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ACTION SPECTRA OF PHOTOSYSTEM I AND PHOTOSYSTEM II IN SPINACH CHLOROPLAST GRANA AND STROMA LAMELLAE

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SUMMARY

Stroma lamellae prepared by the French press method from spinach chloroplasts are similar to other Photosystem I fractions in showing a decline in quantum yield at less than 700 nm. Evidence is presented which suggests that the inactive chlorophyll in stroma lamellae may be in part due to the presence of regions of membrane synthesis in the stroma lamellae fraction.

INTRODUCTION

Previous studies on action spectra of Photosystem I fractions prepared from chloroplasts by detergents showed reduced quantum yields at 680 nm compared with longer wavelengths[1-3]. A similar decline in quantum yields at short wavelengths for a Photosystem I reaction occurs in chloroplasts isolated from variegated regions of a tobacco mutant[4]. These mutant chloroplasts contain mostly stroma lamellae and only Photosystem I. In this communication we report a similar wavelength dependence of quantum yields for $NADP^+$ reduction in a Photosystem I fraction prepared from spinach chloroplasts by the French press method[5]. This Photosystem I fraction corresponds to the stroma lamellae of the intact chloroplasts[5,6].

MATERIALS AND METHODS

Chloroplasts and chloroplast fractions were prepared according to the method described previously[5]. Chlorophylls were determined according to the method of ARNON[7]. Photosystem II activity was measured by monitoring the reduction of dichlorophenolindophenol (DCIP) at 580 nm. The reaction mixture for DCIP Hill reaction contained 0.05 M potassium phosphate (pH 7.4), 0.01 M KCl, neutralized methylamine 100 μmoles/ml. The concentration of DCIP was adjusted such that its absorption at 580 nm was 0.25–0.3. Sufficient chloroplasts or chloroplast fragments were added to give an absorbance of 0.3–0.4 for the actinic wavelength from 650 to 690 nm. For measurements at 700 and 710 nm the absorbance was 0.1–0.2 for the actinic beam. $NADP^+$ reduction was monitored at 340 nm. The conditions of assay were the same as described by ANDERSON AND BOARDMAN[8]. Ferredoxin, $NADP^+$

Abbreviations: DCIP, dichlorophenolindophenol; DPC, diphenylcarbazide, DCMU, 3-(3,4 dichlorophenyl)-1,1-dimethylurea.

reductase and plastocyanin prepared according to the methods of TAGAWA AND ARNON[9], SHIN *et al.*[10] and KATOH *et al.*[11], respectively, were added in saturating amount to the reaction mixture.

All the measurements were carried out on a Cary 14 spectrophotometer equipped with a scattered transmission accessory[12]. Actinic light was supplied by a Bausch and Lomb 500 mm red blaze grating monochrometer operated with 3-mm slits yielding a half band width of 10 nm. A Corning 2–58 filter was placed in the actinic beam. For each wavelength, rate measurements of 6 light intensities in duplicate were taken. The quantum requirements were extrapolated to zero intensity and these zero intensity requirements are reported here. Quantum yields at 700 nm and lower are accurate to ± 10% Quantum yields at 710 nm are accurate to ± 20%.

RESULTS

We showed previously that the 160K fraction centrifugally isolated from French press treated spinach chloroplasts possesses only Photosystem I activity[5]. We also showed that the 160K fraction consists of stroma lamellae[5,6]. Since this fraction did not possess detectable Photosystem II activity we anticipated that the quantum yield for $NADP^+$ photoreduction would be constant with wavelength. The data given in Columns 5 and 6 of Table I indicate that our initial hypothesis is incorrect. Though the quantum yields for $NADP^+$ reduction by the 160K fraction are 0.9–1.0 at 710 nm, quantum yields are invariably less at shorter wavelengths. A minimum is constantly observed between 670 and 680 nm. We observe that the quantum yield at 650 nm is usually higher than the quantum yield at 670 or 680 nm.

TABLE I

QUANTUM YIELDS (ELECTRON EQUIVALENTS PER ABSORBED QUANTUM) OF $NADP^+$ PHOTOREDUCTION FROM ASCORBATE *plus* DCIP COUPLE

Wavelength (nm)	*Original*	*French press fraction*	*10K fraction*	*160K fraction (1500 lb/inch²)*	*160K fraction (1500 lb/inch²)*
650	—	—	0.15	0.48	0.40
660	0.28	0.13	0.21	0.40	0.48
670	—	—	0.24	0.28	0.34
680	0.28	0.12	0.14	0.31	0.36
690	0.45	0.45	0.48	0.36	0.59
700	≃1.00	0.43	—	0.59	0.67
710	—	—	—	0.91	1.00

This result was obtained whether we used the same chlorophyll concentration at all wavelengths or whether we adjusted chlorophyll concentration to give the same absorbed intensities. These results indicate that over half the chlorophylls in the 160K fraction are not involved in $NADP^+$ reduction. At 680 nm only 30–40% of the total absorption is coupled to the Photosystem I reaction. This raises a question regarding the function of the remaining chlorophyll in these isolated stroma lamellae. One could argue that the chlorophylls not coupled to $NADP^+$ reduction may belong only to damaged photosystems. Alternately the inactive chlorophyll may reside in a site of membrane synthesis in which the photosystems are functionally incomplete.

