PROTON TRANSLOCATION INDUCED BY ATPase ACTIVITY IN CHLOROPLASTS

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Proton uptake by chloroplasts was induced by light-triggered ATPase activity. A quotient of two was obtained when the initial rate of proton uptake was divided by the rate of P_i released from ATP. Gramicidin accelerated the rate of ATPase activity and reduced the H^+/P_i ratio to 1.4. The results were found to be consistent with the chemiosmotic theory.

1. Introduction

According to the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation, electron transport induces proton translocation across the coupling membrane [1]. An ATPase system located in the membrane utilizes the proton gradient produced by electron flow for the synthesis of ATP. Hydrolysis of ATP by this ATPase produces proton translocation in the same direction across the membrane as does electron flow. Support for the hypothesis came from the finding of H^+/P_i ratio of two during ATPase activity which was of the same value as the quotient of $H^+/2e^-$ per phosphorylation site during electron transport in mitochondria [1]. In chloroplasts light-induced proton uptake was demonstrated [2] but $H^+/2e^-$ quotient varied with the experimental conditions [2–5]. The finding of an ATPase induced ammonium ion uptake was assumed to indicate the presence of an ATPase driven proton uptake in chloroplasts [6, 7]. In this paper a preliminary finding of proton uptake induced by light-triggered ATPase activity is reported.

2. Methods

Chloroplast fragments were prepared from lettuce leaves as previously described [2]. The reaction medium contained KCl, 50 mM; $MgCl_2$, 3 mM; Dithiothreitol, 5 mM; PMS, 0.01 mM, pH 8 in a total volume of 4 ml at 24°C. When addition of ATP is indicated, a solution containing ATP, 150 nmoles and $MgCl_2$ 150 nmoles, pH 8, in a volume of 5 μl was used. In several experiments PEP (phosphoenolpyruvate) 0.8 mM and PK (pyruvate kinase) 1 I U/ml were added to the reaction medium. PK was freed from $(NH_4)_2SO_4$ by dialysis of 0.2 ml of the enzyme suspension against 2 l of tris-HCl, pH 8, 5 mM and KCl, 2 M for 24 hr at 4°C. PK activity was assayed according to the method of Bücher [8]. Illumination was provided by a 500 W slide projector through a CS 4-96 Corning filter. Proton uptake was measured as a change of pH in a rapidly stirred medium by a GK 20240 Radiometer pH electrode connected to a model 26 Radiometer pH meter and was recorded on a Sergant recorder. ATPase activity was measured as a change in pH of the medium according to the method of Nishimura et al. [9]. In some experiments ATPase activity was also assayed by withdrawing of aliquots from the medium, deproteinization with 3% cold CCl_3COOH and determination of P_i released according to the method of Ames [10].

3. Results and discussion

From fig. 1c it can be seen that the addition of ATP to a chloroplast suspension caused a very slow rate of decrease in the pH of the medium. This proton release accompanies ATP hydrolysis at pH 8 according to Scheme 1.

Scheme 1 : $ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + nH^+$.

A value of n = 0.95 (n = $\Delta H^+/P_i$) was calculated [9]. The rate of ATPase activity calculated from proton released was compared to P_i released from ATP during ATPase activity and the results obtained by the two assays were in good agreement. The well documented light-induced pH shift was recorded (fig. 1A) during light-triggering of ATPase activity in the presence of dithiothreitol. The addition of ATP after light-triggering of ATPase activity should cause a pH drop which accompanies ATP hydrolysis. However, this pH drop was preceded by a pH rise. The pH rise is assumed to be a result of proton uptake energized by ATP hydrolysis. We assumed that the slope of the pH drop at steady state represents the rate of proton released accompanying ATP hydrolysis. The slope of the pH rise resulted from proton uptake energized by ATPase activity minus the rate of proton released due to ATP hydrolysis. Based on these assumptions the initial rate of proton uptake was calculated by adding the rate of proton released at steady state to the observed rate of proton uptake. Calculated in this way a H^+/P_i quotient of two was obtained for the initial rate of proton uptake divided by the rate of ATPase activity (table 1). This ratio indicates that two protons were translocated for each ATP hydrolyzed.

