8,9,14], and have been interpreted to indicate that, in the presence of ATP, the coupling factor (CF_1) assumes a conformation which prevents the outward leakage of protons through a transmembrane proton channel [8].

A similar experiment using the energy transfer inhibitor triphenyltin chloride is shown in fig.2. Like ATP, triphenyltin stimulates the extent of the pH rise while partially inhibiting the rate of basal electron flow. However, in a series of experiments it was repeatedly observed that triphenyltin inhibits basal electron transport to a lower rate than does ATP, although both compounds stimulate the extent of the pH rise to a similar degree. Fig.3 shows the effect of triphenyltin on basal electron transport and the extent of the pH rise in the presence of 5 μM ATP. Surprisingly, triphenyltin reproducibly causes a further inhibition of basal electron flow (by about 30–40%) which is not accompanied by any further increase in the extent of the pH rise. In fact, under

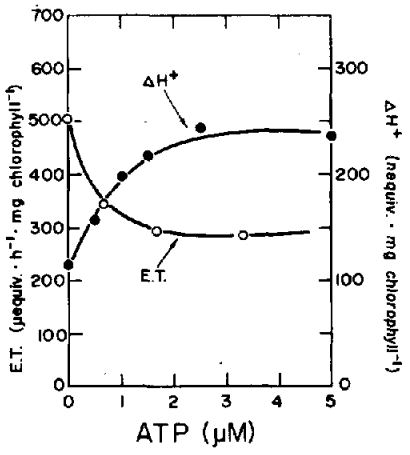

Fig.1. Stimulation of proton uptake (ΔH^+) and inhibition of basal electron transport (E.T.) by ATP. The basic reaction mixture for the measurement of electron transport (3 ml) contained 0.1 M sucrose, 2 mM $MgCl_2$, 50 mM tricine-NaOH (pH 8.2), 0.33 mM $K_3Fe(CN)_6$ and chloroplasts equivalent to 63 μg chlorophyll. The basic reaction mixture for the measurement of proton uptake (3 ml) contained 0.1 M sucrose, 2 mM $MgCl_2$, 1 mM tricine-NaOH (pH 8.2), 0.25 mM methylviologen and chloroplasts equivalent to 63 μg chlorophyll. Note that the data presented in figs.1–3 are taken from the same experiment and can therefore be compared on a quantitative basis.

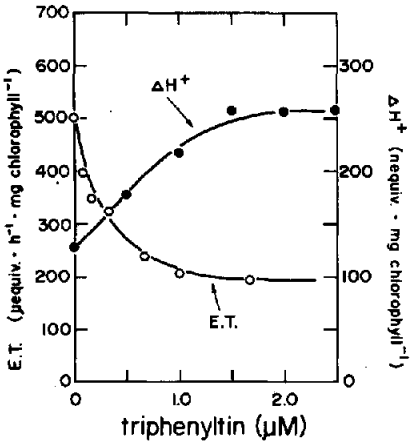

Fig.2. Stimulation of proton uptake (ΔH^+) and inhibition of basal electron transport (E.T.) by triphenyltin chloride. The reaction conditions are given in fig.1. Note that the rate of basal electron transport in the presence of a saturating concentration of triphenyltin is significantly lower than in the presence of a saturating concentration of ATP (fig.1), although proton uptake is stimulated to a similar extent by both compounds.

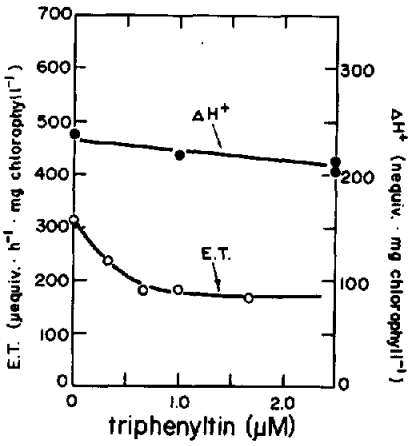

Fig.3. Inhibition of basal electron transport (E.T.) without stimulation of proton uptake (ΔH^+) by triphenyltin in the presence of 5 μM ATP. Reaction conditions were the same as in fig.1, except that 5 μM ATP was present in all samples. Note that triphenyltin causes a further 30–40% inhibition of the rate of basal electron transport over the rate obtained in the presence of ATP alone, and that this inhibition is not accompanied by any further increase in the extent of proton uptake.

