BBA Report

BBA 41190

Photosystem I and II chlorophyll-protein complexes of higher plant chloroplasts

S.D. KUNG and J.P. THORNBER

Department of Botanical Sciences and Molecular Biology Institute, University of California, Los Angeles, Calif. 90024 (U.S.A.)

(Received September 7th, 1971)

SUMMARY

An improved purification procedure which provides large quantities of the Photosystem I and II chlorophyll-protein complexes in a homogeneous form is reported. Only approx. 20% of the chlorophyll in the plants examined is associated with Photosystem I. Preliminary analyses of the properties of the two chlorophyll-proteins are given.

The method available[1,2] for preparing the chlorophyll-protein complexes of higher plant chloroplasts is unsatisfactory. It is a one-step electrophoretic procedure which yields small quantities of material. In addition, the composition of the products varies significantly due to the dissociation which occurs during electrophoresis in sodium dodecyl sulfate-containing buffers[1-4]. If the two chlorophyll-proteins are to be used to extend our knowledge of the composition, structure and function of plant photosystems, and if they are to be used for comparative biochemical studies, then a reproducible isolation procedure has to be found which will yield large quantities of the two complexes in a homogeneous form. Such a procedure has now been devised and is reported here. The technique is an extension of that used for the isolation of the P700-chlorophyll *a*-protein complex of blue-green algae[4,5].

Young leaves of three species of *Nicotiana* (*N. tabacum*, *N. glauca* and *N. glutinosa*) or of jack bean (*Canavalia ensiformis*) were collected from plants grown in a greenhouse. The leaves were homogenized in 50 mM Tris-HCl, pH 8.0, and filtered through four layers of cheesecloth. After centrifugation of the filtrate at 1000 × *g* for 1 min, the supernatant was further centrifuged at 10 000 × *g* for 10 min, and the pellet of photosynthetic lamellar material was resuspended in buffer, and washed twice by high speed centrifugation (100 000 × *g*, 10 min) to remove most of the soluble proteins. The washed material was dispersed in 50 mM Tris–1% (w/v) sodium dodecyl sulfate, pH 8.0 (detergent:chlorophyll = 5:1, w/w), and then centrifuged at 100 000 × *g* for 20 min. The

green supernatant, referred to as the sodium dodecyl sulfate extract, was used as the starting material for the purification procedure.

The sodium dodecyl sulfate extract was chromatographed on hydroxylapatite[6]. A column (1.5 cm X 9 cm) was packed with the adsorbent to a height of 6–7 cm, and then equilibrated with 10 mM sodium phosphate, pH 7.0 (15 ml). Throughout the packing of the column and the subsequent chromatography, the flow rate was maintained at 1.5 ml/min by use of a peristaltic pump. 3 ml of the sodium dodecyl sulfate extract containing approx. 6 mg chlorophyll was run into the column, and washed in with 15 ml of the equilibrium buffer. All the color in the extract was adsorbed. Increasing concentrations of sodium phosphate, pH 7.0 (15 ml each of 0.1, 0.2, and 0.3 M) were then added to the column; sodium phosphate (0.2 and 0.3 M) eluted a small portion of the chlorophyll on the column. The absorption spectrum of this eluate was characteristic of the Photosystem I chlorophyll–protein of higher plants[7] (Fig. 1); in addition, the eluate contained P700, the photochemical reaction center of Photosystem I. The color remaining on the column could not be washed off even by 1.0 M sodium phosphate; however, all the remaining green material can be eluted with 0.4 M sodium phosphate – 1 mM $MgCl_2$ containing 0.05% sodium dodecyl sulfate. Those fractions of eluate which showed a pronounced shoulder at 650 nm were pooled, diluted with equal volume of 10 mM phosphate – 1 mM $MgCl_2$, pH 7.0, and run into a second hydroxylapatite column (1.5 cm X 9 cm) previously equilibrated with 10 mM sodium phosphate - 1 mM $MgCl_2$, pH 7.0. The hydroxylapatite column was eluted with 0.01, 0.1, 0.2 and 0.3 M sodium phosphate - 1 mM $MgCl_2$, pH 7.0. The latter two concentrations eluted green material, the absorption spectrum of which (Fig. 1) showed the same ratio of chlorophyll *a*/*b* previously reported for the Photosystem II chlorophyll-protein[7]. Further purification of both complexes was performed by rechromatography on hydroxylapatite and by ammonium sulfate fractionation. Both chlorophyll-proteins precipitated at an

