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RELATION OF PHOTOPHOSPHORYLATION TO HYDROGEN ION TRANSPORT

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The experiments of Jagendorf and coworkers have provided evidence that a pH gradient across chloroplast membranes can be the driving force for ATP synthesis. Illuminated chloroplasts catalyze a rapid hydrogen ion uptake which is sensitive to uncouplers of photophosphorylation (Neumann and Jagendorf, 1964). Under conditions of maximal H^+ transport, an intermediate or state is formed in the light which is capable of driving the synthesis of ATP in a dark reaction (Hind and Jagendorf, 1963). ATP synthesis in the dark was found more recently to accompany the formation of an artificial pH gradient across chloroplast membranes (Jagendorf and Uribe, 1966a). These results have been interpreted (Jagendorf and Uribe, 1966b) in the light of the chemiosmotic (Mitchell, 1961;1964) and chemical theories of oxidative phosphorylation. The former hypothesis assumes that hydrogen ion uptake is a direct consequence of electron transport and that the pH gradient in itself is a high energy state capable of driving ATP synthesis. In the chemical hypothesis, proton uptake is thought to be dependent upon the formation of a high energy intermediate and to be a side reaction rather than on the main pathway of ATP formation.

The data in this report show that NH_4Cl abolishes the light-induced pH rise in subchloroplast particles at concentrations which have little effect on ATP synthesis. These results suggest that the pH change observed on illumination is not the driving force for ATP synthesis in subchloroplast

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particles under continuous illumination.

Because these findings have been observed with subchloroplast particles only, their preparation is briefly described. Once washed spinach chloroplasts, prepared as described previously (McCarty and Racker, 1967), were taken up in the suspending medium (0.02M Tris (hydroxymethyl) methylglycine-NaOH, pH 8, 0.01M NaCl, 0.4M sucrose) to give a chlorophyll concentration of 2 to 2.5 mg. per ml. The suspension was exposed to sonic oscillation at 0 to 4° for a total of 45 sec with a Branson 17 KC instrument and the particles collected by centrifugation essentially as described by Vambutas and Racker (1965). The particles were washed with 15 to 20 ml of the suspending medium and taken up in a small volume of suspending medium to give a chlorophyll concentration of 3 to 5 mg. per ml. The capacity for photophosphorylation in the subchloroplast particles in the presence of pyocyanine usually ranged from 125 to 350 μmoles P_i esterified/hr/mg. chlorophyll and was stable for months at -90° under nitrogen.

Photophosphorylation catalyzed by subchloroplast particles in the presence of ferricyanide was extremely resistant to inhibition by NH_4Cl (Figure 1). In fact, low concentrations of NH_4Cl stimulated ferricyanide reduction and coupled phosphorylation to a similar extent. Even at 10 mM NH_4Cl, phosphorylation was inhibited only about 10%. In contrast, 2 mM NH_4Cl inhibited phosphorylation in chloroplasts by over 50% but had no effect on the rate of ferricyanide reduction. The effects of NH_4Cl on pyocyanine-dependent phosphorylation in chloroplasts and subchloroplast particles were quite similar to those obtained on phosphorylation with ferricyanide as the electron acceptor. Ethylamine at 10 mM also had little effect on phosphorylation in subchloroplast particles. However, 2 to 4 μM carbonyl cyanide m-chlorophenyl hydrazone inhibited phosphorylation by about 50% in subchloroplast particles and in chloroplasts.

Although NH_4Cl had little effect on photophosphorylation in subchloroplast particles, it affected the light-induced pH rise in a manner similar to that

