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ARE SPECIFIC CHLOROPHYLL–PROTEIN COMPLEXES REQUIRED FOR PHOTOSYNTHESIS?

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SUMMARY

Using analytical polyacrylamide gel electrophoresis of detergent extracts of Scenedesmus chloroplast fragments, we have shown that the electrophoresis pattern of Bishop's mutant 8 (which lacks a functional Photoreaction I) differs from the pattern shown by the wild-type and mutant 11. Gels from mutant 8 extracts do not show a band corresponding to the chlorophyll–protein–detergent complex I nor is a corresponding protein band present. Thus chlorophyll–protein–detergent complex I reflects solubilization of a real and essential chlorophyll–protein complex of System I of photosynthesis, and lack of a functional System I in this mutant is due to absence of this chlorophyll–protein complex. This implies that at least this chlorophyll–protein complex is required for photosynthesis.

INTRODUCTION

The photochemical processes of photosynthesis are found only in lamellar systems which are composed of lipoprotein in intimate association with chlorophyll. To what extent does this association involve specific complexes which are required for operation of the photochemical system? Several workers have approached this problem by solubilizing the lamellae with anionic detergents, and subsequently isolating the resulting detergent micelles which contain chlorophyll associated with protein[1–5].

Using anionic detergents such as sodium dodecyl sulphate[2] and sodium dodecyl benzene sulphonate[1,4,5] only two kinds of chlorophyll–protein–detergent complexes have been observed after separation by electrophoresis on polyacrylamide gels. One of these complexes, chlorophyll–protein–detergent complex II, contains equal amounts of chlorophyll *a* and chlorophyll *b*. The other complex, chlorophyll–protein–detergent complex I, contains chlorophyll *a* with less than 10 % chlorophyll *b*. Using fractions of digitonin-solubilized chloroplasts prepared by the methods of ANDERSON

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AND BOARDMAN[6], THORNBER *et al.*[1] found that the fraction showing high Photoreaction I activity was enriched in chlorophyll–protein–detergent complex I, while chlorophyll–protein–detergent complex II was enriched in the fraction showing high Photoreaction II activity. This suggested to them the association of chlorophyll–protein–detergent complexes I and II with, respectively, Photoreactions I and II of photosynthesis[1], as these photoreactions were defined by DUYSENS *et al.*[7]. BAILEY AND KREUTZ[5] found chlorophyll–protein–detergent complex I to show light-induced absorption changes at 700, 682, and 435 nm. We now report that chlorophyll–protein–detergent complex I is absent in detergent extracts of a mutant green alga which lacks activity in Photoreaction I. These two observations make it clear that the detergent–protein–chlorophyll complex I micelle is derived from a pre-existing complex which is required *in vivo* for operation of Photoreaction I.

MATERIALS AND METHODS

Three strains of *Scenedesmus obliquus*, wild-type, mutant 8 and mutant 11, originally isolated by BISHOP[8,9] were cultured in the dark in a New Brunswick Psycro-Therm gyrotory shaker at 25° in the liquid media described by BISHOP[8]. Mutant 8 lacks activity in Photoreaction I, and mutant 11 lacks activity in Photoreaction II[8–12]. Since the wild-type and each of the mutants show a characteristic delayed light emission[13], the delayed light emitted from 1 to 20 msec after excitation was checked on each culture before it was used. This eliminated the possibility that the mutants had reverted to wild-type.

5-day-old cultures were harvested and washed once with distilled water. The cells from 1 l of culture medium were then suspended in 20 ml of 0.1 M Tris–acetate buffer (pH 7.5). All of the following operations, except where noted, were carried out in an ice bath in subdued light. The 20 ml of cells were sonicated for 5 min in a 10-kHz Raytheon Sonic Oscillator Model DF-101. Unbroken cells were removed by centrifugation at 500 × *g* for 5 min. Chloroplast fragments were isolated by centrifuging the 500 × *g* supernatant at 104000 × *g* for 30 min. Virtually all the green material sedimented. The pellet was resuspended in approx. 2 ml of the same buffer. Sufficient amounts of a 2 % (w/v) stock solution of sodium dodecyl benzene sulphonate were added to give a concentration of 50 mg sodium dodecyl benzene sulphonate per mg chlorophyll. This was diluted with the same buffer to a total volume of 10 ml and the suspension was immediately centrifuged for 45 min at 144000 × *g*. The resulting supernatant is referred to as "Extract 1". This extract was applied to polyacrylamide gels as described by THORNBER *et al.*[4]. The pellet from this centrifugation always contains a small amount of chlorophyll, less than 5 % of the total. This pale green pellet was resuspended in 10 ml of a solution containing 0.1 % (w/v) sodium dodecyl benzene sulphonate, 0.01 M Tris–acetate (pH 7.5), and was centrifuged for 45 min at 144000 × *g*. The resulting supernatant contains all the green color and is referred to as "Extract 2". This extract was also applied to polyacrylamide gels.

The gels contained 9 % (w/v) polyacrylamide (cross-linked 1.7 %) in 0.1 % (w/v) sodium dodecyl benzene sulphonate, 0.02 M Tris–acetate (pH 7.5). The gels were prepared in glass tubing 6 cm long and 0.5 cm inside diameter. The surrounding buffer contained 0.05 M Tris–acetate (pH 7.5) *plus* 0.1 % sodium dodecyl benzene sulphonate. The gels were loaded with 0.02–0.1 ml of either extract 1 or 2, 10 % (w/v)

sucrose having first been added to the extracts. Sucrose was added to stabilize the samples on the gels, thus minimizing diffusion back onto the surrounding buffer. Extract 1 contained approx. 0.5 mg chlorophyll per ml, and Extract 2 contained approx. 20 times less chlorophyll concentration. A Model 6 Canalco disc gel electrophoresis apparatus was adapted for use with the system described here. A voltage of 50 V (about 8 V/cm) giving a current of 1.5 mA per gel was applied for 45–60 min.