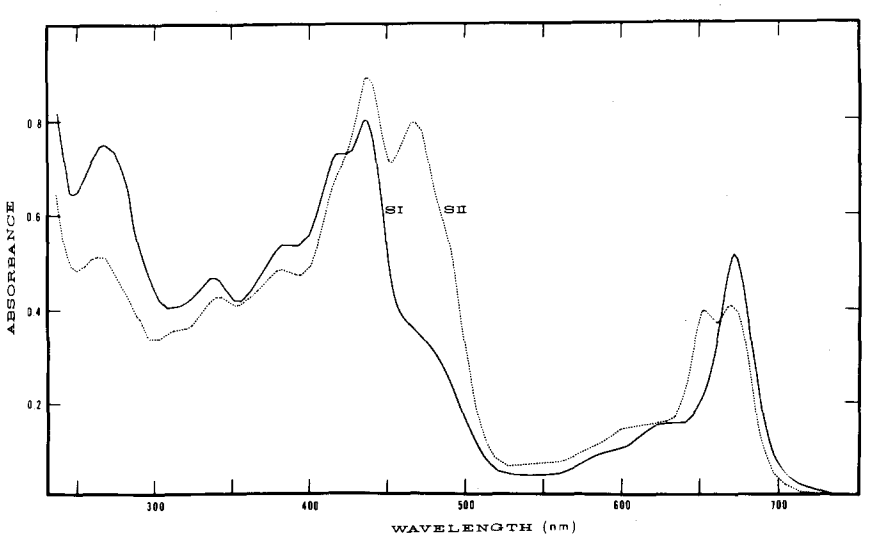

Fig. 1. Absorption spectrum of the Photosystem I chlorophyll-protein (———) and Photosystem II chlorophyll-protein (· · · ·) of *N. tabacum* in 50 mM Tris-HCl, pH 8.0.

ammonium sulfate concentration of 15% (w/v); the precipitates were dissolved and stored in 50 mM Tris - 1 mM $MgCl_2$, pH 8.0.

Polyacrylamide gel electrophoresis, performed as described elsewhere[8], showed that the sodium dodecyl sulfate extract of the chloroplasts of all the plants used gave three major pigmented zones (SI, SII and FP of Fig. 2) equivalent to the Photosystem I chlorophyll-protein, the Photosystem II chlorophyll-protein and sodium dodecyl sulfate-complexed free pigment, respectively, of other chloroplasts[1]. The electrophoretic mobility of the material isolated from the first hydroxylapatite column coincided with that of the Photosystem I complex, whereas that of the material from the second column coincided with that of the Photosystem II complex (Fig. 2), thereby substantiating the identity ascribed above to the two green solutions eluted from the hydroxylapatite columns. Both complexes were obtained in a pure form as judged by polyacrylamide gel electrophoresis. Calibrated sodium dodecyl sulfate–polyacrylamide gels[9] gave molecular weights for the Photosystem I and II chlorophyll-proteins of 100 000 and 35 000, respectively. The chlorophyll *a*/*b* ratio, determined by Arnon's[10] equations, is 5.0 ± 0.3/1.0 for the Photosystem I complex, and 1.0 ± 0.1/1.0 for the Photosystem II complex. A boundary of 3.1 ± 0.1 S was measured upon analytical centrifugation of several samples of the Photosystem II chlorophyll-protein in 50 mM Tris - 1 mM $MgCl_2$, pH 8.0; the color in the samples sedimented with this boundary. No impurity was observed during ultracentrifugation, thereby confirming the purity observed by polyacrylamide gel electrophoresis.

The new purification procedure has been applied succesfully to several different higher plants, and has yielded homogeneous entities of the same basic characteristics in each case. For *N. tabacum* the yields of the Photosystem I and Photosystem II complexes

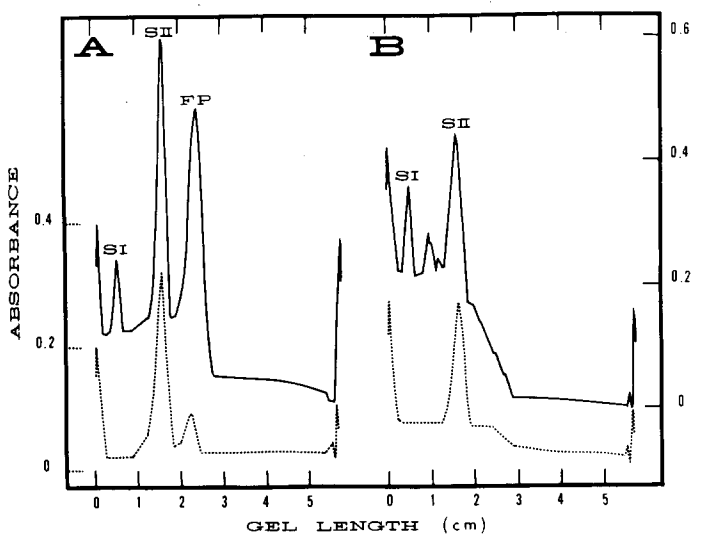

Fig. 2. Densitometric tracings recorded by Cary 15 spectrophotometer of polyacrylamide gels following electrophoresis of the sodium dodecyl sulfate extract (———) and the isolated Photosystem II chlorophyll-protein (· · · · ·) of *N. tabacum*. Tracings were obtained before (A) and after (B) staining with Amido black; lights of 670 and 600 nm was used in A and B, respectively. SI: Photosystem I chlorophyll-protein, SII: Photosystem II chlorophyll-protein, and FP: sodium dodecyl sulfate-complexed free pigment.

