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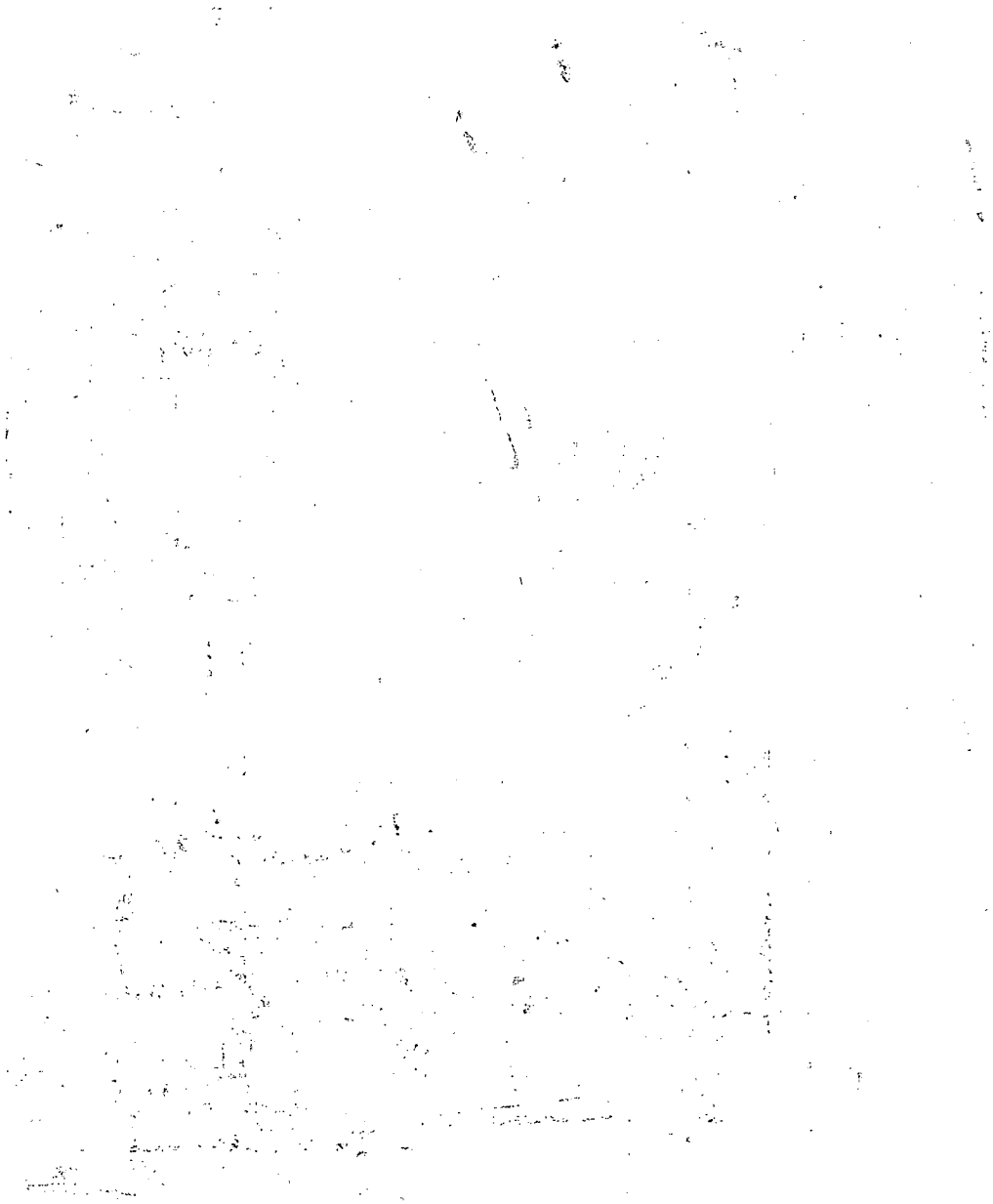

PURIFICATION OF PHOTOSYSTEM I REACTION CENTERS FROM SPINACH STROMA LAMELLAE[1]

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SUMMARY

A procedure for the preparation of partially purified photosystem I reaction centers is described. This procedure gives a preparation with a very high chl a/chl b[2] ratio which contains 1 P-700 per 16 chlorophylls. Starting material is a stroma lamella fraction obtained from spinach chloroplasts.

Kok (1) first reported partial purification of a pigment complex containing P-700. Vernon and his coworkers (2,3) have recently described a procedure for partial purification of these reaction centers from plant tissues using acetone and Triton X-100 extraction. Their preparation from spinach chloroplasts contains 1 P-700 per 28 chlorophylls. Ogawa and Vernon (4) have further characterized the reaction centers obtained from Anabaena variabilis and spinach.

We recently showed that there are two kinds of photosystem I in spinach chloroplasts (5). One is located in the stroma lamellae. It has high P-700 content and is physically separated from photosystem 2. The second is present in grana regions. It has low P-700 content and is physically in close association with system 2. In this paper we describe a procedure for obtaining partially purified photosystem I reaction centers from these fractions without the use of detergents.

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[2]The abbreviations used are: EDTA, ethylenediaminetetra-acetate; chl a, chlorophyll a; chl b, chlorophyll b.