After electrophoresis, the gels were photographed. The gels were then removed from the glass tubing, stained for protein, and photographed again. The staining, which also fixed the protein, was carried out at room temperature in subdued light. The stain consisted of 0.1 % (w/v) Amido black (Amido schwartz) dissolved in a solvent of methanol–water–acetic acid (45:45:10, by vol.). The gels were immersed in this stain for 30 min, then exhaustively washed in the solvent (3–7 days with 10–20 solvent changes) to remove stain from those areas of the gel which contained no protein. The other stains which we used, nigrosin and coomasie brilliant blue, gave results similar to those obtained with amido black, except that the amido black resulted in better resolution on the gels.

Using the methods described above, solubilization of lamellar fragments with sodium dodecyl sulphate yielded results similar to those found with sodium dodecyl benzene sulphonate. Protein staining of the sodium dodecyl sulphate gels was, however, unreliable with the stains we used. One advantage of sodium dodecyl sulphate over sodium dodecyl benzene sulphonate is that no green color is present in the first 144000 × *g* pellet, implying that at the concentration used, the sodium dodecyl sulphate more completely solubilizes the lamellae than does the sodium dodecyl benzene sulphonate.

Chlorophyll determinations were carried out according to the method of MACKINNEY[14].

RESULTS AND DISCUSSION

Fig. 1 is a diagram of a typical separation on our gels of chlorophyll–protein–sodium dodecyl benzene sulphonate complexes, and of stained protein bands, in Extract 1 of the three algal strains. Similar results were obtained with Extract 2. The green complex I is absent in extracts of mutant 8. The corresponding protein is also absent, as indicated by the clear area at this position after protein staining. Furthermore, we found no new protein band at a different position in gels of extracts from mutant 8. The green complex I, and its associated band of stained protein, are both present in about equal amounts in extracts of wild-type and mutant 11. Complex I could have been distinguished by eye in the gels of mutant 8 extracts if the complex had been present in concentrations as small as 5 % of the wild-type or mutant 11.

The absence of chlorophyll–protein–detergent complex I in extracts of mutant 8 cannot be due to resistance of the mutant chlorophyll–protein complex to solubilization by sodium dodecyl benzene sulphonate, because electrophoresis of Extract 2 showed an absence of complex I in mutant 8, and the pellet from this supernatant lacks chlorophyll. Furthermore, the lack of complex I in mutant 8 cannot be due to a lack of chlorophyll because mutant 8 has the same amount of chlorophyll *a* and *b* as the wild-type strain and mutant 11. If P700 is chlorophyll in a special environment,

then in mutant 8 the complex I protein component is either completely missing, or else altered so as to prevent the linkage with chlorophyll which would result in a Photosystem I reaction center.

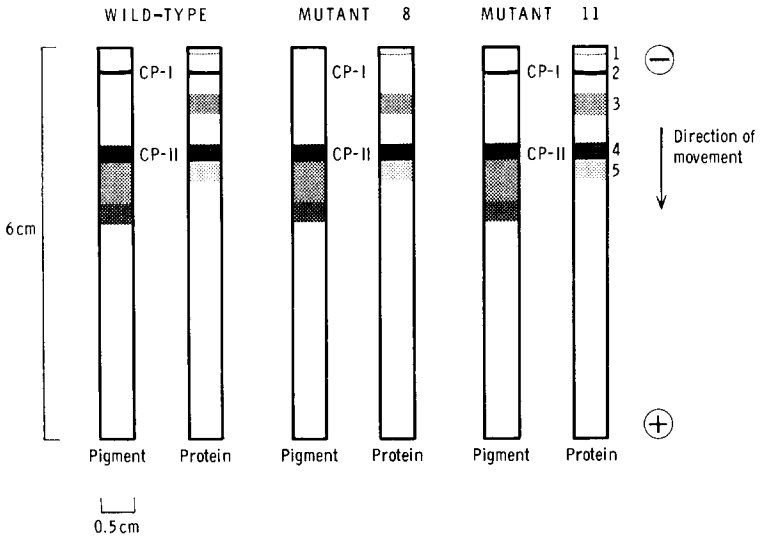

Fig. 1. Diagram of a typical separation on polyacrylamide gels of chlorophyll–protein–sodium dodecylbenzene sulphonate complexes. Both the pigment and protein of the chlorophyll–protein–detergent complex I were missing in mutant 8. CP-I, chlorophyll–protein–detergent complex I; CP-II, chlorophyll–protein–detergent complex II.

Considering the green complex of the chlorophyll–protein–detergent complex II region in unstained gels, we find no difference between extracts of wild-type, mutant 8 and mutant 11. Resolution in this region of the gels is, however, impaired by a diffuse zone of free pigment which is not present in the chlorophyll–protein–detergent complex I region of the gels. Nevertheless, the appearance of the unstained gels in this region was similar in extracts of all three algal strains, and we can say that complex II was probably present in all three strains.

It has been known for some time that mutant 8 does not show the light-induced absorption shifts, or the low temperature absorption band, which have both been associated with P700[10]. Our results show that the chlorophyll–protein–detergent complex, complex I, is also absent from the Scenedesmus mutants. However, complex I has recently been shown to contain P700 by BAILEY AND KREUTZ[5] and by DIETRICH AND THORNBER[15]. It thus appears that Photoreaction I requires a specific chlorophyll–protein complex for photochemical competence, and that complex I represents a detergent-solubilized form of this complex.

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