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Institute of Botany, Azerbaijan Academy of Sciences, Baku (U.S.S.R.), and Department of Botany, University of Illinois, Urbana, Illinois (U.S.A.)

Excitation Energy Transfer in Photosystems I and II from Grana and in Photosystem I from Stroma Lamellae, and Identification of Emission Bands with Pigment-Protein Complexes at 77 K[1]

R. GASANOV[2]) Z. K. ABILOV[2]), R. M. GAZANCHYAN[2]), U. M. KURBONOVA[2]), RITA KHANNA[3]) and GOVINDJEE[3])

With 14 figures

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Summary

Parallel measurements on action spectra of chlorophyll *a* fluorescence and absorption (direct and first derivative) spectra, at 77 K, are presented for grana, photosystem I from stroma lamellae, grana photosystem I and grana photosystem II. Analysis of quantum yield action spectra of chlorophyll *a* fluorescence show, *in all cases:* (1) the absence of «red drop»; (2) ~ 100 % efficiency of energy transfer from chlorophyll *b* to chlorophyll *a*; (3) a wavelength dependent variable efficiency of energy transfer from carotenoids to chlorophyll *a*, about 50 % in the 460 nm region, and about 25 % in the 490 nm region.

Examination of 77 K emission spectra, obtained with a slit width of ~1 nm, of preparations enriched in the light-harvesting chlorophyll *b* – chlorophyll *a* protein complex (LHPP) and the pigment system II core chlorophyll *a* (Ch a_{II}) from grana photosystem II shows that F 696 (fluorescence band at 696 nm) clearly belongs to Ch a_{II} and F 680–683 to LHPP. Further measurements reveal that F 685 is in a distinct physically separable complex (labelled as II b); it thus appears that F 685 and F 696, belonging to Chl a_{II}, may be in separate complexes. A complex, labelled II a, showing F 681 is, perhaps, derived from LHPP.

Key words: photosynthesis, energy transfer, action spectra of chlorophyll a, fluorescence, pigment-protein complexes.

Introduction

Low temperature (77 K) emission spectra of particles enriched in photosystem I (PS I) or II (PS II) (BOARDMAN et al., 1966; BRIL et al., 1969; BRODY et al., 1965; BROWN, 1969; CEDARSTRAND and GOVINDJEE, 1966; GASANOV and GOVINDJEE, 1974;

[1]) Dedicated to the memory of Prof. Dr. G. JACOBI of Göttingen.

[2]) Institute of Botany, Azerbaijan Academy of Sciences, Patamdarskoe 40, Baku 370073 (U.S.S.R.).

[3]) Departments of Physiology and Biophysics, and Botany, 289 Morrill Hall, University of Illinois, Urbana, Illinois 61801 (U.S.A.).

Govindjee, 1965; Ke and Vernon, 1967; Kok and Rurainski, 1966; Shimony et al., 1967; Vredenberg and Slooten, 1967) and of pigment-protein complexes isolated from these particles (Gazanchyan et al., 1975; Mohanty et al., 1972; Satoh and Butler, 1978; Strasser and Butler, 1977; Thornber, 1975; Vernon et al., 1971; Wessels and Borchert, 1975) are well documented in the literature. It appears that there are five emission bands: F 680, F 685, F 696, F 720 and F 730, where F stands for «fluorescence band at», and numbers indicate the approximate wavelengths of the maxima, in nanometers. Although emissions of PS I and PS II overlap at almost all the wavelengths, the present assignment is as follows: F 680 (a small band) is from light harvesting chlorophyll (Chl) *a*/Chl *b* complex LHPP (Rijgersberg et al., 1979); F 685 (a major band) is either from LHPP or from a Chl *a* complex closely associated with Chl a_{II}, or both (Cho and Govindjee, 1970; Mohanty et al., 1972), F 696 belongs to Chl a_{II} (this paper), and F 720 and F 730 (Cho and Govindjee, 1970) belong to Chl *a* complexes in PS I (Govindjee and Yang, 1966). In this paper, the identification of these various fluorescence bands with distinct pigment-protein complexes is established or confirmed. In particular, it is shown that F 681 is located on complex II *a*, and F 685 and F 696 are located on Chl a_{II} complex; the complex II *b* contains F 685.

