UPTAKE OF AMMONIUM ION BY CHLOROPLASTS, AND ITS RELATION TO PHOTOPHOSPHORYLATION *

Antony R. Crofts
Department of Physiology, University of California, Berkeley, California, U.S.A.

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Uncoupling of photophosphorylation in isolated chloroplasts by ammonium (Krogmann, Jagendorf and Avron, 1959) and monosubstituted amines (Good, 1960) has been well characterized. Recently, a light dependent uptake of ammonium ions by spinach chloroplasts accompanying uncoupling of electron flow has been described, and an ionic mechanism of amine uncoupling has been proposed (Crofts, 1966).

The relations between ammonium uptake, photophosphorylation and light-activated ATPase (Petrack and Lipmann, 1961) have now been investigated. It has been found that light-induced ammonium uptake is partially inhibited under phosphorylating conditions in a manner which suggests that the two processes are competing for a common energy supply. Ammonium uptake can also be driven in the dark, under conditions of ATP hydrolysis, and ammonium uptake is associated with a stimulation of ATPase activity.

RESULTS

Ammonium uptake and photophosphorylation.

Competition between photophosphorylation and NH_4-uptake by chloroplasts (for a common energy supply) is apparent from the experiment in Figure. 1. The traces show simultaneous recordings of photophosphorylation, as measured by loss of H^+ from the medium (Nishimura, Ito and Chance, 1962), and changes in cation concentration, in a choline chloride medium containing chloroplasts, N-methyl

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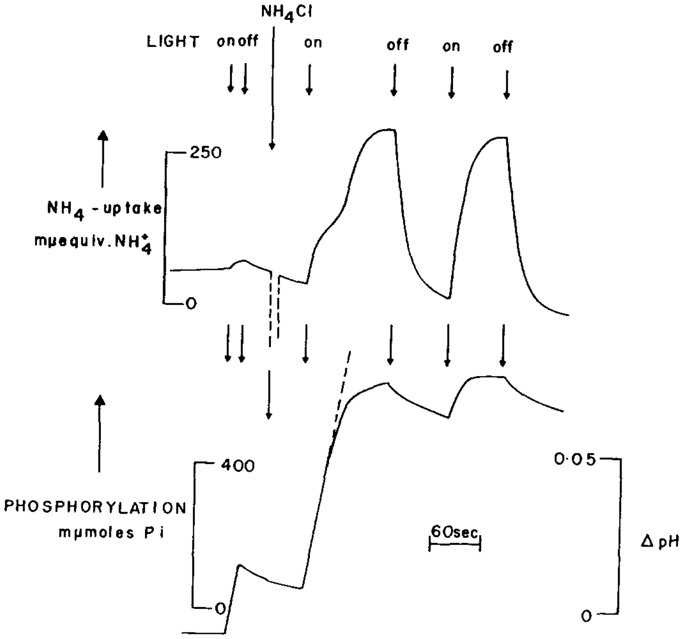

Figure 1. Competition between photophosphorylation and NH_4-uptake by chloroplasts.

Chloroplasts (28 μg chlorophyll/ml) were suspended in 5 ml of a medium containing 100 mM choline chloride, 2.5 mM $MgCl_2$, 2.5 mM phosphate (as Tris-salt), 1 mM NaCl, 20 μM PMS and 0.77 μmoles ADP (as Na-salt), at pH 7.15, 25°. NH_4Cl was added where indicated to a concentration of 0.1 mM. Measurement of cation and H^+ concentrations, and preparation of spinach chloroplasts in choline chloride were as described previously (Crofts et al., 1966). The cation electrode used (GKN33, Electronic Instruments Limited) was 3.35 times as sensitive to K^+ as to NH_4^+. Illumination was with broad-band red light equivalent to 1,000 foot-candles. The upper trace shows cation electrode response, the scale is for change in NH_4^+ concentration. The lower trace shows the pH change associated with phosphorylation, the scale is calculated from a known addition of H^+, using the data of Nishimura et al. (1962) for phosphorylation of Mg-ADP. ADP added was assayed enzymically.

