A NATURALLY OCCURRING COFACTOR FOR PHOTOSYNTHETIC PHOSPHORYLATION

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With the discovery of photosynthetic phosphorylation, it was immediately recognized that chloroplasts isolated from spinach leaves must be supplemented with water soluble constituents if a vigorous rate of ATP synthesis is to be observed (1). The water soluble cofactors are presumably washed out of the chloroplast during isolation of this particulate fraction from the whole leaf homogenate. More recently, a method for the preparation of chloroplasts from lyophilized leaves using nonaqueous solvents has been described (2). While such nonaqueous chloroplast preparations show neither Hill activity nor photophosphorylation activity, they have been found to contain a cofactor which will support the photophosphorylation activity of chloroplasts isolated in the conventional aqueous preparative media.

Chloroplasts isolated in nonaqueous media served as the source of cofactor, and were prepared according to the procedure of Stocking (2). Chloroplasts used in the assay of cofactor activity were prepared and washed once in aqueous, isotonic sucrose solution according to the procedure of Jagendorf and Avron (3). The conditions for measurement of photophosphorylation are essentially those of Jagendorf and Avron (3) with the exception that all phosphorylation assays were carried out aerobically. Inorganic phosphate was measured by the method of Fiske and SubbaRow (4) and chlorophyll by the method of Arnon (5). FMN, catechin and caffeic acid were purchased from

the Nutritional Biochemical Corporation, and quercetin and quercitrin from the California Corporation for Biochemical Research. Chlorogenic acid was supplied by the Mann Research Laboratories.

The data in Table I show that the chloroplasts prepared and washed once in aqueous, isotonic sucrose are incapable of photophosphorylation in the absence of an added cofactor. On addition of FMN, phosphorylation is observed. Similarly, an aqueous extract of nonaqueous chloroplasts is seen to elicit photophosphorylation. This extract was prepared by suspending dry, nonaqueous chloroplasts in cold water at a concentration giving one milligram chlorophyll per ml, then centrifuging at 20, 000 x g. for twenty minutes to remove the chloroplasts and chloroplast fragments. That the cofactor activity is heat stable is indicated by the retention of activity after incubation of the extract in a boiling water bath for twenty minutes. In other experiments, a nitrogen atmosphere was found to surpress photophosphorylation at all concentrations of the extract tested.

The cofactor activity was not absorbed on Amberlite MB3 mixed bed ion exchange resin and could be extracted from an aqueous solution into

TABLE I

PHOTOPHOSPHORYLATION WITH NONAQUEOUS CHLOROPLAST EXTRACT

Cofactor	μ moles P_i taken up per mg. chlorophyll per hour
None	0
FMN	92
Extract	55
Boiled Extract	54

The amount of extract is equivalent to 0.1 mg. chlorophyll in nonaqueous chloroplasts.

butanol or ethyl acetate. Further purification was accomplished by chromatography on Whatman 3 MM paper using butanol-acetic acid-water (4:1:5) as ascending solvent. Eighty percent of the cofactor activity recovered from this chromatogram was associated with an area showing a visible yellow color, a purple fluorescence and an Rf of 0.43. An Rf of 0.83 was obtained with fifteen percent acetic acid as the solvent. Spectra of a partially purified cofactor preparation are presented in Figure 1. A double peaked spectrum with maxima at 270 mμ and 350 mμ is seen at neutral pH, while a pronounced shift of the 350 mμ peak to a longer wave length (390) is observed on the addition of alkali. Similar spectral shifts are induced by $AlCl_3$ and H_3BO_3 and all of these responses are suggestive of a flavone type compound (6).

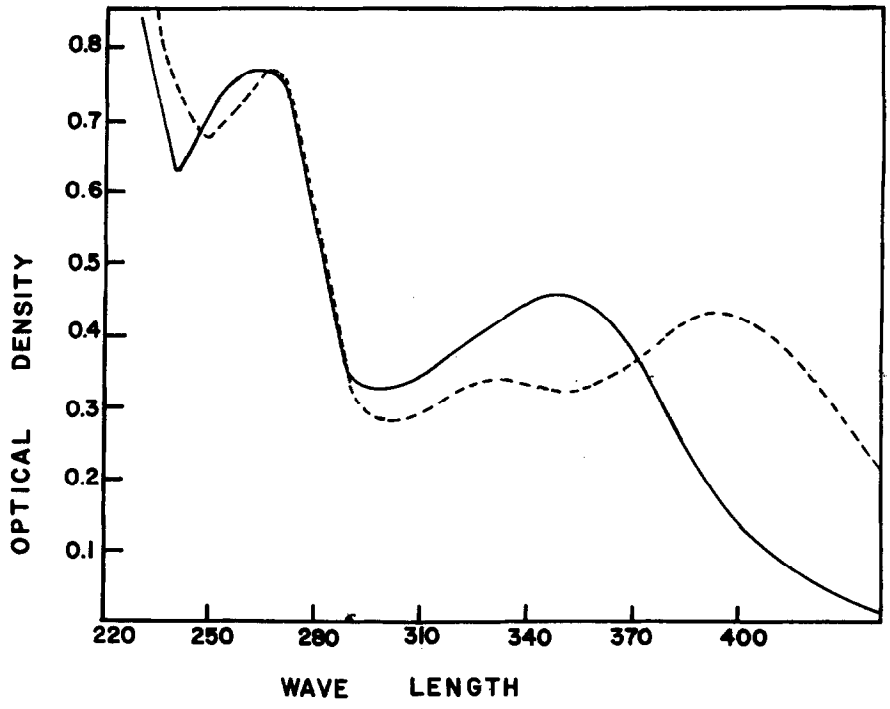

FIG. 1

To further support the notion that the cofactor activity found in chloroplasts isolated in nonaqueous media is due to a flavone, several commercially available flavones and flavone like compounds were tested for

activity as cofactors for photophosphorylation. Table II gives date obtained with these compounds as well as the optimum concentrations for activity. As with the cofactor activity found in nonqueous chloroplasts, a nitrogen atmosphere surpressed photophosphorylation at all concentrations of these cofactors tested.

TABLE II

PHOTOPHOSPHORYLATION WITH VARIOUS COFACTORS

Cofactor	Optimum Concentration	μ moles P_i taken up per mg. chlorophyll per hour
Chlorogenic acid	6.7×10^{-4} M	93
Caffeic acid	4.0×10^{-5} M	52
Quercetin	5.2×10^{-5} M	50
Quercitrin	2.7×10^{-5} M	26
Catechin	4.0×10^{-5} M	70

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