ENHANCEMENT OF PHOTOPHOSPHORYLATION AND PHOTOREDUCTION BY A CHLOROPLAST FACTOR FROM SPINACH LEAVES

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SUMMARY

A factor from spinach chloroplasts stimulates both photoreduction of ferricyanide and photophosphorylation where assayed using isolated chloroplasts. This factor consists of a low molecular weight chromophore attached by non-covalent bonds to a protein. Spectral and chemical evidence suggests that the factor is a direct link in the photosynthetic electron transport chain and does not act as an uncoupling agent nor in pseudo-cyclic phosphorylation.

INTRODUCTION

A variety of factors have been investigated from plant tissues particularly from chloroplasts which are able to enhance photophosphorylation (1,2,3,4) or photoreduction (4,5) by isolated chloroplasts exposed to light. There are a number of chemical and physical similarities among these factors. They exhibit distinctive properties of light absorption and fluorescence particularly an absorbancy maximum at 315 nm and an excitation at approximately 320 nm with fluorescence at 360 - 460 nm. It appears as if these

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factors are a family consisting of a chromophoric group(s) bound to protein(s) of the lamellar membranes of the chloroplasts. None of these factors has been characterized with respect to its mechanism of action or the nature of the chromophoric group. We wish to report that a protein chromophore complex is able to enhance simultaneously both photophosphorylation as measured by ATP synthesis and photoreduction as measured using ferricyanide. Further, this factor in the presence of illuminated chloroplasts undergoes spectral changes which suggest that the factor itself may directly participate in the electron transport pathway.

METHODS

The protein bound chromophore was isolated from spinach chloroplasts following the procedures of sonification and heat purification to remove inactive proteins as originally described (3). The buffer Tricine (N-Tris hydroxymethyl [methyl glycine]) was used instead of Tris.

A further purification step was carried out using a DEAE cellulose column with 50 mM Tris pH 7.8 and a KCl salt gradient as eluent. The factor is eluted from the column at 0.3 M KCl. The factor at the stage of purification is homogenous as determined by sedimentation velocity and acrylamide gel electrophoresis. The chloroplasts used in the assay procedures were isolated as described previously (6). Tricine buffer was used instead of Tris. Chlorophyll was measured as described by Arnon (7). Photophosphorylation was measured by the method of Avron (8). Photoreduction was measured by the absorbancy change at 420 nm using Fe

$(CN)_6^{3-}$ as the electron acceptor as described by Izawa and Good (9). Measurement of spectral changes in the factor were made in the presence of substrate amounts of factor added to chloroplast suspensions under photophosphorylating conditions, ADP and P_i present. Following exposure periods to light the chloroplasts were centrifuged at 10,000 x g for 10 min and the absorbancy difference at 315 nm was measured using the supernatant from light exposed chloroplasts as the reference and reading the supernatant from the dark control.

RESULTS

The ability of the factor to stimulate $Fe(CN)_6^{3-}$ reduction

TABLE I

STIMULATION OF PHOTOREDUCTION AND PHOTOPHOSPHORYLATION BY THE FACTOR

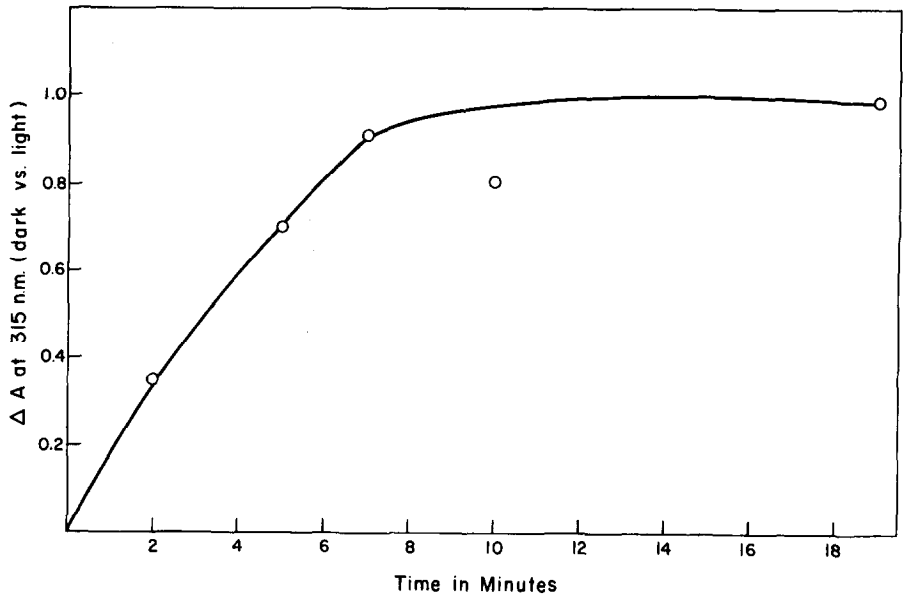
Conditions	* μ moles $Fe(CN)^{3-}$ reduced	* μ moles P esterified	P/2e$^-$
ADP + P_i		20	
+ Factor (44 μg).		155	
+ $Fe(CN)_6^{3-}$	255	146	1.14
+ $Fe(CN)_6^{3-}$ + Factor (44 μg).	360	190	1.10
+ $Fe(CN)_6^{3-}$ + Factor (88 μg).	420	294	1.40