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phenazonium methosulfate (PMS), Mg^{++}, phosphate and a small amount of ADP. On illumination, a rapid rate of phosphorylation was observed, accompanied by a small uptake of cation (Crofts, Deamer and Packer, 1966). On addition of a low concentration of NH_4Cl and subsequent illumination, a slightly slower rate of phosphorylation was observed, together with a rapid rate of NH_4-uptake which declined as the external concentration of NH_4^+ fell. However, as the rate of phosphorylation fell on exhaustion of ADP, a concomitant increase in the rate and extent of NH_4-uptake was observed. This reached a maximum after phosphorylation had ceased, indicating that additional energy had become available for NH_4-uptake. NH_4^+ was released from the chloroplasts in the dark, and taken up again during a subsequent illumination cycle. Since ADP had been exhausted in the first illumination cycle, no inhibitory phase was observed during the second cycle of NH_4-uptake, but the extent of uptake was the same as that after phosphorylation had ceased in the first cycle. In other experiments in which a higher initial concentration of ADP was added to ensure continued phosphorylation, the biphasic kinetics of NH_4-uptake were more pronounced, and the second phase was always delayed until exhaustion of the added ADP.

Phlorizin at a concentration of 1.5 mM, which completely inhibited phosphorylation (Izawa, Winget and Good, 1966), did not inhibit the initial

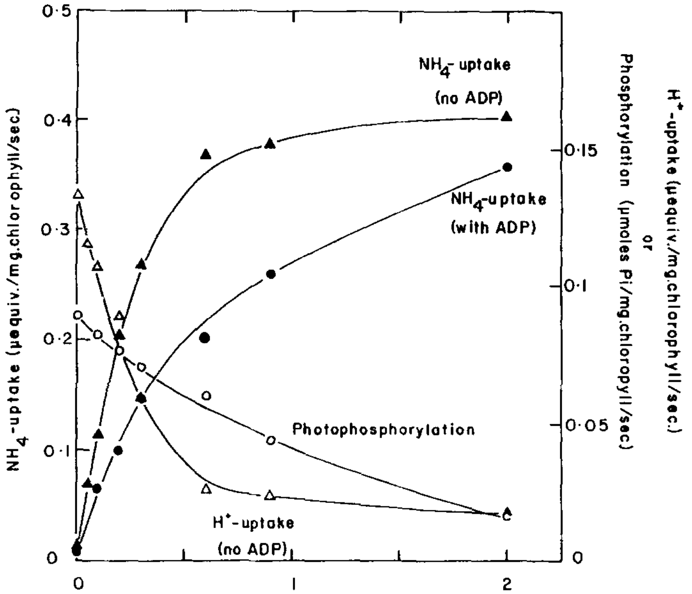

Figure 2. Competitive inhibition of ammonium uptake by photophosphorylation.

Conditions as in Figure 1, except that ADP was ommitted where indicated. Rates of phosphorylation were calculated from the pH change. Rates of H^+-uptake in the absence of ADP were recorded at ten times sensitivity. Kinetic data from double reciprocal plots are as follows.

NH_4-uptake in absence of ADP: Km = 0.44 mM; Vmax = 0.54 μeq/mg chlorophyll/sec.
NH_4-uptake in presence of ADP: Km = 1.0 mM; Vmax = 0.54 μeq/mg chlorophyll/sec.

rate of NH_4- or H^+-uptake, though a partial inhibition of the extent of NH_4-uptake was observed. In the absence of phosphate, ADP had no inhibitory effect on NH_4-uptake.

In experiments similar to those described above, the initial rates of NH_4-uptake in the presence and absence of ADP were measured as shown in Figure 2. Here the rates of NH_4-uptake, H^+-uptake in the absence of ADP, and photophosphorylation, are plotted as a function of NH_4Cl concentration. Both the graph and the Km and Vmax values given show that the inhibition of NH_4-uptake in the presence of ADP is more nearly competitive than non-competitive. It is also of interest that the maximal rate of NH_4-uptake is five times greater than that of photophosphorylation, while the rate of H^+-uptake in the absence of NH_4Cl is 1.5 times that of phosphorylation. The results reported above indicate that NH_4-uptake and phosphorylation compete for a common energy supply at a level closer to electron flow than the terminal phosphorylation reactions.

