Phlorizin, a Specific Inhibitor of Photophosphorylation and Phosphorylation-Coupled Electron Transport in Chloroplasts[1]

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We wish to report that phlorizin inhibits the phosphorylation reaction in chloroplasts. It also inhibits the enhancement of electron transport by phosphate and ADP. Neither the non-phosphorylating electron transport which occurs in the absence of phosphate nor the more rapid electron transport which occurs in the presence of uncouplers is affected. Indeed, the addition of an inhibitory concentration of phlorizin has the same effect as the omission of phosphate. Consequently it must be concluded that phlorizin acts by blocking a phosphorylation reaction, not by dissociating electron transport from phosphorylation as do the many known uncouplers. Substances which inhibit mitochondrial ATP synthesis in a similar manner have been called "energy transfer inhibitors" (e.g., oligomycin). Heretofore there have been no reports of this type of inhibition of chloroplast photophosphorylation.[2]

Figure 1a illustrates the effects of phlorizin on rates of ferricyanide reduction and photophosphorylation in spinach chloroplasts. ATP synthesis and that part of the electron transport stimulated by the phosphorylation conditions are inhibited. (Enhancement of electron transport by arsenate

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2 During the preparation of this manuscript, the authors were informed that Dio-9, an antibiotic and a known inhibitor of oxidative phosphorylation, also acts as an "energy transfer inhibitor" in chloroplast photophosphorylation reactions (R. E. McCarty, personal communication).

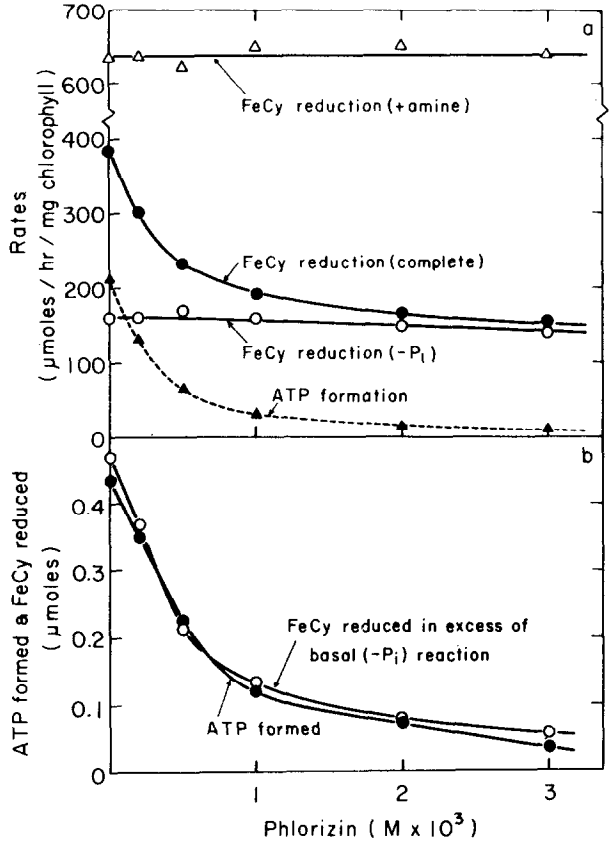

Fig. 1a. Effect of phlorizin on rates of ferricyanide reduction and rates of ATP formation in illuminated spinach chloroplasts. The reaction mixture (2.0 ml) contained in μmoles: sucrose 300, Tricine buffer (pH 8.4) 100, potassium ferricyanide 0.8, $MgCl_2$ 2, ADP 1, $Na_2HP^*O_4$ 30, methylamine-HCl 20 (if added). Chloroplasts (40 μg chlorophyll). Ferricyanide reduction was measured by the method of Izawa and Good (1965). ATP formation was measured by the method of Avron (1960). Saturating orange light (> 560 mμ) was used. Temperature was 19°. For method of preparation of chloroplasts, see Winget et al. (1965).

Fig. 1b. Relation of the amount of ATP formed to the extra amount of ferricyanide reduced as a consequence of the phosphorylation conditions (i.e., the amount of ferricyanide reduced in the presence of P_i minus the amount reduced in the same length of time in the absence of P_i). Computed from the data of Fig. 1a. Note that the P/2e ratio thus calculated is essentially constant and close to 2.0 at all levels of inhibition of phosphorylation.

and ADP is likewise inhibited.) The basal ($-P_i$) electron transport is insensitive to phlorizin, as is the amine-uncoupled electron transport. We have also found that chloroplasts uncoupled by atebrin or EDTA-treatment are insensitive.

Similar concentrations of phlorizin inhibit non-cyclic and PMS-catalyzed cyclic photophosphorylation to the same extent.

Phloretin, the aglucone of phlorizin, inhibits electron transport whether it is uncoupled or not.

There are several reasons for thinking that phlorizin may inhibit reactions close to the final phosphorylation of ADP. Phlorizin is known to inhibit the phosphorolysis of α-1,4-glucosides by muscle phosphorylase (Cori et al., 1943) and, by analogy, might be suspected of inhibiting a reaction involving phosphate. A much more significant consideration is the release of phlorizin inhibition by a variety of uncouplers including EDTA. EDTA-uncoupling involves the removal of a "coupling factor" (Avron, 1963) which is a latent ATPase (Vambutas and Racker, 1965). Since the site of phlorizin action cannot precede the site of EDTA action in the energy transfer chain, it seems probable that both EDTA-uncoupling and phlorizin inhibition occur at the level of ATP synthesis.

Figure 1b shows that the number of moles of ATP formed agrees almost exactly with the number of moles of ferricyanide reduced in excess of the amount which would have been reduced by the basal, non-phosphorylating electron transport. This is true regardless of the extent of the inhibition. The implication is that the non-phosphorylating process continues unchanged during phosphorylation; under the conditions of our experiment the phosphorylating electron transport seems to be superimposed on the basal electron transport. If so, the P/2e ratio of the coupled part of the electron transport is 2.0. This is in accord with our earlier observation that the overall P/2e ratio (uncorrected for basal electron transport) is well over 1.2 (Winget et al., 1965).

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