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Chlorophyll *a* forms in partially purified photosynthetic reaction centers

Previous studies have shown that fragments enriched in the reaction center chlorophyll, P700, may be isolated from Scenedesmus mutant 6E (carotenoidless mutant) and *Anabaena variabilis* grown in the presence of diphenylamine (designated diphenylamine-Anabaena) by the use of Triton X-100 (ref. 1). A similar particle has been obtained from four plants species (normally cultured *A. variabilis*, spinach chloroplast *etc.*) by treatment with Triton X-100 after extracting the pigments by the organic solvents[2-4]. These fragments, denoted "high P700" or HP700 fragments, contain one P700 to approx. 30 chlorophyll molecules. These 30 chlorophyll molecules which are inaccessible to the detergent are in a different environment from the remainder of the chlorophyll molecules which are solubilized by the action of Triton X-100 when carotenoids are missing or have been removed.

Samples used in this study were prepared according to the procedures described previously[1-4]. The absorption spectra obtained at liquid nitrogen temperature were measured with a Cary 14 spectrophotometer modified for the low temperature absorption spectra measurements. In Fig. 1 are given the absorption spectra measured at 77 °K for the HP700 (curve A) and membrane (curve B) fragments of diphenylamine-Anabaena. Similar data were also obtained for the HP700 and membrane fragments, respectively, prepared from normally cultured *A. variabilis*. Curve B in Fig. 1 shows the presence of at least five forms of chlorophyll *a*, designated C-672, C-678, C-685, C-695 and C-707, in the membrane fragment. The presence of these forms of chlorophyll *a* has been reported by BROWN AND FRENCH[5] and BUTLER[6]. It appears that the Anabaena C-707 is the same as C-705 of BUTLER[6] and C-700 of KOK[7]. C-707 was barely observable in the HP700 fragment, as shown by curve A, and the relative contents of C-685 and C-695 to C-677 (C-678 in the membrane fragment) are less in the HP700 fragment than in the original membrane fragment. Neither C-695 nor C-707 is identical with P700, since the relative contents of the C-695 and C-707 forms of chlorophyll *a* were lower in the HP700 fragment in spite of the increased P700 content. Furthermore, the addition of dithionite did not change the

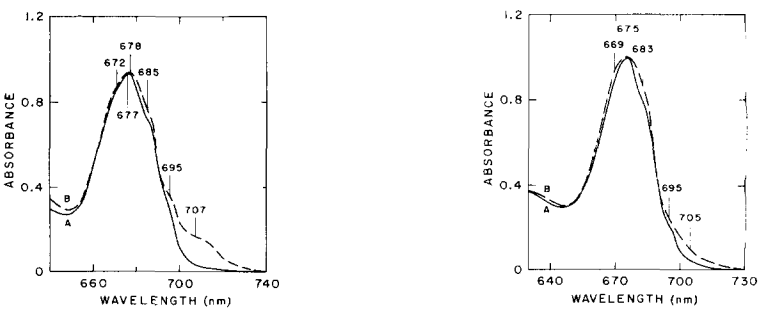

Fig. 1. Absorption spectra measured at 77° K for the HP700 (curve A) and membrane (curve B) fragments prepared from diphenylamine-Anabaena cells. The fragments were suspended in 0.01 M Tris–HCl buffer, pH 7.5.

Fig. 2. Absorption spectra measured at 77 °K for the HP700 fragment (curve A) and whole cells (curve B) of Scenedesmus mutant 6E. The fragment and cells were suspended in 0.01 M Tris–HCl buffer, pH 7.5, containing 1 mM ascorbate.

spectrum of C-695 and C-707. Only a slight increase of absorbance around 700 nm was observed for the HP700 fragment by the addition of dithionite (the increase corresponds to a reduction of P700 which is present in the oxidized form).

A similar decrease in the content of a far-red-absorbing form of chlorophyll *a* was observed for the HP700 fragments prepared from Scenedesmus mutant 6E and spinach chloroplasts. Fig. 2 shows the 77 °K absorption spectra of the HP700 fragment (curve A) and whole cells (curve B) of Scenedesmus mutant 6E. As seen from curve B, this mutant has at least four forms of chlorophyll *a*, C-675, C-683, C-695 and C-705. The shoulder around 669 nm is due to a degradation product of chlorophyll *a*, since the increase of the absorbance at 669 nm was observed accompanied by the decrease of the absorbances between 680 and 730 nm when the cells were exposed to light for long time. Curve A in Fig. 2 shows that the content of C-683, C-695 and C-705 relative to C-675 are less in the HP700 fragment.

