PLASTOCYANIN-DEPENDENT PHOTOREDUCTION OF NADP BY AGRANAL CHLOROPLASTS FROM MAIZE

Robert M. SMILLIE, Kirsten S. ANDERSEN and D.G. BISHOP

Plant Physiology Unit, C.S.I.R.O. Division of Food Research, and School of Biological Sciences, Macquarie University, North Ryde, 2113, Sydney, Australia

Received 16 February 1971

1. Introduction

It is well known that isolated chloroplasts containing grana can carry out the photoreduction of NADP coupled to the evolution of oxygen. In contrast, it has been recently shown that higher plant chloroplasts containing either no grana or a few small grana ('agranal' chloroplasts), such as those found in the bundle-sheath cells of leaves of certain C_4 plants including maize, sugar cane and *Sorghum*, have a different photosynthetic electron transfer pathway. Isolated 'agranal' chloroplasts are unable to photoreduce NADP [1–3] even though photosystems I and II are active [2, 3] as the two photosystems do not appear to be linked by a membrane-bound electron transfer pathway. Evidence that photosystems I and II are in fact linked in the intact bundle-sheath cells [3] has led us to postulate the existence of a soluble linking protein(s) which is lost during isolation of the chloroplasts.

In this paper we show that the copper-protein, plastocyanin, functions as a linking protein to permit photoreduction of NADP by 'agranal' chloroplasts isolated from bundle-sheath cells of maize. The rates of NADP photoreduction obtained in this reconstituted system are comparable with those obtained from grana-containing mesophyll chloroplasts.

2. Materials and methods

Chloroplasts from mesophyll cells (grana chloroplasts) and bundle-sheath cells ('agranal' chloroplasts) were prepared according to the procedure of Woo et al. [1] from 15-day-old greenhouse-grown maize (*Zea mays* var. DS 606A). The chloroplast preparations were washed once before use. Photochemical activities were assayed as described [2, 3]. Plastocyanin was purified from leaves of Swiss Chard (*Beta vulgaris*) [4].

3. Results

3.1. *NADP photoreduction by 'agranal' chloroplasts in the presence of plastocyanin*

Granal chloroplasts isolated from the mesophyll cells of maize photoreduced NADP from water at rates of 0.5 to 2.0 μmoles NADPH/min/mg chlorophyll. In contrast, 'agranal' chloroplasts isolated from the bundle-sheath cells showed little or no activity for photoreduction of NADP. Upon the addition of plastocyanin photoreduction of NADP occurred. In the experiment shown in fig. 1, a rate of 0.79 μmole NADPH/min/mg chlorophyll was obtained and rates of up to 1.5 have been obtained in other experiments, The reduction was abolished by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) showing that the reaction was dependent upon photosystem II activity. Maximum activity was obtained at a plastocyanin concentration of 6 to 10 μM (fig. 2). The 'agranal' chloroplast preparations also contain photosystem I activity which is greatly stimulated by the addition of plastocyanin (fig. 1). Maximum activity was obtained at a plastocyanin concentration of around 10 μM.

3.2. *Photoreduction and photooxidation of plastocyanin by 'agranal' chloroplasts*

Evidence that plastocyanin is reduced by photosystem II and oxidized by photosystem I is shown in