ATP-induced ammonium uptake by chloroplasts.

A light-activated, thiol requiring ATPase of chloroplasts (Petrack and Lipmann, 1961) has been characterized by a number of workers (Marchant and Packer, 1963; Hoch and Martin, 1963; Petrack, Craston, Sheppy and Farron, 1965; Bennun and Avron, 1965). It has been recently shown that the antibiotic Dio-9, and a partially purified antibody preparation for the isolated ATPase of chloroplasts (McCarty and Racker, 1966), and also phlorizin (Izawa et al., 1966) are able to inhibit both photophosphorylation and the light-activated ATPase, suggesting that these reactions have a common active site at the level of the phosphorylation reactions. Hoch and Martin (1963) have reported that NH_4Cl added during the dark phase stimulated the rate of ATPase, and Packer and Marchant (1964) have shown that ATP is able to support an increased light scattering of chloroplasts under conditions of ATP hydrolysis, and that NH_4Cl reverses this effect. It seemed possible from these results that the energy of ATP hydrolysis might be available to drive NH_4-uptake.

The measurement of NH_4-uptake accompanying ATP hydrolysis in the dark is complicated by the preincubation requirements for activation of the ATPase reaction. The presence of NH_4Cl inhibits activation (Hoch and Martin, 1963) so that the amine must be added after preillumination, and although ATP may be added after activation (Marchant and Packer, 1963) its addition gives rise to a chelation of Mg^{++} from the medium which results in an electrode artifact. For these reasons it has not been possible to measure initial rates of NH_4-uptake accurately. However, addition of Triton X-100 in lytic quantities after NH_4-uptake had reached a maximum, led to the release of accumulated cation, thus affording a means for estimating the extent of NH_4-uptake under conditions of ATP hydrolysis. The results of such experiments (Figure 3) show that the extent of NH_4-uptake increased with higher NH_4Cl concentrations, and that the uptake was associated with a stimulation of ATP hydrolysis.

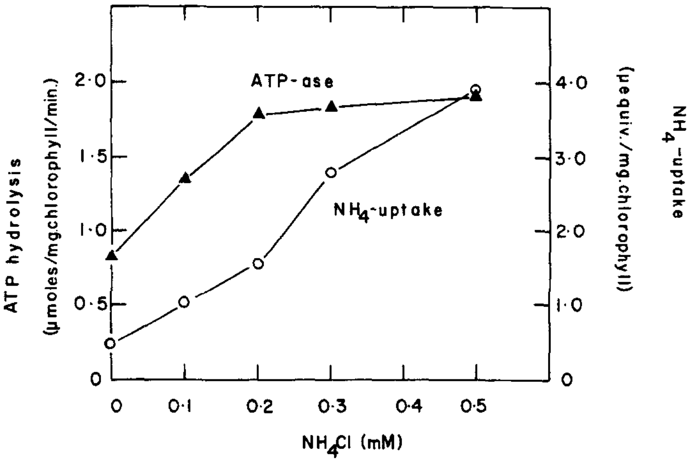

Figure 3. Ammonium uptake in the dark under conditions of ATP hydrolysis (light-activated).

Chloroplasts (56.7 μg chlorophyll/ml) were suspended in 5 ml of a medium containing 100 mM choline chloride, 0.5 mM KCl, 3.1 mM ATP (as Tris-salt), 2.5 mM $MgCl_2$, 3.7 mM dithioerythritol, 20 μM PMS and 10 mM Tris at pH 8.1, 25°. The chloroplasts were illuminated for 6 minutes with broad band red light at approximately 1,000 foot-candles. NH_4Cl at the indicated concentrations was added 30 seconds after switching off the light. The rate of ATP hydrolysis was calculated from the steady state rate of H^+ production. NH_4-uptake reached a maximum after 2-3 minutes, then Triton X-100 was added to a concentration of 0.01%, and the extent of NH_4^+ released estimated by comparison with a known addition of NH_4Cl. The small cation uptake shown by the point at zero [NH_4Cl] probably represents binding of K^+ and Mg^{++}; the point given is the equivalent electrode response for NH_4^+.