In contrast to a vast number of studies on emission spectra, 77 K action (excitation) spectra of Chl *a* fluorescence of PS I and PS II particles are rare. Still more rare are quantum yield action spectra of Chl *a* fluorescence – that require the availability of both action spectra and absorption spectra for the same sample measured under the same experimental conditions. Only such measurements allow calculations of the efficiency of excitation energy transfer from various pigments to Chl *a*. Boardman et al. (1966) reported the action spectra of Chl *a* fluorescence in 10,000 ×g (enriched in PS II) and 144,000 ×g (enriched in PS I) fractions from chloroplasts at 20 °C and 77 K, and concluded that both Chl *b* and carotenoids are active in harvesting light quanta for the photochemistry of PS I and II; no quantum yield action spectra were presented. Satoh and Butler (1978) reported the incomplete action spectra of Chl *a* fluorescence for Chl a_{II} (in the 600–700 nm region, and even without the Chl *a* peak), for Chl a_I (only in blue and red regions, separately) and for fractions (labelled F–IV and F–V) enriched in carotenoids (only in the blue region); fraction IV showed energy transfer from carotenoids (at 460 and 490 nm) to Chl *a*, but fraction V did not. The quantum yield action spectra were not reported and thus quantitative conclusions were not possible. In this paper, we present systematic measurements on the action spectra of Chl *a* fluorescence, the absorption spectra and the quantum yield action spectra, at 77 K, for grana, grana PS I, grana PS II and PS I from stroma lamallae. It is shown that the efficiency of energy transfer from Ch *b* to Chl *a* is 100 %, and from carotenoids to Chl *a* is ~ 50 % (at 460 nm) and ~ 25 % (at 490 nm) *in all cases*. Furthermore a digitonin/Chl *a* ratio of 20 is needed to obtain «pure» PS II from grana; a digitonin/Chl *a* ratio of 7 is enough to yield «pure» PS I but the yield is low.

Materials and Methods

Chloroplasts were isolated from spinach leaves (purchased at the market) after blending cut leaves in phosphate buffer (0.06 M phosphate, 0.4 M sucrose and 0.01 M NaCl, pH 7.8), followed by differential centrifugation. Grana were isolated according to JACOBI and LEHMANN (1969) by sonication (50 W/cm^2; 0.6 A on the sonicator) for 20 seconds at 0 °C in high salt medium. The supernatant of a 3,000 ×g 10 min step was centrifuged at 10,000 ×g for 30 min. The sediment contained grana and supernatant was centrifuged at 70,000 ×g for 30 min, the supernatant of which was recentrifuged at 144,000 ×g for 60 min to obtain PS I from stroma lamellae.

Light and heavy fractions enriched in photosystem I and II, respectively, were obtained from grana by digitonin treatment of grana with gentle stirring (either at a digitonin/Chl ratio of 7 or 20) at 0 °C for 30 min (ARNTZEN et al., 1972; GASANOV and GOVINDJEE, 1974; GAZANCHYAN et al., 1975; OHKI and TAKAMIYA, 1970). Grana treated with digitonin were centrifuged at 10,000 ×g for 15 min, its supernatant was centrifuged at 20,000 ×g for 45 min to yield grana PS II in the sediment – the supernatant of the previous step was treated exactly as mentioned for the preparation of PS I from stroma lamellae to yield grana PS I.

Chlorophyll complexes enriched in LHPP and Chl a_{II} were prepared by the methods of THORNBER (1975) and of VERNON et al. (1971), respectively. In some experiments, chlorophyll protein complexes were prepared by SDS-gel electrophoresis. Chloroplast membranes, obtained from wheat leaves, were solubilized at room temperature in 1 % (w/v) SDS and electrophoresed immediately after the addition of the detergent (SDS/Chl = 2.5). Gels were made in 5 mm diameter and 70 mm long glass tubes and consisted of 20 % acrylamide – 0.6 % bis-acrylamide (w/v) made up in 80 ml of Tris-glycine (pH 8.3) containing 0.1 % SDS (w/v). Solubilized preparations (30 μg Chl per gel) were applied immediately on the gel, which were run at 4 °C with a starting current of 0.5 mA/gel for 15 min and then 4 mA/gel for the next 30–45 min. Gels were scanned with a microphotometer, using the gel scanning attachment.