these basal conditions triphenyltin causes a slight but reproducible *decrease* in the extent of the pH rise. The concentration of triphenyltin required to inhibit basal electron transport in the presence of ATP (fig.3) is the same as in its absence (fig.2), and is stoichiometrically dependent upon the chlorophyll concentration. Indeed, the stoichiometry is the same as that found earlier for the inhibition of phosphorylation by triphenyltin, with a ratio of 2–3 triphenyltin molecules/100 chlorophyll molecules at half-maximal inhibition [6]. This suggests that all of these effects are related to a single inhibitory function of the triphenyltin molecule; very likely the blocking of a transmembrane proton channel gated by CF_1 [6].

That the 'extra' inhibition of basal electron transport by triphenyltin is not due to a direct inhibition by triphenyltin of one of the electron carriers is shown by the fact that the uncoupler gramicidin D, which greatly increases the membrane permeability to H^+ ions, effectively relieves the triphenyltin inhibition of electron transport (table 1) [6]. The possibility that triphenyltin may function as an energy-dependent electron transport inhibitor [15] can also be eliminated, since preillumination of chloroplasts in the presence of triphenyltin had no effect on the subsequent rate of electron transport when gramicidin was present (data not shown). Increasing the membrane permeability to K^+ by adding valinomycin is also ineffective in relieving triphenyltin's inhibition of basal electron flow, indicating that triphenyltin's effects are also not related to counterion fluxes.

The effects of ATP and triphenyltin on proton movements in chloroplasts are shown in a different way in fig.4 and 5. As shown earlier [9] 5 μM ATP causes the normally exponential dark decay of the light-induced pH rise to become biphasic. Addition of 1.5 μM triphenyltin not only restores the decay to a monophasic, exponential form, but also markedly accelerates the rate of decay: the first order rate constant being about twice the control value. Note,

Table 1
Effect of gramicidin and valinomycin plus K^+ on the rate of basal electron transport in chloroplasts in the presence of triphenyltin chloride

Addition	Electron transport rate (μequiv. · h^{-1} · mg chlorophyll^{-1})
None	448
ATP	292
Triphenyltin	178
Triphenyltin, KCl	169
Triphenyltin, KCl, valinomycin	165
Gramicidin D	990
Triphenyltin, gramicidin D	905

Details of the basic reaction mixture are given in the legend to fig.1. When added, ATP was 5 μM, triphenyltin was 1.3 μM; KCl was 20 mM, valinomycin was 0.1 μM and gramicidin D was 8 μg/ml.

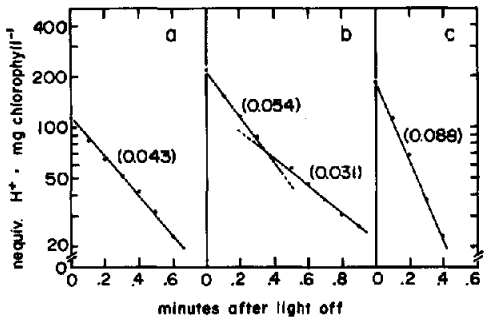

Fig.4. Kinetics of proton efflux from chloroplasts in the dark after an illumination. The basic reaction mixture was as given in fig.1 with the following additions: (a) none (control), (b) 5 μM ATP, and (c) 5 μM ATP plus 1.5 μM triphenyltin. The numbers in parentheses are the first order rate constants in sec^{-1}. Note that ATP causes the decay to become biphasic, and that triphenyltin reverses this effect and accelerates the decay considerably.

however, that the steady-state extent of the pH rise (i.e., at time = 0) is not further increased by triphenyltin over the ATP stimulated extent (compare figs.4b and 4c), even though the rate of basal electron transport is markedly inhibited (fig.3).