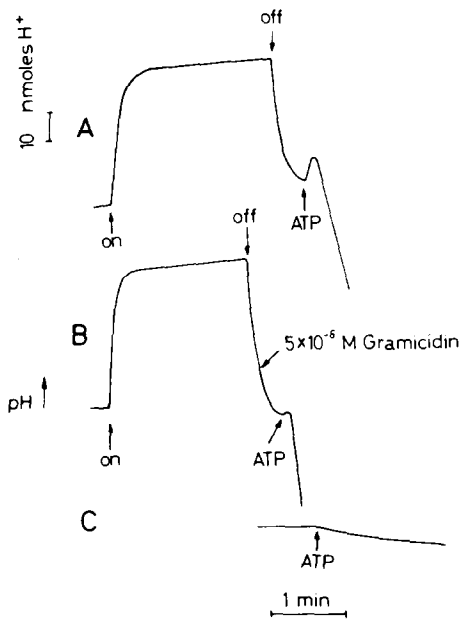

Fig. 1. pH Changes induced by light-triggered ATPase activity. A: the effect of ATP. B: the effect of gramicidin on ATPase induced pH change. C: the effect of ATP added before light-triggering of ATPase activity. Experimental conditions as described under Methods. The medium contained 46 μg chlorophyll/ml.

Gramicidin, an uncoupler of photophosphorylation, caused a decrease in the yield of light-induced proton uptake as well as in the yield (defined as the maximum in pH increase following the addition of ATP) of proton uptake induced by ATPase activity (table 1). The decrease in yield of proton uptake following the addition of ATP was not a result of a lower ATPase activity, since gramicidin stimulated this activity (fig. 1B). The decrease in H^+/P_i quotient (table 1) suggests that proton permeability is raised by gramicidin. These data support the suggestion that gramicidin stimulates ATPase activity by increasing the proton permeability properties of the chloroplast membrane.

Gramicidin decreased the extent of the initial proton uptake (fig. 1) without altering the extent of the pH drop accompanying complete hydrolysis of a limiting amount of ATP (not shown). This indicates that the initial proton uptake process accompanying ATPase is reversible.

In order to obtain a more exact measurement of the initial rate of proton uptake, proton release which accompanies ATP hydrolysis was eliminated by the rephosphorylation of ADP with PEP and PK. The phosphorylation of ADP by PEP and PK at pH 8 involves proton uptake from the medium according to Scheme 2.

Scheme 2: $PEP^{3-} + ADP^{3-} + nH^+ \rightarrow ATP^{4-} + Pyruvate^{1-}$.

Before light-triggering only a very small increase in pH followed the addition of ATP (fig. 2B). Addition of ATP after light-triggering of ATPase activity initiated a fast proton uptake followed by a much slower rate (fig. 2A). The second slow rate of proton uptake is believed to be a result of the difference

Table 1
Effect of PK and gramicidin on proton translocation induced by ATPase activity and by light*.

Additions	Rate of activity (μmoles/mg Chl/hr)			H^+/P_i	Yield (nmoles H^+/mg Chl)	
	Observed H^+ uptake	Calculated H^+ uptake	P_i released		Light** H^+ uptake	ATPase H^+uptake
None	24.7	46.8	22.1	2.1	184	31
Gramicidin 5×10^{-6} M	17.5	49.9	32.4	1.46	97	23
PK, PEP	72	–	35	2.06	196	117

* Experimental conditions as described under methods. The medium contained 45 μg chlorophyll/ml.
** Yield before the addition of ATP.

between $\Delta H^+/P_i$ released during ATP hydrolysis to the ΔH^+/PEP during the phosphorylation of ADP by PK. A higher ΔH^+/PEP ratio is expected at pH 8 because the pK_a of PEP is lower than that of P_i. In calculation of both the initial rate and the yield of proton uptake, a correction was made for this second slow rate of proton uptake. It can be seen from fig. 2 that