North-Holland Publishing Company – Amsterdam

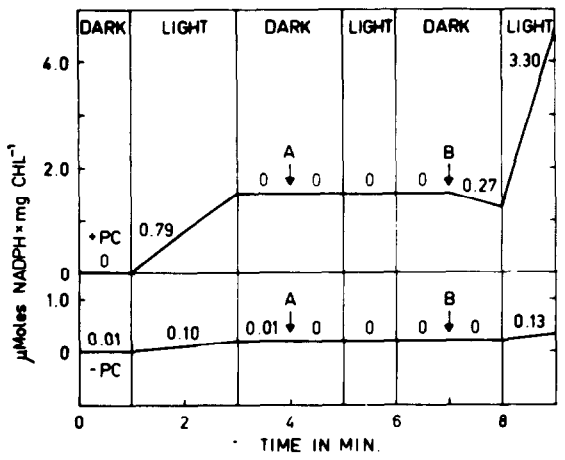

Fig. 1. Effect of 6.2 μM plastocyanin (PC) on photoreduction of NADP by 'agranal' chloroplasts. NADP photoreduction was followed using an Aminco Chance Dual Wavelength Spectrophotometer fitted with a cross-illumination attachment. The reaction mixture (0.75 ml) contained chloroplasts (3.5 μg chlorophyll), sorbitol 300 mM, phosphate buffer pH 7.4 10 mM, $MgCl_2$ 1 mM, NADP 0.67 mM, and ferredoxin (from *Anacystis nidulans*) 3.3 μM. (A) DCMU 2.5 μM was added; (B) ascorbate 2.5 mM and 2,6-dichlorophenol-indophenol (DCIP) 67 μM was added. The assay temperature was 25°. Actinic light was provided by a tungsten light filtered with a Corning 2-60 red filter and two 1-69 infra-red filters; energy incident on sample was 6×10^4 ergs $cm^{-2}sec^{-1}$. The rates shown on the graphs are in μmoles NADP reduced/min/mg of chlorophyll.

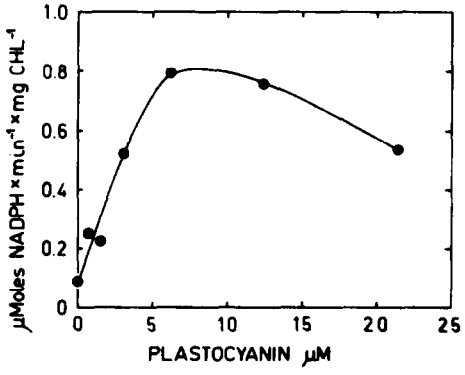

Fig. 2. Rate of photoreduction of NADP as a function of plastocyanin concentration. The reaction mixture is given in fig. 1. (DCMU, ascorbate and DCIP omitted.)

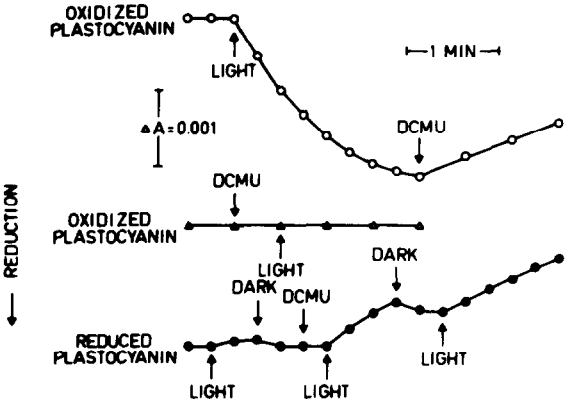

Fig. 3. Photoreduction and photooxidation of plastocyanin by 'agranal' chloroplasts. The absorbancy changes were followed using an Aminco Chance Dual Wavelength Spectrophotometer with the measuring wavelength set at 597 nm and the reference wavelength at 570 nm. Actinic light was as described in fig. 1. The reaction mixture is given in fig. 1 and reduced or oxidized plastocyanin, 3.1 μM, was included as indicated. DCMU was added to a final concentration of 2.5 μM.

fig. 3. Illumination of a reaction mixture containing oxidized plastocyanin resulted in reduction of the plastocyanin. DCMU inhibited this reduction. If DCMU was added after reduction had been allowed to proceed for some minutes, photooxidation of plastocyanin occurred. Illumination in the presence of reduced plastocyanin resulted in a slight oxidation of plastocyanin only, but if DCMU was added a steady rate of oxidation took place.

In the experiment shown in fig. 1, reduced plastocyanin was added. With oxidized plastocyanin there was a lag of several minutes before maximal rates of NADP photoreduction were reached.

4. Discussion

Hitherto only one photosynthetic electron transfer pathway associated with oxygen evolution has been recognized as occurring in the chloroplasts of higher plants, namely a membrane-bound pathway containing two distinct light reactions leading to a reduction of ferredoxin. Ferredoxin in turn reduces NADP via the enzyme ferredoxin NADP reductase.