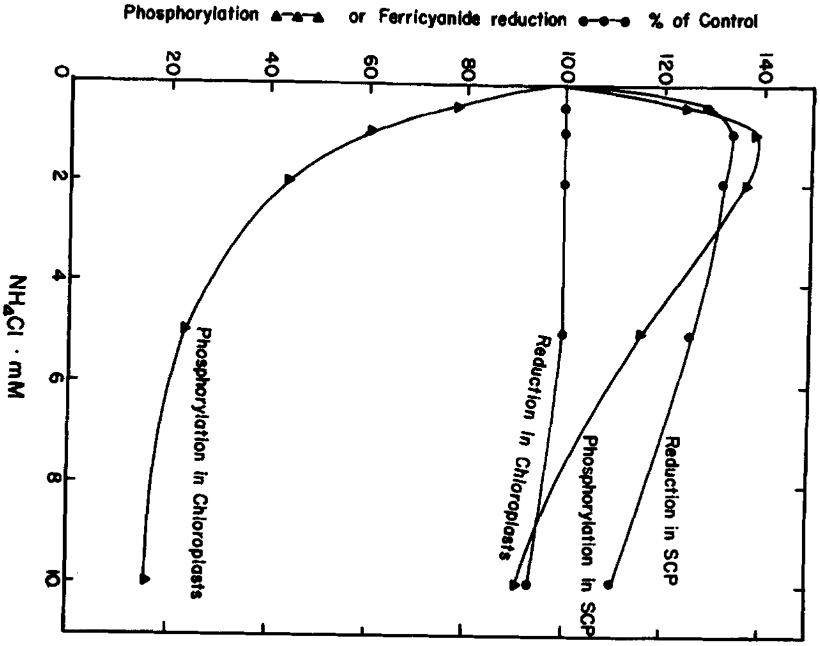

Figure 1. Effects of NH$_4$Cl on Ferricyanide Reduction and Coupled Phosphorylation. The reaction mixture contained, in a volume of 1.0 ml, 50 mM Tris (hydroxymethyl) methyl glycine-NaOH, pH 8, 50 mM NaCl, 5 mM MgCl$_2$, 1 mM K$_3$Fe(CN)$_6$, 3 mM ADP, 2 mM potassium phosphate buffer, pH 8.0, containing 5 X 10^5 cpm ^{32}P$_i$, 1 mg defatted bovine serum albumin and chloroplasts or subchloroplast particles equivalent to 50 μg of chlorophyll. The gas phase was nitrogen. Ferricyanide reduction was assayed by a direct spectrophotometric method (Jagendorf and Smith, 1962) and ^{32}P$_i$ esterification, as described previously (McCarty and Racker, 1967). [1]The control rates, expressed as μmoles per hour per mg. chlorophyll, were (a) chloroplasts, ferricyanide reduction 185, phosphorylation 84 (b) subchloroplast particles, ferricyanide reduction 202, phosphorylation 51. "SCP" stands for subchloroplast particles.

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observed in chloroplasts (Table I). In both chloroplasts and subchloroplast particles, NH$_4$Cl markedly increased the apparent first order rate constants for both the pH rise and its decay in the dark. Total H$^+$ uptake in the light was lower in the subchloroplast particles than in the chloroplasts and was more sensitive to NH$_4$Cl.

TABLE I

EFFECTS OF NH_4Cl ON THE LIGHT-INDUCED pH RISE IN CHLOROPLASTS AND SUBCHLOROPLAST PARTICLES

The reaction mixture contained in a volume of 1.0 ml. 50 mM NaCl, 0.05 mM pyocyanine and chloroplasts or subchloroplast particles equivalent to 60 to 100 μg of chlorophyll. The pH of the mixture was adjusted to 6.05 ± 0.02 prior to illumination. The temperature was 2° and the light intensity 1.9 X 10^6 ergs/cm^2/sec. Apparent first order rate constants (K_1) for the pH rise and its decay were calculated as previously described (McCarty and Racker, 1967).

	Chloroplasts			Subchloroplast particles		
NH_4Cl (mM)	K_1 rise (sec^{-1})	K_1 decay (sec^{-1})	H^+ accumulated (μeq/mg chloro)	K_1 rise (sec^{-1})	K_1 decay (sec^{-1})	H^+ accumula (μeq/mg chlo
0	0.091	-0.033	0.73	0.089	-0.061	0.30
1	0.138	-0.086	0.79	0.166	-0.129	0.20
2	0.270	-0.157	0.78	0.182	-0.168	0.16
5	0.323	-0.254	0.62	0.186	-0.199	0.12
10	0.318	-0.330	0.46	0.198	-0.23	0.08

Since NH_3 rather than NH_4^+ appears to be the uncoupling species (Hind and Whittingham, 1963), the inhibition of the pH rise at pH 7 should be greater than at pH 6. As predicted, 2 mM NH_4Cl virtually abolished the pH rise in subchloroplast particles at pH 7.2 whereas only a 50% inhibition of H^+ uptake was observed at pH 6.

To confirm that the pH rise in subchloroplast particles was more sensitive to NH_4Cl than phosphorylation, the pH rise was assayed under phosphorylating conditions (Figure 2). As expected, illumination of chloroplasts or subchloroplast particles at pH 7.2 in the absence of ADP and/or P_i caused a pH increase which reversed in the dark. NH_4Cl at 5 mM abolished the pH rise under these conditions (experiments A and C). Under conditions of phosphorylation, in the absence of NH_4Cl, light caused a rapid pH increase which was linear after a few seconds and which was only partially reversible in the dark (experiments B and D). The linear pH increase is probably

