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The Function of Plastoquinone in Photosynthetic Phosphorylation

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The effect of plastoquinone on the cyclic photophosphorylations catalyzed by phenazine methosulfate, vitamin K_3 and flavin mononucleotide was examined. The extraction of plastoquinone from lyophilized chloroplasts by heptane treatment resulted in an almost complete loss of phosphorylating ability; the readdition of plastoquinone restored cyclic photophosphorylation in all three systems. The significance of this plastoquinone requirement for the mechanism of photosynthetic phosphorylation is discussed.

The preceding article[1] discussed the role of plastoquinone in the noncyclic electron transport of photosynthesis. Krogmann[2] has shown that the extraction of plastoquinone impairs a type of light-induced ATP formation in chloroplasts (cyclic photophosphorylation) that is catalyzed by phenazine methosulfate (PMS). The present communication extends the investigation of the effect of plastoquinone to cyclic photophosphorylation of the types catalyzed by vitamin K_3 (menadione) and flavin mononucleotide (FMN).

There is evidence that details of the electron flow pathway involved in cyclic photophosphorylation catalyzed by vitamin K_3 and FMN differ not only from that of noncyclic electron flow[3] but also from the electron flow pathway in cyclic photophosphorylation catalyzed by PMS. This investigation (a preliminary report of which has already been made[4]) has shown that plastoquinone is needed for the cyclic photophosphorylation catalyzed by vitamin K_3 and FMN, as well as that catalyzed by PMS.

METHODS

The chloroplast fragments were prepared, and plastoquinone was extracted and added back by the methods described in the preceding paper[1]. ATP formation was measured with ^{32}P as described previously[5,6]. The plastoquinone used in these experiments was a gift from Dr. O. Isler.

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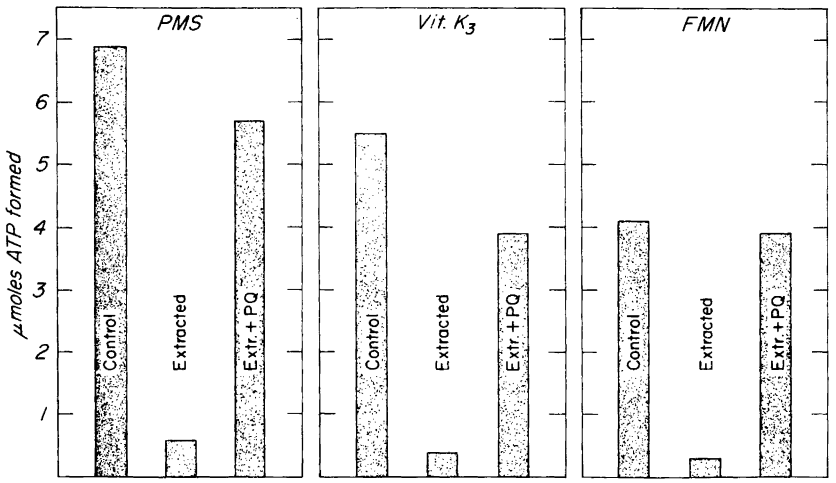

Fig. 1. Requirement of plastoquinone for cyclic photophosphorylation. The "control" vessel received lyophilized chloroplasts, the "extracted" vessel received lyophilized chloroplasts extracted with heptane, and the "extracted + PQ" vessel received extracted lyophilized chloroplasts to which plastoquinone (PQ) had been added (.05 mg/.5 mg chlorophyll). The reaction mixture contained, in a total volume of 3 ml., the following in micromoles: tris (hydroxymethyl) aminomethane buffer, pH 7.8, 80; $MgCl_2$, 10; ADP, 10; $K_2H^{32}PO_4$, 10; and one of the following cofactor combinations, as indicated (1) *PMS,* phenazine methosulfate, 0.1 (2) *Vit. K*$_3$, menadione, 0.3; and ascorbate, 10; (3) *FMN,* flavin mononucleotide, 0.3; and ascorbate, 10. Each vessel received chloroplasts containing 0.5 mg. chlorophyll. The experiment was carried out in Warburg manometer vessels at 15°C in a nitrogen atmosphere. Illumination at 25,000 lux was for 10 min. in the *PMS* series, 20 min. in the *Vit. K*$_3$ series and 30 min. in the *FMN* series. ATP formation was measured as described earlier[4,5].