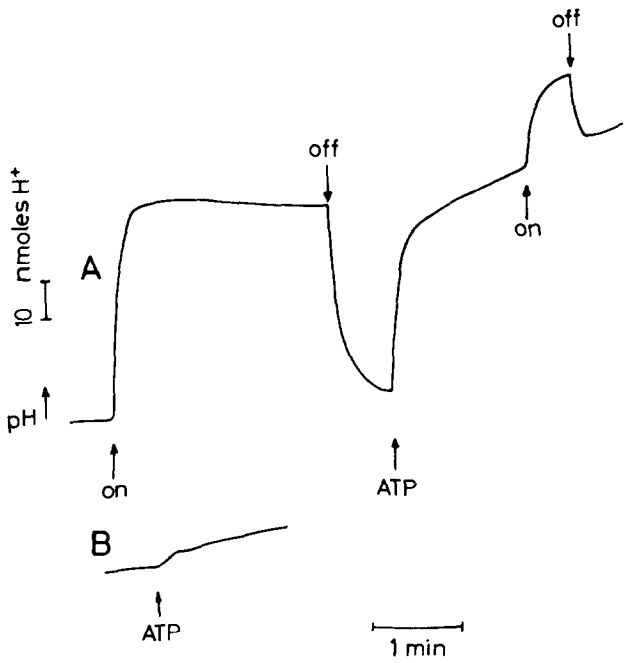

Fig. 2. The effect of PK plus PEP on ATPase induced pH changes. A: the effect of illumination on pH changes during ATPase activity. B: ATP was added before light-triggering of ATPase. Experimental conditions as described under Methods. Chlorophyll content 80 μg/ml.

because of the elimination of proton release which accompanies ATP hydrolysis by PK plus PEP, both the yield and the apparent rate of proton uptake were increased (table 1). The H^+/P_i quotient remains two in the presence of PK and PEP, thus confining the results obtained without PK.

The function of ATPase system which translocates protons across the chloroplast membrane with H^+/P_i quotient of two is compatible with the chemiosmotic theory. A $H^+/2e^-$ ratio of 2.7 was obtained at pH 8 during ferricyanide reduction by chloroplasts although a variation with pH was observed [5]. From $P_i/2e^-$ ratio of one, most commonly obtained during photophosphorylation with ferricyanide as an electron acceptor [11], a H^+/P_i ratio of 2.7 is calculated. A H^+/P_i quotient of two during ATPase activity and of 2.7 during photophosphorylation are sufficiently close to call for more precise measurements of H^+/P_i ratio in the two reactions under exact experimental conditions. Further support for the chemiosmotic hypothesis can be obtained if the same H^+/P_i ratio during photophosphorylation and ATPase activity are found at each pH.

The relation between light and ATPase induced proton translocation was demonstrated by the effect of light after proton translocation induced by ATPase activity. The yield of extra light induced proton uptake was smaller than the light-induced proton uptake before addition of ATP (figs. 2 and 3). These results were obtained whether ATP was added prior to light-triggering (fig. 3A) or illumination followed proton uptake induced by ATPase activity (fig. 3B). The greater the yield of proton uptake induced by ATPase

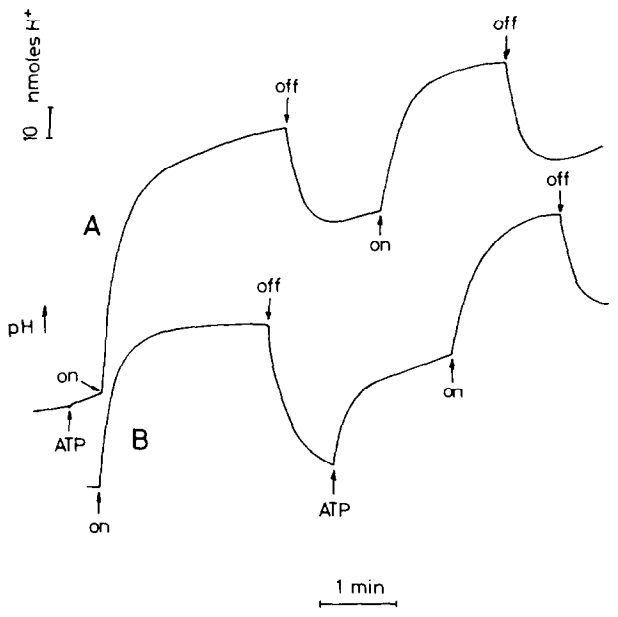

Fig. 3. Interaction between pH changes induced by light and by ATPase activity. A: ATP was added before light-triggering. B: ATP was added after light-triggering of ATPase activity. Experimental conditions as described under Methods. The reaction mixture contained PK plus PEP and 55 μg chlorophyll/ml.

activity, the smaller was the yield of extra light-induced proton uptake (compare fig. 2A with fig. 3B). Based on this finding, it is reasonable that H^+ uptake produced by light and ATP occur in the same compartment. The same rate of light-induced electron transport should give a smaller yield of proton uptake when working against an established proton gradient. Another possibility that is presently under investigation, is that light-induced proton gradient inhibits ATPase activity, or that ATPase activity inhibits electron transport. Although the results presented above support the chemiosmotic hypothesis,they can be equally well explained by the assumption that a proton pump is energized by a high energy intermediate which is generated by either electron transport or ATPase activity.

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