DISCUSSION

The observation that ATP hydrolysis by chloroplasts in the dark is able to support NH_4-uptake, and that the uptake is associated with an increased ATPase activity, shows that the ATPase is able to interact with the energy conserving system of chloroplasts at a level closer to electron flow than the phosphorylation reactions.

The competitive inhibition of NH_4-uptake observed under phosphorylating conditions, and the fact that ATP hydrolysis is able to drive NH_4-uptake strongly suggest that NH_4-uptake and the reactions of phosphorylation are interacting with a common high energy state. It has been previously proposed (Crofts, 1966) that NH_4-uptake reflects a displacement of the equilibrium of NH_3 across the chloroplast membrane in response to the acidification of the chloroplast interior following H^+-uptake. The demonstration of competitive inhibition of NH_4-uptake by photophosphorylation suggests that the availability of H^+ within the chloroplast is reduced under phosphorylating conditions. Evidence that H^+-uptake is a primary energetic process has been advanced by a number of workers (Neumann and Jagendorf, 1964; Hind and Jagendorf, 1965; Jagendorf and Uribe, 1966; Deamer, Crofts and Packer, 1966; Crofts et al., 1966) and Mitchell (1961, 1966) has proposed a mechanism whereby ATP synthesis could be coupled to a trans-membrane potential of H^+-activity. The observations reported above suggest that H^+-uptake is equivalent to the energetic state leading to ATP synthesis, and it is of interest that the maximal rate of H^+-uptake as indicated by the Vmax for NH_4-uptake is more than adequate to provide the $2H^+$/ATP suggested as being necessary by Mitchell (1966). However, either a mechanism in which H^+-uptake is directly coupled to electron-flow or ATP hydrolysis, or one in which both H^+-uptake and photophosphorylation are driven by a common chemical intermediate, would be compatible with the experiments reported above.

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REFERENCES

Bennun, A. and Avron, M., Biochim. Biophys. Acta 109, 117 (1965).
Crofts, A. R., Biochem. Biophys. Res. Commun. 24, 127 (1966).
Crofts, A. R., Deamer, D. W. and Packer, L., Biochim. Biophys. Acta, submitted (1966).
Deamer, D. W., Crofts, A. R. and Packer, L., Biochim. Biophys. Acta, submitted (1966).
Good, N. E., Biochim. Biophys. Acta, 40, 502 (1960).
Hind, G. and Jagendorf, A. T., J. Biol. Chem. 240, 3202 (1965).
Hoch, G. and Martin, I., Biochem. Biophys. Res. Commun. 12, 223 (1963).
Izawa, S., Winget, G. and Good, N. E., J. Biol. Chem., submitted (1966).
Jagendorf, A. T. and Uribe, E., Proc. Nat. Acad. Sci. U.S. 55, 170 (1966).
Krogmann, D. W., Jagendorf, A. T. and Avron, M., Plant Physiol. 34, 272 (1959).
Marchant, R. H. and Packer, L., Biochim. Biophys. Acta 75, 458 (1963).
McCarty, R. E. and Racker, E., in Energy Conversion in the Photosynthetic Apparatus, Brookhaven Symposium, June 1966.
Mitchell, P., Nature, Lond. 191, 144 (1961).
Mitchell, P., Publication No. 66/1 of Glynn Research Ltd. (1966).
Neumann, J. and Jagendorf, A. T., Arch. Biochem. Biophys. 107, 109 (1964).
Nishimura, M., Ito, T. and Chance, B., Biochim. Biophys. Acta 59, 177 (1962).
Packer, L. and Marchant, R. H., J. Biol. Chem. 239, 2061 (1964).
Petrack, B., Craston, A., Sheppy, F. and Farron, F., J. Biol. Chem. 240, 906 (1965).
Petrack, B. and Lipmann, F., in Light and Life, Ed. by W. D. McElroy and H. B. Glass, John Hopkins Press, Baltimore, 1961, p. 621.