RESULTS AND DISCUSSION

As shown in Fig. 1, all three types of cyclic photophosphorylation responded similarly to the experimental treatment. The thorough extraction of plastoquinone from the lyophilized chloroplast fragments by heptane resulted in an almost complete loss of phosphorylating ability, and the readdition of pure plastoquinone (by evaporation of isooctane from a solution of plastoquinone in isooctane) restored the phosphorylating ability in each case. Similar results were also recently reported by Krogmann and Olivero[7]. In individual experiments the degree of restoration by adding back plastoquinone was variable; sometimes the "restored" rate was low compared with the unextracted chloroplasts (only 50 %), but in several experiments the "restored" rate was greater (up to 150 %) than that of the unextracted chloroplasts. In any one experiment all three systems behaved similarly. The data shown in Fig. 1 demonstrate a requirement for plastoquinone in all three types of cyclic photophosphorylation, but these data do not by themselves distinguish between a requirement for plastoquinone (a) for the photophosphorylation steps proper and (b) for the cyclic electron transport with which the photophosphorylation steps are coupled. However, in view of the evidence for the participation of plastoquinone in the noncyclic electron flow, as discussed

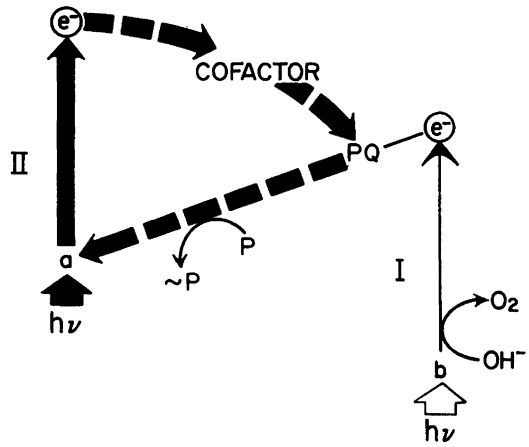

Fig. 2. A scheme for electron flow in cyclic photophosphorylation. Chlorophyll *a* (bound to a protein in the chloroplast) becomes excited by the absorption of a photon. The excited chlorophyll donates an electron, via the cofactor, to plastoquinone. The cycle is completed when the plastoquinone donates an electron (via cytochrome) to chlorophyll *a*. The phosphorylation step is shown to be linked with the transfer of the electron between plastoquinone and chlorophyll *a*.

in the preceding paper[1], the simplest interpretation is to assign plastoquinone to a site in the photosynthetic electron transport chain that is common to both cyclic and noncyclic electron flow (Fig. 2).

This interpretation, although stressing the similarities in the different photosynthetic electron transport pathways, is not in conflict with some important differences which have been observed among the three cases of cyclic photophosphorylation discussed here. These differences in cyclic photophosphorylation were first observed in their strikingly different responses to temperature[8] and light intensity[3]. Hall and Arnon[8], using a low light intensity (which at 15°C was rate-limiting), showed that the PMS-catalyzed system was not temperature-sensitive down to −5°C, whereas the FMN- and vitamin K_3-catalyzed systems were markedly temperature sensitive. In addition, as shown in Table 1, at 9 000 lux (white light) the amount of ATP formed was much less with PMS than with

Table 1. Effect of light intensity on cyclic photophosphorylation with various cofactors. (Tsujimoto, Hall and Arnon[3])

Light intensity lux	Cofactor system PMS	Vit. K_3	FMN
	micromoles ATP formed		
9 000	1.3	3.5	3.9
25 000	4.5	4.6	4.2
50 000	9.8	5.0	5.1

vitamin K_3 or FMN, at 25 000 lux there was no significant difference between the three systems, but at 50 000 lux the vitamin K_3- and FMN-catalyzed systems gave no additional phosphorylation (light saturation), while in the PMS-catalyzed system ATP formation continued to increase in proportion to the light intensity.

These differences have been explained [3,8] on the premise that the vitamin K_3- and FMN-catalyzed systems differ from the PMS system in having an additional phosphorylation site which, however, becomes rate-limiting at high light intensity. Since we have concluded that electron flow in all three systems proceeds through plastoquinone it would appear that the additional phosphorylating site envisaged for the vit. K_3- and FMN-catalyzed systems must be located in that segment of the cyclic electron pathway which is shown (by the heavy line) between "e⁻" and "PQ" in Fig. 2.

Although plastoquinone seems to be more probably a component of the electron transport chain rather than of the phosphorylation reaction proper, this conclusion must still remain tentative[4] because of the experimental difficulties, encountered also by other investigators[7,9], in extracting and restoring a fat-soluble constituent to chloroplasts, and in maintaining a high level of phosphorylating activity. It is still possible, as was suggested earlier[4], that plastoquinone may play a dual role in photosynthesis, both as an electron carrier and as a catalyst of the phosphorylation reaction proper.

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