* Values are presented in terms of per mg chlorophyll per hour.

Reaction mixture contains: Tricine, 250 μmoles, pH 7.8; $MgCl_2$, 12.5 μmoles; KCl, 50 μmoles; $K_3Fe(CN)_6$, 2 μmoles; ADP, 1 μmoles; $^{32}P_i$, 10 μmoles. Total volume was 3.0 ml containing 55 μg chlorophyll, temperature 22° and illumination of 2,000 fc. at the outside of the experimental cuvettes using a Sylvania "Sun Gun°" Model 1, through a dilute copper sulfate filter. Time of illumination, 5.0 min.

is presented in Table I. There is an increase of 41% and 64% in the rate of reduction with the addition of 44 µg and 88 µg of the factor, respectively. This enhancement is not a function of uncoupling of photophosphorylation from electron transport as shown in the data regarding phosphorylation rates. The factor increases phosphate esterification almost 8 times in the absence of electron acceptor. Further, in the presence of $Fe(CN)_6^{3-}$ there is almost a doubling of the rate of photophosphorylation. The $P/2e^-$ ratios are actually increased slightly in the presence of the factor. This observation indicates that the factor does not stimulate cyclic photophosphorylation.

When about 400 µg of the factor is present with illuminated chloroplasts there is a decrease in the absorbancy at 315 nm as a function of time, Fig. 1. Within approximately 8 min. an asymptote is reached. It is tempting to speculate that this change is due to reduction of the chromophore. Chemical reduction using sodium dithionite or sodium borohydride produces similar spectral changes; unfortunately addition of sodium borate also alters the visible spectra in a similar manner. The addition of 5×10^{-6} M DCMU blocks the loss of absorbancy as well as the stimulation of phosphorylation by the factor and suggests that electrons are not flowing through or into this factor in the presence of DCMU. It is important to recognize that prior to the measurement of absorbancy following illumination the factor is kept in the dark at low temperature during centrifugation. Oxygen is present but oxidation does not occur to a significant extent. If the factor is first reduced in the light and then $Fe(CN)_6^{3-}$ added in the dark,



LEGEND FOR FIGURE I

The decrease in absorbancy at 315 mm of a solution containing the factor (400 μg) as a function of time of exposure to light in the presence of chloroplasts. Experimental conditions are the same as given in Table I with the exception that no $Fe(CN)_6^{3-}$ was present. Chloroplasts were removed by centrifugation. A dark control was used as the reference.

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no appreciable reduction of the $Fe(CN)_6^{3-}$ is observed.

DISCUSSION

The factor can enhance both phosphate esterification and electron transport by isolated chloroplasts, and may also participate directly in the noncyclic flow of electrons between Photosystem II and I. It is unlikekly that the factor is operative in pseudo-cyclic phosphorylation. The chemical nature of the chromophore is only partially characterized (R. Gee unpublished). The spectrum of the chromophore and its dependence on pH and the spectra in the

presence of borate suggest that it is a flavinoid similar to quercetin, and also related to the factors isolated by Krogman and Stiller (1), Black, et. al. (2), and Wu and Myers (4). The chromophore of our factor is linked by non-covalent bonds to the protein. Addition of organic solvents such as acetone or propanol, or acidification caused release of the chromophore and loss of the biological activity. Dialysis or gel filtration on Sephadex will also result in the release of the chromophore. The variability noted in the molecular weight of the factors isolated by various investigators is probably a function of the mode of rupture of the chloroplast membranes and the isolation procedure.

We have been able to isolate similar factors from algae and other plants which can stimulate photophosphorylation and reduction in chloroplasts from spinach and Codium fragile. Our present efforts are directed toward elucidating the precise position of these factors in the electron transport pathways of the chloroplasts (10).

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