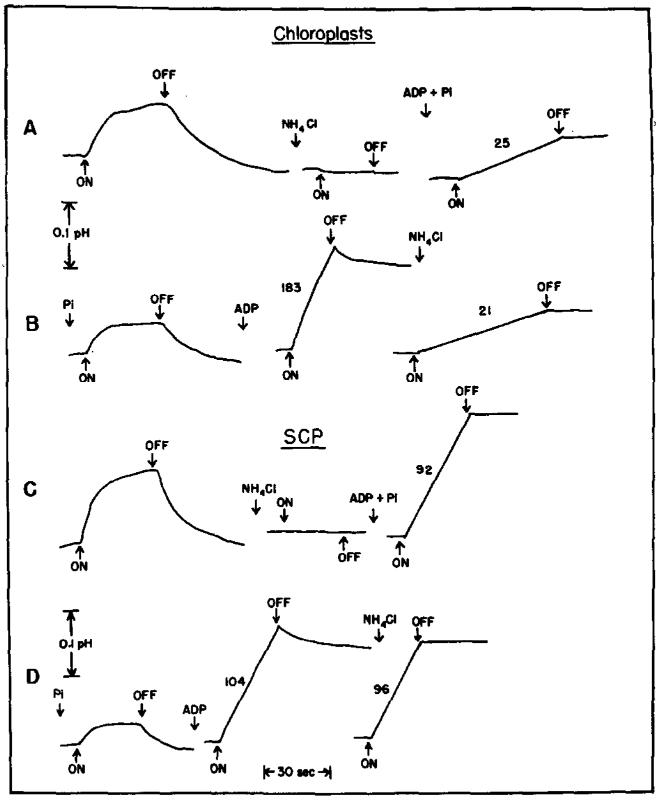

Figure 2. Effects of NH_4Cl on the pH Rise Under Conditions of Phosphorylation. The stirred reaction mixture contained, in 1.0 ml, 50 mM NaCl, 5 mM $MgCl_2$, 0.05 mM pyocyanine and chloroplasts or subchloroplast particles equivalent to 65 to 100 µg of chlorophyll. P_i and ADP were added to a final concentration of 0.5 mM and NH_4Cl was 5 mM. The pH was adjusted to 7.2 ± 0.05 prior to illumination. The temperature was 5° and the light intensity about 2 X 10^6 ergs/cm^2/sec. The numbers shown in the figure are phosphorylation rates calculated from the linear portion of the curves assuming 1 µeq of H^+ was consumed per µeq ATP formed. "SCP" stands for subchloroplast particles. "On" and "Off" refer to light on and light off.

caused by H^+ uptake during phosphorylation (Nishimura, et.al., 1962), whereas the small pH decrease, which occurred on cessation of illumination, is most likely the decay of the light-induced H^+ uptake (Karlish and Avron, 1967). Phosphorylation in chloroplasts, as calculated from the linear pH change, was inhibited over 80% by 5 mM NH_4Cl, whereas 5 mM NH_4Cl inhibited phosphorylation in subchloroplast particles by only about 10%. However, 5 mM NH_4Cl did appear to abolish H^+ uptake in chloroplasts as well as subchloroplast particles under phosphorylating conditions since no decrease in the pH was detected on

TABLE II

EFFECT OF NH_4Cl ON POST-ILLUMINATION AND ACID-BASE TRANSITION ATP SYNTHESIS IN SUBCHLOROPLAST PARTICLES

For postillumination ATP synthesis, the light reaction mixture contained in 0.5 ml, 10 mM morpholinoethane sulfonate-NaOH, pH 6.0, 0.05 mM pyocyanine, 50 mM NaCl and subchloroplast particles equivalent to 0.1 mg of chlorophyll. The mixture was illuminated (2 X 10^6 ergs/cm^2/sec) at 4° in a 0.5 ml glass syringe. After 60 sec, the mixture was rapidly injected into a mixture in the dark which contained in 0.5 ml, 100 mM Tris (hydroxymethyl) methyl glycine-NaOH, pH 8, 10 mM $MgCl_2$, 6 mM ADP and 2 mM potassium phosphate buffer, pH 8 containing 2 X 10^6 cpm $^{32}P_i$. After 15 sec, 0.05 ml of 40% trichloroacetic acid was added to terminate the reaction.

Acid-base induced phosphorylation was assayed essentially as described previously (McCarty and Racker, 1966) except that Tris (hydroxymethyl) methyl glycine buffer was used in place of Tris and the temperature was 2°. The NH_4Cl was added prior to illumination or the pH transition.

	$^{32}P_i$ esterified (mμmoles/mg. chloro)	
	Post-illumination	Acid-Base Induced
Control	24.5	40.1
+ 2 mM NH_4Cl	5.3	5.4

turning out the light in the presence of this uncoupler. Results similar to those obtained with NH_4Cl were obtained with 10 mM ethylamine-HCl.

ATP synthesis in chloroplasts takes place in the dark after a prior illumination (Hind and Jagendorf, 1963) or after a rapid pH transition (Jagendorf and Uribe, 1966). If phosphorylation under these conditions in subchloroplast particles depends upon the formation of a pH gradient, it should be inhibited by NH_4Cl. ATP synthesis in subchloroplast particles caused either by a prior illumination or by a pH transition was strongly inhibited by 2 mM NH_4Cl, a concentration which actually stimulated phosphorylation in continuous light (Table 2).

These results strongly indicate that light-induced H^+ movements, which are measured by pH changes in the suspending medium, are not directly involved in ATP formation in subchloroplast particles in the presence of ammonia. However, the pH gradient formed by proton uptake in the light can be utilized under some conditions to drive the synthesis of ATP. This conclusion is supported by the observation that nigericin inhibited the light-induced pH rise in R. rubrum chromatophores at concentrations which little affected phosphorylation (Shavit, et.al., 1968). The causes of the resistance of phosphorylation in subchloroplast particles to NH_4Cl are not known and are currently under study.

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