A REQUIREMENT FOR PLASTOQUINONE IN PHOTOSYNTHETIC PHOSPHORYLATION*

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Bishop (1959) has presented evidence to show that a lipid soluble benzoquinone is essential for the Hill reaction activity of isolated chloroplasts. The structure of this compound has been defined as 2, 3-dimethyl-5-solanosyl benzoquinone by Trenner et al., (1959). Crane has called this compound plastoquinone (PQ) since he has found it localized in the chloroplasts (1959).

Although photophosphorylation is under many circumstances contingent on the occurence of a Hill reaction, the light dependent ATP synthesis elicited by phenazine methosulfate (PMS) has been shown to be independent of the Hill reaction (Jagendorf and Avron, 1959 and Krall et al., 1960). Hence the participation of PQ in PMS catalyzed photophosphorylation is a matter of some importance. Experiments are reported here which establish PQ as an essential participant in PMS supported photophosphorylation.

In all the experiments, lyophilized chloroplasts were used. The chloroplasts were prepared, lyophilized and resuspended in 0.4 M sucrose 0.01 M NaCl. Lyophilization of the chloroplasts entailed a loss of from thirty to fifty per cent of the activities measured by comparison with fresh chloroplasts. PQ was extracted by dispersing the lyphilized chloroplasts

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in spectral grade heptane with a Potter Elvejhem homogenizer, then stirring at room temperature for one hour. The chloroplasts were then recovered by filtration and portions of the extracted chloroplasts were reactivated by suspension in iso-octane containing PQ. The solvent was then removed by evaporation under vacuum. Crystalline PQ was obtained from spinach chloroplasts using the procedure described by Crane (1959). The assays for Hill reaction activity with indophenol dye and for photophosphorylation with PMS have been described elsewhere (Avron et al., 1958, Jagendorf and Avron, 1958).

The table shows the results of a typical experiment in which heptane extraction of lyophilized chloroplasts causes concomitant loss of Hill

TABLE I

The effect of PQ depletion and readdition on photosynthetic phosphorylation and Hill activity

Chloroplast Preparation	μ moles 2, 3', 6-trichloro-phenol indophenol reduced per mg chlorophyll per hour	μ moles ATP synthesized per mg. chlorophyll per hour
control	126	159
extracted	36	38
extracted plus 0.05 mg PQ per mg chlorophyll	57	66
extracted plus 0.1 mg PQ per mg chlorophyll	78	112

reaction activity to the dye 2, 3', 6-trichlorophenol indophenol and of photophosphorylation with PMS as the catalytic cofactor. On readdition of PQ there is an approximately parallel restoration of both Hill reaction activity

and photophosphorylation. In six similar experiments, the maximum restoration varied between fourty and eight per cent of the activities in the unextracted chloroplasts. In every case, both the Hill reaction and photophosphorylation responded in a similar manner.

These experiments show that PQ is essential for photosynthetic phosphorylation. PMS supported photophosphorylation is a unique case in that ATP synthesis can be elicited by the cofactor from preparations in which the Hill reaction has been selectively inhibited (Jagendorf and Avron, 1959). This selective inhibition of the Hill reaction has been interpreted as a blockage of the terminal steps in the oxygen evolving pathway. The experiments reported here indicate that PQ functions at a point internal to these terminal stage of oxygen evolution.

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