ELECTRON TRANSPORT IN CHLOROPLASTS: A NEW REDOX PROTEIN, RUBIMEDIN

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During studies of the role of plastoquinones in electron transport in chloroplasts we have found a red protein which is released from chloroplasts by heptane treatment. When freeze-dried spinach chloroplasts are extracted with heptane plastoquinones and other lipids are removed. After heptane extraction the chloroplasts are washed with salt solution and centrifuged to remove residual heptane. After centrifugation, the aqueous supernatant contains a red protein which does not show any characteristics of other proteins which have been extracted from chloroplasts. We wish to report here evidence that this red protein is involved in electron transport in chloroplasts and may influence the recovery of activity achieved by adding back plastoquinones and other lipids extracted by the hydrocarbon. A role between the second and first light reaction (cf. Clayton, 1965) is indicated so that an appropriate name for this material would be rubimedin.

Spinach chloroplasts are prepared in 0.5 M sucrose as previously described (Crane, 1959). After washing to remove most of the sucrose the chloroplasts are freeze-dried. The dried chloroplasts are then extracted by shaking with ten volumes of redistilled heptane for four hours at room temperature. The extracted chloroplasts are filtered from

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0.015 M NaCl and centrifuged at 50,000 xg for 30 min. The supernatant contains the red protein.

Quinones and other lipids are added back to the dried chloroplasts in heptane and the heptane is evaporated before the chloroplasts are resuspended in 0.015 M NaCl. Purified plastoquinones are prepared as previously described (Henninger et al., 1966).

After heptane extraction a large part of various photoreductase activities can be restored by addition of the lipid from the heptane extract or by addition of purified plastoquinones A and C. Photoreduction of NADP mediated by ascorbate and NNN'N'tetramethylphenylenediamine (TMPD) is not restored by addition of lipid but is restored by addition of the red protein to the resuspended chloroplasts.

After more thorough heptane extraction (4 hours) the red protein does not fully restore this latter activity and addition of lipid from the heptane extract is also required to restore activity. Plastoquinones A and C do not stimulate the TMPD-NADP reductase system even though they do restore indophenol photoreduction and partially restore the reduction of NADP from water or in presence of ascorbate and indophenol with CMU present. Restoration of various activities is shown in Table I. After washing extracted chloroplasts, rubimedin increases the amount of NADP photoreduction from water when added along with plastoquinones A and C.

Rubimedin can be prepared from the aqueous chloroplast extract by fractionation of the extract with ammonium sulfate. Active material is obtained in the fraction which precipitates between 60 and 70% saturated ammonium sulfate. This precipitate is collected by centrifugation and dialysed against 0.01 M phosphate buffer at pH 7.2 for 8 hours. After dialysis it is centrifuged at 144,000 xg to remove insoluble material. The clear solution is then concentrated by dialysis against carbowax. After concentration, the red solution is passed through Sephadex G100 and the red material which passes directly through is collected and passed through

Sephadex G200. Again the red protein is eluted immediately off the gel in 0.01 M phosphate buffer. This preparation shows only one major red band in polyacrylamide gel electrophoresis. The red protein will not pass through a DEAE cellulose column but remains as a brownish red band at the top after spinach ferridoxin, flavoprotein and plastocyanin are eluted from the column

A broad absorption band from 600 to 400 mμ with a maximum at 470 mμ is characteristic of the protein. This band disappears on reduction with dithionite (cf. figure 1). Activity is destroyed by boiling for five minutes or by standing at 0° for one or two days. Our best preparation shows an absorbancy of 1.0 per mg protein in one ml at 470 mμ in a 1.0 cm path length and a ratio of A_{470}: A_{520} of 2.31.

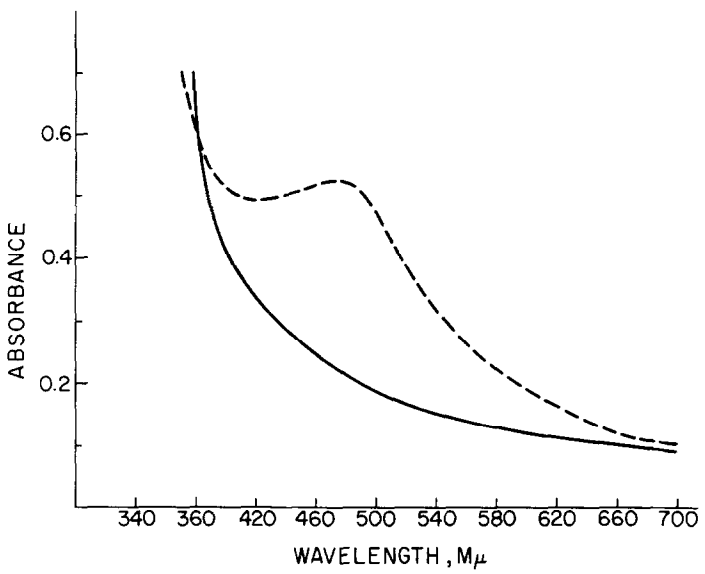

Fig. 1. Spectrum of the red protein. 0.52 mg protein in one ml phosphate buffer pH 7.2. Dashed line - oxidized form, solid line - reduced with dithionite.

The properties and enzymatic effects of the protein do not resemble other proteins which have been extracted from chloroplasts. It has no ferridoxin activity and ferridoxin is required in addition to the red protein for NADP reduction. It also does not show the same spectrum or

TABLE I

Restoration of NADP Photoreductase Activity
by Red Protein after Heptane Extraction

Preparations and Additions	Photoreductase activity μmoles/min/mg chlorophyll		
	ASC-DCIP NADP	ASC-TMPD NADP	H_2O NADP
Washed chloroplasts	3.41	4.31	4.50
Dried chloroplasts	3.70	4.24	4.14
Extracted chloroplasts	0.29	0.42	0.31
Extracted + rubimedin 0.04 mg	0.46	1.30	0.52
Extracted + lipids* + rubimedin 0.04 mg	1.10	3.41	2.79
Extracted + lipids*	1.35	1.13	1.40
Extracted + 0.1 μmole PQ A + 0.01 μmole PQ C	1.20	0.37	1.60
Extracted + 0.1 μmole PQ A + 0.01 μmole PQ C + rubimedin 0.04 mg	1.40	1.36	4.13
Extracted + rubimedin (no ferridoxin)		0.0	0.0

*extracted lipids added at the level in original chloroplasts.

Activity determined by the following assay procedures: reduction of NADP by ascorbate-indophenol (ASC-DCIP) by method of Keister et al., (1962). Reduction of NADP by ascorbate-NNN'N' tetramethyl-p-phenylene-diamine (ASC-TMPD) by method of Vernon and Shaw (1965).

activity as phytoflavin (Smillie, 1965). It shows no significant NADPH-cytochrome C reductase activity and on acidification does not yield material with a flavin spectrum. The spectral properties do not resemble plastocyanin (Katoh et al., 1962) and it does not restore NADP reduction by ascorbate in absence of indophenol which is characteristic of plastocyanin. (Davenport, 1965)

The activity of this protein in increasing NADP reduction in addition

to the effects of plastoquinones A and C suggests alternative pathways of photosynthetic electron transport between the first and second light reactions and that this protein is involved between the second light reaction and cytochrome f.

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