were 10% and 30–40%, respectively, of the chlorophyll in the sodium dodecyl sulfate extract loaded onto the first hydroxylapatite column; gel electrophoresis of the extract (Fig. 2) revealed that the proportion of chlorophyll in SI:SII:FP was approx. 1:5:4. Since most of the FP appears to originate from the Photosystem II complex (see later), it is estimated that less than 20% of the chlorophyll in *N. tabacum* (and in the other species studied) is associated with Photosystem I, whereas in spinach beet leaves there was an approximate 1:1 ratio of chlorophyll in the two photosystems[11]. This variation in the division of chlorophyll between the two photosystems in different plants is under investigation.

In this report more information is given on the Photosystem II than on the Photosystem I chlorophyll-protein, since the latter is analogous[4] to the blue-green algal chlorophyll *a*-protein which has been described extensively elsewhere[5], whereas few details are available on the Photosystem II complex. The isolated Photosystem II chlorophyll-protein most probably contains one mole each of chlorophylls *a* and *b* per 35 000 g of complex (*cf.* ref. 11). This chlorophyll *a*/*b* ratio is lower than that (1.2–2.3/1) occurring in any other isolated Photosystem II material[12]. We believe that each molecule of the Photosystem II chlorophyll-protein has an additional chlorophyll *a* molecule associated with it *in vivo* (*i.e.* its chlorophyll *a*/*b* ratio is 2/1 *in vivo*); this second chlorophyll *a* molecule is more accessible to the solvent than the other two chlorophyll molecules, and can be displaced reasonably readily by ionic detergent. With respect to this hypothesis, R.S. Alberte and J.P. Thornber (unpublished results) have observed that during gel electrophoresis of sodium dodecyl sulfate extracts of higher plant chloroplasts, the SII zone continuously changes its chlorophyll *a*/*b* ratio from approx. 2/1 to 1/1 and lower (*cf.* also ref. 3), and the pigment released from the SII zone subsequently electrophoreses in the FP zone. It is thus possible to rationalize the variation in the chlorophyll *a*/*b* ratio of the previously isolated Photosystem II material[1,12]. We think that the Photosystem II chlorophyll-protein loses its second chlorophyll *a* molecule during the extensive washing of the first hydroxylapatite column with 0.4 M sodium phosphate - 1 mM $MgCl_2$ - 0.05% sodium dodecyl sulfate, and that it is only after displacement of this chlorophyll molecule that the Photosystem II complex will chromatograph on the adsorbent; $MgCl_2$ is essential for the chromatography of the Photosystem II chlorophyll-protein, but the reason is not clear.

We wish to thank Dr. S.G. Wildman for his advice and encouragement during the course of this work. Research was supported by a grant AI00536 from the National Institutes of Health, U.S. Public Health Service, and Contract AT(11-1)-34, Project 8, from the Division of Biology and Medicine, U.S. Atomic Energy Commission, and by a grant from the Committee on Research, University of California.

REFERENCES

1 J.P. Thornber, R.P.F. Gregory, C.A. Smith and J.L. Bailey, *Biochemistry*, 6 (1967) 391.
2 T. Ogawa, F. Obata and K. Shibata, *Biochim. Biophys. Acta*, 112 (1966) 223.
3 J.L. Bailey and W. Kreutz, in H. Metzner, *Progress in Photosynthetic Research*, Vol. I, Tübingen, (1969), p. 149.
4 J.P. Thornber, *Biochim. Biophys. Acta*, 172 (1969) 230.
5 W.E. Dietrich, Jr. and J.P. Thornber, *Biochim. Biophys. Acta*, 245 (1971) 482.

6 H.W. Siegelman, G.A. Wieczorek and B.C. Turner, *Anal. Biochem.*, 13 (1965) 402.
7 J.P. Thornber and J.M. Olson, *Photochem. Photobiol.*, in the press.
8 J.P. Thornber, *Biochemistry*, 9 (1970) 2688.
9 K. Weber and M. Osborn, *J. Biol. Chem.*, 244 (1969) 4406.
10 D.I. Arnon, *Plant Physiol.*, 24 (1949) 1.
11 J.P. Thornber, J.C. Stewart, M.W.C. Hatton and J.L. Bailey, *Biochemistry*, 6 (1967) 2006.
12 N.K. Boardman, *Annu. Rev. Plant Phys.*, 21 (1970) 115.

Biochim Biophys. Acta, 253 (1971) 285–289