The 77 °K absorption spectra of the spinach HP700 fragment (curve A) and spinach chloroplasts (curve B) are shown in Fig. 3. Four forms of chlorophyll *a*, C-672, C-677, C-685 and C-705, were observed in the absorption spectrum of spinach chloroplasts measured at 77 °K (curve B). A shoulder was not observed around 695 nm, which indicates the presence of little or no C-695. The peak at 650 nm is due to chlorophyll *b* absorption. It has been shown by KOK AND RURAINSKI[8] that C-705 is concentrated in the Photosystem I particles prepared from spinach chloroplasts by the use of digitonin[9]. However, as seen from curve A in Fig. 3, the content of C-705 in the HP700 fragment was less than that in the chloroplasts. In the case of spinach, however, the relative content of C-685 and C-672 to C-677 was higher in the HP700 fragment than in the chloroplasts.

It was demonstrated in the previous paper[1] that the fluorescence intensity of the HP700 fragment at 730 nm at 77 °K is much less than that of the membrane fragment. This was explained in terms of the removal of most of the chlorophyll molecule, C-705, from the membrane fragment by treatment with Triton X-100 during the isolation of the HP700 fragment. The present observations strongly support this suggestion, since in all cases there is less C-705 and also a corresponding decrease in fluorescence in the HP700 fragments prepared from the three sources.

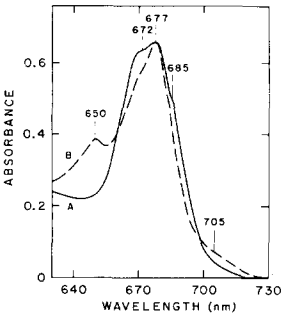

Fig. 3. Absorption spectra measured at 77 °K for the spinach HP700 fragment (curve A) and spinach chloroplasts (curve B). The fragment and chloroplasts were suspended in 0.01 M Tris–HCl buffer, pH 7.5.

KOK observed strong 730 nm fluorescence at 77 °K in Scenedesmus mutant No. 8, which lacks P700 activity and in which the absorbance at 700 nm is much

smaller than that of the wild type strain of Scenedesmus at 77 °K. These data are in apparent contradiction to our concept that the 730 nm fluorescence arises from C-705. It appears from the absorption spectrum of the Scenedesmus mutant No. 8 at 77 °K (*cf.* ref. 7), however, that sufficient amounts of the far-red-absorbing forms of chlorophyll *a* still exists in this mutant to explain the fluorescence data of KOK. Since the chlorophyll molecule, C-707, is barely observable in the diphenylamine-Anabaena HP700 fragment which has high $NADP^+$ photoreduction activity (*cf.* ref. 1), it seems unlikely that C-707 has a direct role in the photoreduction of $NADP^+$ as does P700. The role of C-707 as a "lens" to collect the energy for P700, as proposed by KOK[7], is also unlikely. There remains the possibility that some of the C-695 or C-685 may act as a lens.

Further study is necessary on the role of these far-red-absorbing forms of chlorophyll *a* on photosynthesis.

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1 T. OGAWA AND L. P. VERNON, *Biochim. Biophys. Acta*, 197 (1970) 292.
2 T. OGAWA AND L. P. VERNON, *Biochim. Biophys. Acta*, 180 (1969) 334.
3 L. P. VERNON, H. Y. YAMAMOTO AND T. OGAWA, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 911.
4 H. Y. YAMAMOTO AND L. P. VERNON, *Biochemistry*, 8 (1969) 4131.
5 J. S. BROWN AND C. S. FRENCH, *Plant Physiol.*, 34 (1959) 305.
6 W. L. BUTLER, *Arch. Biochem. Biophys.*, 93 (1961) 413.
7 B. KOK, in B. KOK AND A. T. JAGENDORF, *Photosynthetic Mechanisms of Green Plants*, Natl. Acad. Sci. – Natl. Res. Council, Washington, 1963, p. 45.
8 B. KOK AND H. J. RURAINSKI, *Biochim. Biophys. Acta*, 126 (1966) 584.
9 N. K. BOARDMAN AND J. M. ANDERSON, *Nature*, 203 (1964) 166.

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