We here report the existence of another type or variant of the photosynthetic electron transport pathway. This second pathway predominates in chloroplasts which lack extensive grana development. 'Agranal' chloroplasts isolated from maize or *Sorghum* are unable to photoreduce NADP at appreciable rates [1, 2], although it is possible to demonstrate the presence of both photosystem II and photosystem I [2, 3]. Part of the membrane-bound electron transfer pathway between the two photosystems appears to be missing and it is perhaps significant that these chloroplasts lack cytochrome *b*-559 and fluorescence bands associated with photosystem II [1]. When the 'agranal' chloroplasts are supplemented with the soluble chloroplast protein plastocyanin, NADP is photoreduced at rates comparable to those found with granal chloroplasts.

Various chloroplast preparations showing a requirement for plastocyanin have been reported in the literature. Particles prepared by treating chloroplasts with high concentrations of detergents which destroy photosystem II activity often require added plastocyanin for photosystem I activity [5, 6]. Arnon et al. [7] have recently used detergents to extract from spinach chloroplasts a particle which shows plastocyanin-dependent photoreduction of NADP from water. The particle appears to be devoid of photosystem I activity although the effect of plastocyanin on photosystem I activity was not reported. It is emphasized that our preparative methods are based on procedures which are commonly used for isolating chloroplasts and chloroplast fragments and that no harsh mechanical or chemical treatments have been employed.

Our data could also be interpreted in terms of the scheme for photosynthetic electron transfer recently promulgated by Arnon et al. [7, 8]. Photoreduction of NADP in 'agranal' chloroplasts, a reaction requiring plastocyanin would then not involve photosystem I, but would consist of two photosystems (photosystem IIa and IIb) which show light requirements characteristic of photosystem II. Some modification of the scheme for photosystem II would be required as cytochrome *b*-559 is absent. Photosystem I would be a separate photosystem whose main function presumably is the generation of ATP by ferredoxin-mediated cyclic photophosphorylation. However, we prefer the explanation that plastocyanin links photosystem II with photosystem I since (a) plastocyanin is reduced by a DCMU-sensitive photoreaction and oxidized by a DCMU-insensitive photoreaction (fig. 2) and (b) NADP reduction from DCIP and ascorbate (photosystem I) also shows a requirement for plastocyanin (fig. 1). It would seem reasonable to suppose that plastocyanin is acting at the same site in both this latter reaction and the photoreduction of NADP from water. The fact that maximum activity is obtained in both reactions at a similar concentration of plastocyanin is consistent with this theory.

Acknowledgements

We wish to thank Mrs. A.Batsch, Mrs. J.Conroy and Mr. N.F.Tobin for valuable assistance.

References

[1] K.C.Woo, J.M.Anderson, N.K.Boardman, W.J.S.Downton, C.B.Osmond and S.W.Thorne, Proc. Natl. Acad. Sci. U.S. 67 (1970) 18.
[2] D.G.Bishop, K.S.Andersen and R.M.Smillie, Biochem. Biophys. Res. Commun. 42 (1971) 74.
[3] D.G.Bishop, K.S.Andersen and R.M.Smillie, Lamellar structure and composition in relation to photochemical activity, in: Photosynthesis and Photorespiration, eds. M.D.Hatch, C.B.Osmond and R.O.Slayer. (John Wiley, Interscience, New York, 1971) in press.
[4] S.Katoh, I.Shiratori and A.Takamiya, J. Biochem. (Tokyo) 51 (1962) 32.
[5] D.I.Arnon, H.Y.Tsujimoto, B.D.McSwain and R.K.Chain, in: Comparative Biochemistry and Biophysics of Photosynthesis, eds. K.Shibata, A.Takamiya, A.T.Jagendorf and R.C.Fuller (University of Tokyo Press, Tokyo, 1968) p. 113.
[6] J.C.S.Wessels, Biochim. Biophys. Acta 126 (1966) 581.
[7] D.I.Arnon, R.K.Chain, B.D.McSwain, H.Y.Tsujimoto and D.B.Knaff, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1404.
[8] D.I.Arnon, H.Y.Tsujimoto and B.D.McSwain, Nature 207 (1965) 1367.