TABLE II

QUANTUM YIELDS (ELECTRON EQUIVALENTS PER ABSORBED QUANTUM) OF DCIP PHOTOREDUCTION BY FRENCH PRESS FRACTIONS

Wavelength (nm)	*Original*	*Quantum yield*		
		French press fraction (6000 lb/inch²)	*10K fraction (6000 lb/inch²)*	*10K fraction (1500 lb/inch²)*
650	0.43	0.17	0.14	0.20
660	—	—	—	0.22
670	—	—	—	0.19
680	0.34	0.09	0.11	0.20
690	—	—	—	0.18
700	0.23	0.15	0.22	0.12
710	0.12	0.15	0.25	—

In order to investigate the first possibility, the damage caused by the French press was assayed by determining quantum yields for $NADP^+$ reduction and DCIP reduction before and after treatment. The 2nd and 3rd columns of Tables I and II show the quantum yields for Photosystem I and Photosystem II reactions before and after French press disruption. Data for the original chloroplasts before disruption are similar to those already published[12–16]. There is 50% loss in the quantum yield for $NADP^+$ reduction after the treatment particularly at shorter wavelengths (660 and 680 nm). A much greater loss in quantum yield of DCIP reduction occurs due to French press disruption. The fact that long wavelength quantum yields of the 160K fraction approach 1 suggests that French press treatment does not destroy Photosystem I reaction centers of this fraction. However, uncoupling of the Photosystem I chlorophylls absorbing at short wavelengths from the Photosystem I reaction center may have occurred due to the French press treatment.

The quantum yield of DCIP reduction was studied in a 10K fraction obtained by using high (6000 lb/inch²) or low pressures (1500 lb/inch²) during French press disruption. Higher pressure treatment causes decreased efficiency of a component absorbing in the 680-nm region. Values as low as 0.07 for the quantum yield of DCIP reduction at 680 nm were observed for the 10K fraction after high pressure treatment. The facts that the 10K fraction shows lower quantum yields than original chloroplasts for DCIP Hill reaction and in addition is unable to completely photoreduce the quencher of Photosystem II fluorescence (Q)[17] indicate that damage to Photosystem II has occurred. The 10K fraction is also quite inefficient in $NADP^+$ reduction. The sum of the quantum yields of Photosystem I and Photosystem II reactions in the 10K fraction is less than 0.5 at wavelengths 680 nm and lower indicating that over 50% of the absorption is not coupled to either reaction. On the basis of these data we conclude that the uncoupled chlorophyll in the 10K fraction originates from damaged photosystems.

DISCUSSION

The quantum yield studies of French press fractions show that the treatment of chloroplasts with the French press results in damage to both Photosystem I and Photo-

system II in the total homogenate. These studies also show that the 160K fraction which possesses no Photosystem II activity possesses efficient Photosystem I at long wavelengths. However, at shorter wavelengths over half the chlorophyll is not coupled to Photosystem I. Our results and those in the literature enable us to eliminate some possible explanations for the uncoupled chlorophylls in the 160K fraction.

First, several experiments indicate that these inactive chlorophylls do not result from a damaged Photosystem II. Our study of variable fluorescence reported in the accompanying paper[17] shows that while the total amount of quencher Q in a chloroplast preparation is not altered by French press treatment, Q is present in negligible amounts in the 160K fraction. Another observation supporting our contention that the inactive chlorophylls of the 160K fraction do not result from a damaged Photosystem II is that the diphenyl carbazide (DPC) to DCIP reaction[18] is not only very slow, but is relatively insensitive to high concentrations of 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU). On the other hand, electrophoresis and cytochrome data indicate that some components of Photosystem II are present in the 160K fraction[5]. These observations can be explained if stroma lamellae contain a developing Photosystem II which is completed upon folding of the lamellae to form grana[19].

Second, there is some evidence in the literature which may indicate the uncoupled chlorophyll in the 160 K fraction does not result from a damaged Photosystem I. HOMANN AND SCHMID[4] have isolated chloroplasts containing primarily stroma lamellae from the variegated regions of a tobacco mutant. These stroma lamellae show a similar decline in quantum yield for Photosystem I at shorter wavelengths. The mutant chloroplasts were subjected only to ordinary chloroplast isolation procedures and neither to the French press nor to detergents. If these stroma lamellae isolated from the mutant are suitable models for the stroma lamellae of normal grana containing chloroplasts, the decline in Photosystem I quantum yields at short wavelengths suggests that the presence of uncoupled chlorophylls is an inherent characteristic of stroma lamellae.

Preparations by detergent techniques of what we interpret to be stroma lamellae fractions also demonstrate a decline in quantum yields at shorter wavelenghts. The studies by SCHWARTZ[2], VREDENBERG AND SLOOTEN[1] have shown that quantum yields for Photosystem I in digitonin Photosystem I fractions decline at shorter wavelengths. VERNON *et al.*[3] also reported a decline in quantum yield of $NADP^+$ reduction at shorter wavelengths in a Photosystem I fraction obtained by triton treatment observing lowest efficiencies between 670 and 680 nm. We conclude that the uncoupled chlorophylls in the 160K fraction are not due primarily to damage but are part of a developing membrane system. It is conceivable that the incompletely developed photosystems in the stroma lamellae may be completed on folding to form grana. Such a scheme for development fits the data on development of structure and function during greening in which stroma lamellae containing primarily Photosystem I activity precede the formation of grana and Photosystem II (refs. 20–22).

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