THE MECHANISM OF PHOTOPHOSPHORYLATION I. INHIBITION OF THE LIGHT-INDUCED PROTON TRANSLOCATION BY INORGANIC PHOSPHATE

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SUMMARY

Inorganic phosphate, in the absence of added ADP, inhibits the steady state level of the light-induced proton uptake by isolated chloroplasts. Fifty percent inhibition is obtained at a phosphate concentration of 2.0 mM, but precise determinations at higher phosphate concentrations are complicated by considerable buffering. The inhibition is dependent upon the presence of Mg^{++}, indicating that energy transfer may be involved. The results are consistent with the formation of a high energy, labile, phosphorylated intermediate prior to ATP in the energy transfer chain.

INTRODUCTION

Under conditions favoring electron transport, chloroplasts exhibit a rapid, light-dependent proton accumulation (1) coupled with a metal cation extrusion (2). According to the chemiosmotic hypothesis (3), the resulting hydrogen ion gradient represents the first intermediate in the energy conservation reactions leading to the formation of ATP (4). However, the exact molecular mechanism of the subsequent reaction steps has remained largely unresolved.

Evidence has accumulated for the existence of a second, phosphorylated intermediate (5,6). Such an intermediate has recently been tentatively detected in oxidative phosphorylation (7), and studies with pyrophosphate have indicated participation of a phosphorylated intermediate in bacterial photophosphorylation (6).

If we assume that the proton gradient represents the first, nonphosphorylated intermediate of photophosphorylation (see, for example, 4), or is equilibrium with one (8), then we would expect the steady state level of this intermediate to be lower in the presence of phosphorylating reagents (9). Such a decrease in the extent of the ion gradient due to ATP synthesis has previously been noted (9,10). We wish to report that inorganic phosphate, in the absence of added ADP, exhibits a similar inhibition of the steady state level of the proton gradient.

MATERIALS AND METHODS

Spinach chloroplasts were isolated by conventional methods (11) and suspended in a medium consisting of 0.2 M sucrose and 10 mM NaCl. Chlorophyll concentration was determined by the method of Arnon (12). Light-induced hydrogen ion translocation was measured by a method similar to that of Izawa (13) using a Radiometer TTT11 automatic titrator and micrometer buret, and a Sargent miniature combination pH electrode. Reactions were run in a water-jacketed test tube (12 x 100 mm) at 14°C. The actinic light (>560 nm) was supplied by a 1000 watt projector bulb and the appropriate filters. For details of the reaction mixture, see figures.

RESULTS

Figure 1 shows the effect of increasing phosphate concentration on the light-induced proton uptake by spinach chloroplasts and the concommitant pH rise of the reaction medium. The technique for titrating the illuminated chloroplast suspension back to the initial (dark) pH required at least a small change in the pH of the medium, making, precise quantitative titrations extremely difficult at higher phosphate concentrations (> 2mM),

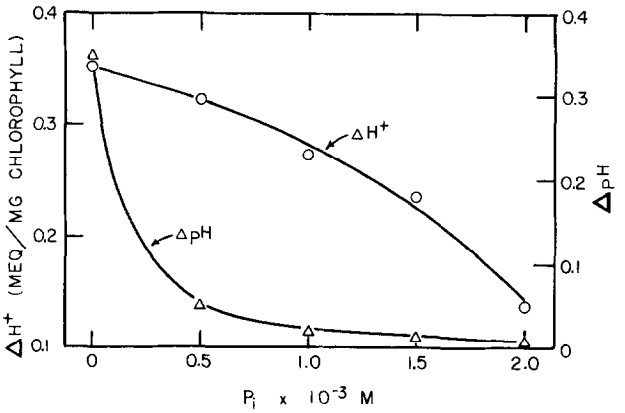

Figure 1. Effect of phosphate on the light-induced proton uptake (○) and pH rise (△) of an illuminated chloroplast suspension. The reaction mixture (3.0 ml.) consisted of 0.1 M sucrose, 1.0 mM $MgCl_2$, 0.05 M NaCl, 0.1 M phenazine methosulfate, Na_2HPO_4 as indicated, and chloroplasts equal to 150 μgrams of chlorophyll. The initial (dark) pH was adjusted to 6.3. After 40 seconds of illumination the chloroplast suspension was titrated back down to pH 6.3 in the light (17) with 0.001 M HCl. The intensity of the actinic light was 2.5×10^5 ergs/cm^2-sec at the surface of the reaction vessel. All reactions were carried out with constant stirring.