In the absence of ATP triphenyltin both increases the extent of the pH rise and accelerates the rate of the dark decay (fig.5). It should be pointed out that the acceleration of the decay of the pH rise is not

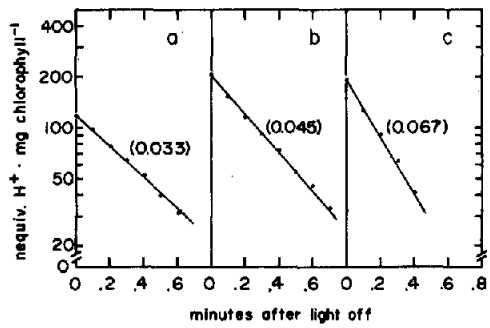

Fig.5. Effect of triphenyltin on the kinetics of proton efflux from chloroplasts. Details of the basic reaction mixture are given in fig.1, with the following additions: (a) none (control), (b) 1 μM triphenyltin, and (c) 1.5 μM triphenyltin. Numbers in parentheses are the first order rate constants in sec^{-1}. Note that the rate of proton efflux is about twice as fast in the presence of 1.5 μM triphenyltin as in the control, even though the extent of proton uptake is also nearly twice as large.

saturated by 1.5 μM triphenyltin, suggesting that this effect probably results from a weak, triphenyltin-dependent increase in counter-ion permeability. In fact, triphenyltin is known to catalyze $Cl^- \rightleftharpoons OH^-$ antiport across certain membrane systems [16,17] and this activity has been suggested to occur to a limited extent in chloroplasts [6,16]. In experiments with valinomycin plus K^+ present, the rates of decay of the pH rise in the control were indeed much faster, and no further increase by triphenyltin could be detected (data not shown). However, the presence of valinomycin plus K^+ did not alter the effects of triphenyltin on either the steady-state extent of the pH rise or the rate of basal electron transport (table 1).

4. Discussion

The effects of ATP on the rate of basal electron transport and on the steady-state extent of the proton gradient have been attributed to an interaction of the nucleotide with the CF_1 molecule in such a way as to cause the permeability of the proton channel gated by CF_1 to decrease, perhaps due to a conformational transition [9]. This decrease in the permeability of the membrane to protons allows a higher steady-state pH gradient to be maintained, and this in turn results in a lowered rate of electron transport between the photosystems, since, at the steady-state, the rate of proton-translocating electron transport must equal the rate of proton efflux [4].

The effects of triphenyltin chloride cannot be as easily understood. Triphenyltin blocks proton movement through the same CF_1-gated membrane channel as ATP, but the presence of CF_1 on the membrane is not even required for triphenyltin's inhibitory function [6]. Indeed, the effects of triphenyltin and ATP are clearly different in both qualitative and quantitative aspects. The effects of triphenyltin supersede the effects of ATP, since, in the presence of a saturating concentration of ATP, addition of triphenyltin causes an additional 40% inhibition of basal electron flow. Addition of ATP to chloroplasts in the presence of a saturating concentration of triphenyltin is without further effect, however.

The most interesting observation is that in the presence of 5 μM ATP, triphenyltin causes an inhibi-

tion of basal electron flow which is not accompanied by an increase in the steady-state extent of proton uptake. At present the reasons for this phenomenon are not at all understood. Nevertheless, it can be speculated that the difference in the effects of ATP and triphenyltin may be related to the different sites at which these compounds interfere with proton movement through the same transmembrane channel. ATP almost certainly affects CF_1, acting as a regulated gate on the outside end of the channel, whereas triphenyltin almost certainly affects a component *within* the membrane itself, perhaps at a site closer to the middle or the inside end of the channel. It is possible that, in the presence of triphenyltin, protons released into the membrane by the electron transport reaction are somehow trapped inside the membrane, thereby greatly increasing a localized proton concentration and inhibiting electron transport, but not necessarily increasing the transmembrane pH gradient. While highly speculative, such a model is consistent with the recent data of Ort et al., [18,19], who showed that the initial time-lag in ATP formation is unaffected by the presence of internal hydrogen-ion buffers, suggesting that the protons involved in ATP formation are actually extracted from localized regions of high proton activity within the thylakoid membrane [20,21].

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