MATERIALS AND METHODS

Chloroplasts isolated from market spinach were fractionated by a passage through a French pressure cell as described earlier (5). The FP homogenate was centrifuged for 10 min at 3000 x g, 30 min at 10,000 x g (grana lamella fraction), 30 min at 30,000 x g, 30 min at 40,000 x g, and 60 min at 160,000 x g (stroma lamella fraction). The precipitates obtained from 10,000 x g (10K), 40,000 x g (40K), and 160,000 x g (160K) centrifugations were used for preparing photosystem I reaction centers. These fractions were diluted 50-fold with 0.75 mM EDTA (pH 8.0) and centrifuged to obtain precipitates. The procedure was repeated once more to remove soluble proteins. The precipitates after the last EDTA wash were dropped into 10 ml of 100% acetone which was precooled to -20°C. The final concentration of acetone was more than 99.5%. These suspensions were stirred with a teflon rod and were then centrifuged on a clinical centrifuge to obtain precipitates. The supernatants containing chlorophylls and other acetone soluble material were discarded. The precipitates were extracted two more times with 10 ml of cold acetone each time. The precipitates obtained at the end of third acetone extraction were dried under vacuum and resuspended in 0.75 mM EDTA (pH 8.0) by brief sonication or homogenization. The suspensions were centrifuged at 30,000 x g for 10 min. The precipitates were washed once with 0.05 M phosphate buffer (pH 7.4) containing 0.01 M KCl. The precipitates were finally resuspended in a small volume of the same buffer. The preparations thus obtained from 160K, 40K, and 10K have been designated RC 160, RC 40, and RC 10 respectively.

P-700 content of these preparations was determined from reduced minus oxidized difference spectra according to the procedure of Vernon *et al.* (2) using a Cary 14 spectrophotometer equipped with a scattering attachment.

Chlorophyll was determined according to Arnon's method (6). Nitrogen was estimated by a standard microkjeldahl procedure using urea as a standard. Electrophoresis was conducted according to the method of Clarke (7). Prior to electrophoresis the material was solubilized in 0.2% sodium dodecyl sulfate.

RESULTS

Table I shows the characteristics of photosystem I reaction center preparations obtained from different fractions.

Material balance studies on the RC 160 preparation showed that less than 10% of the P-700 was destroyed by acetone extraction even though about 90% of the total chlorophyll was removed. This indicates that we are observing 90% of the reaction centers initially present. Based on 10 experiments, the P-700 content of RC 160 ranged from 1 P-700 per 12 chlorophylls to 1 P-700 per 18 chlorophylls. Thus this preparation is approximately two times as purified for P-700 content as the HP700 preparation of Yamamoto and Vernon (3). The chl a/chl b ratio for RC 160 is very high (Table I). We were unable to measure accurately chl a/chl b ratio beyond 10 by our procedure. The chl a/chl b ratio of HP700 observed by Yamamoto and Vernon (3) is 4, a value much less than the one observed for RC 160. The P-700 content of RC 10 is very similar to HP700 preparation of Vernon *et al.* (2) for spinach. Based on 4 experiments, the P-700 content of RC 10 ranges from 1 P-700 per 28 to 1 P-700 per 32 chlorophylls. The chl a/chl b ratio of this preparation is lower than the RC 160. As expected, from the work of Sane *et al.* (5), the RC 40 preparation has characteristics intermediate between RC 160 and RC 10. The lower P-700/chlorophyll and chl a/chl b ratios of the RC 10 preparation may be due to residual photosystem 2 chlorophylls, which are not present in the RC 160 preparation. For this reason it appears that the

RC 160 preparation may be more useful than RC 10 or preparations made from whole chloroplasts for characterization of photosystem I reaction centers. The HP 700 preparation of Yamamoto and Vernon (3) was prepared from whole chloroplasts and one would expect that such a preparation would have characteristics of our most abundant fraction, namely, RC 10. The red absorption maximum of our preparation, like that of Yamamoto and Vernon(3), occurs at 676 nm though our difference spectrum maximum is shifted 1-2 nm toward the blue compared with the HP 700 preparation.

If the material is not washed with EDTA prior to acetone extraction, RC preparations of lower chl a/chl b ratio are obtained. It appears that prior EDTA washing permits a more complete extraction of chlorophyll b. The nitrogen content of different RC preparations is given in Table I. The nitrogen content is generally lowered by washing with EDTA. This purification is evident from electrophoresis of these preparations. The electrophoresis of all the RC preparations gives only one protein-chlorophyll complex. There are, however, other protein bands in material not washed thoroughly with EDTA prior to acetone extraction.

The absence of detergent treatment, the high P-700 content, very high chl a/chl b ratio, and ease of resuspension of the RC 160 suggest that this material will be extremely useful for further characterization of this kind of reaction center.

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Table I

CHLOROPHYLL, P-700, AND NITROGEN CONTENT OF RC PREPARATIONS

The figures in brackets show Chl a/Chl b and P-700 content prior to acetone extraction.

Preparation	Chl a/Chl b	$\frac{\text{Moles of Chl a + Chl b}}{\text{Moles of P-700}}$	$\frac{\mu g \text{ of N}}{m\mu mole \text{ of P-700}}$
RC 160*	> 10 (5-6)	16** (100 to 130)	150
RC 40	8-9 (3-4)	20 (200 to 250)	160
RC 10	7 (2.2-2.4)	32 (500 to 650)	380

*Red absorption maximum for all preparations, 676 nm.
**Difference spectrum maximum, 696-697 nm.

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