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where the buffering of the phosphate obscured the pH change. With 150 μgrams of chlorophyll, the steady state level of the ion gradient was inhibited approximately 50 percent by 2.0 mM phosphate.

The possibility that the observed inhibition is an instrumental artifact resulting from the buffering of the phosphate (pKa=6.8) was also investigated. Figure 2 shows the result of an experiment in which the zwitterionic buffer N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES, pKa=7.15) (14) was substituted for phosphate. The inhibition of the pH rise (due to buffering) is still observed, but no significant inhibition of proton accumulation was detected at concentrations which nearly completely buffered the pH rise of the medium.

The effect of 1.0 mM $MgCl_2$ on phosphate's inhibition of the

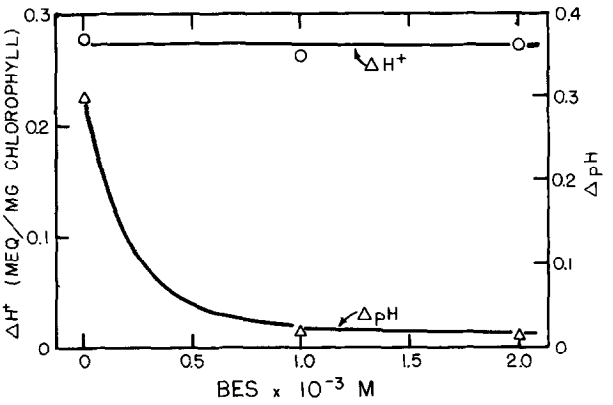

Figure 2. Effect of N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) (14) on the light-induced proton translocation (○) and pH rise (△) of an illuminated chloroplast suspension. Reaction conditions as in figure 1.

TABLE I

EFFECT OF Mg^{++} AND PHOSPHATE ON HYDROGEN ION TRANSLOCATION IN CHLOROPLASTS*

Treatment		Net Proton Uptake (micromoles/mg chlorophyll)	% Inhibition ($-Mg^{++}$)	% Inhibition ($+Mg^{++}$)
1.0 mM $MgCl_2$	1.5 mM Pi			
+	-	0.351	----	0
-	-	0.273	0	22.2
+	+	0.236	13.7	32.8
-	+	0.266	2.7	24.3

* Reaction conditions as in figure 1a, except for Mg^{++}, which was varied as indicated.

proton gradient is shown in Table I. In the presence of 1.5 mM phosphate, ommission of Mg^{++} ion effectively relieves phosphate's inhibition. Stimulation of ion translocation by Mg^{++} has been noted elsewhere (9).

Packer et al. (15) have found that somewhat higher concentrations of phosphate (0.1 M) in the absence of Mg^{++} can induce chloroplast shrinkage and thereby inhibit the overall extent of the proton gradient. Zwitterionic buffers (such as BES) were without effect. It is possible that Mg^{++} may screen the negative charges on the phosphate anion resulting in conformational changes in the grana at lower phosphate concentrations (16). However, this seems unlikely in view of our failure to detect any significant coordination interactions between Mg^{++} and inorganic phosphate at millimolar concentrations.

DISCUSSION

During photophosphorylation, the formation of ATP would require the utilization of a portion of the potential energy manifested in the steady state level of the proton gradient (or a high energy chemical intermediate in equilibrium with an ion gradient) according to the chemiosmotic hypothesis (3). Thus, if a phosphorylated intermediate prior to ATP is present, we should not be surprised to observe a similar inhibition of the steady state level of the proton gradient in the presence of only Mg^{++} and inorganic phosphate. The magnitude of the inhibition reported here rules out ATP formation from endogenous (bound) pools of ADP, yet the requirement of Mg^{++} ion suggests energy transfer is involved. The data presented are therefore consistent with a scheme of energy transfer involving a phosphorylation prior to that of ADP. It is suggested that phosphate's inhibition of the proton gradient in illuminated chloroplasts may represent the utilization of a portion of the potential energy of the electrochemical gradient for the synthesis of a highly labile phosphorylated intermediate prior to ATP in the energy transfer chain.

ACKNOWLEDGEMENTS

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