The low temperature (77 K) absorption spectra and their first derivatives, as well as the room temperature absorption spectra, were recorded on a spectrophotometer equipped with an integrating sphere for light-scattering samples. The path length was 0.08 mm, and the chlorophyll concentration, as measured by the method of ARNON (1949), was 5 or 10 μg/ml suspension. In this low optical density range, the percent absorption spectrum (needed for the quantum yield calculations) parallels the absorbance (O.D.) spectrum. Thus, we could use directly the absorbance spectra, reported here, for quantum yield calculations. *For ease in comparison*, all curves were normalized to 1.0 at their red absorption maxima.

Action spectra of Chl *a* fluorescence, at 77 K, were recorded on a home-made spectrofluorometer described by ABILOV et al. (1967). The wavelength for the observation of Chl *a* fluorescence was 740 nm; in grana, both the direct excitation of PS I and energy transfer from PS II contributed to 740 nm emission; in PS I particles it was excitation of PS I, and in PS II particles, it was excitation of PS II. The half-band width of the observation monochromator's slit was 7 nm, and that of the excitation monochromator varied from 1 to 4 nm in the 400–730 nm region. The chlorophyll concentration and the path length of the excitation beam were the same as for absorption measurements. The action spectra of Chl *a* fluorescence were corrected for the variations in the incident quanta at different wavelengths and were plotted as $I_{fl}/I_{max} \cdot n$, where I_{fl} = intensity of fluorescence in relative units, I_{max} = intensity of fluorescence at the main peak, and n = number of incident light quanta.

Emission spectra were measured with a spectrofluorimeter similar to that described by SHIMONY et al. (1967) with 1 nm observation slits. These spectra were corrected for the transmission characteristics of the monochromator and the photomultiplier.

Results and Discussion

1. Absorption Spectra

The absorption spectra provide information for the location and the relative absorbance by various pigments. Since the pigment composition of PS I and PS II are different, the absorption spectra aid in judging the quality of separation of PS I and PS II; these spectra are also needed for the calculations on the efficiency of the excitation energy transfer from accessory pigments to Chl *a*.

Figures 1 and 2 show the absorption spectra of different chloroplast fragments (grana, PS I and PS II – enriched particles) measured at 20 °C and 77 K, respectively. Fig. 3 shows an expanded plot of the absorption spectra of PS I from stroma lamellae and grana PS II (obtained with digitonin/Chl ratio of 20) in the 600–720 nm region. The ratio of Chl *b*/Cl *a* absorbance is clearly greater (0.55) in grana PS II than in stroma PS I (0.41), the absorption maximum is at 676 nm in grana PS II and at 680 nm in PS I from stroma lamellae, and the long wavelength absorbing forms of Chl *a* are clearly missing in grana PS II. These data show that our preparations are indeed enriched in PS I and PS II, confirmed by photochemical activity measurements (not shown).

Table 1 lists the various absorbance peaks in the different samples along with their possible major origins. The differences in various spectral forms of Chl *a* in the red region of various samples, however, can be best seen in the first derivative absorbance spectra at 77 K (see Fig. 4 and Table 2). The long wavelength forms (Chl *a* 696–698, Chl *a* 702–706, and Chl *a* 709–712) are clearly localized only in PS I, not in PS II. A digitonin/Chl ratio of 20 was needed to obtain PS II particles free from the long wavelength forms of Chl *a*; with digitonin/Chl ratio of 7, PS II of grana still contained these long wavelength forms of Chl *a* (Fig. 4, B).

The PS I from stroma lamellae did not show any sign of absorption band at 640 nm [associated by LEPPINK and THOMAS (1973) to PS II], and at 662 nm (associated with monomeric Chl *a*) (Fig. 4, Table 2); it had the least amount of Chl *b*, as noted above, and it showed a drastic reduction in PS II activity (DPC → DCIP reaction, not shown). Stroma lamellae may be the most primitive lamellae – that can be associated with the primary thylakoids formed from prolamellar bodies (ARGYROUDI-AKOYUNOGLOU et al., 1977 b; SHYLK et al., 1967). The grana PS I had features intermediate between grana PS II and PS I from stroma lamellae. This could be due to true differences between PS I of grana and stroma lamellae (see GASANOV and GOVINDJEE, 1974; GASANOV and FRENCH, 1973) or due to some contamination with grana PS II. Increasing the digitonin/Chl ratio from 7 to 20 did not significantly effect the absorption spectrum of this system in contrast to the observations with PS II; there was only a slight decrease in the carotenoid region as if some carotenoids were removed from the preparation.

Fig. 5 (bottom) shows the densitometric tracing of Chl-proteins on polyacrylamide gels. The electrophoresis reveals, in addition to the classical three colored bands

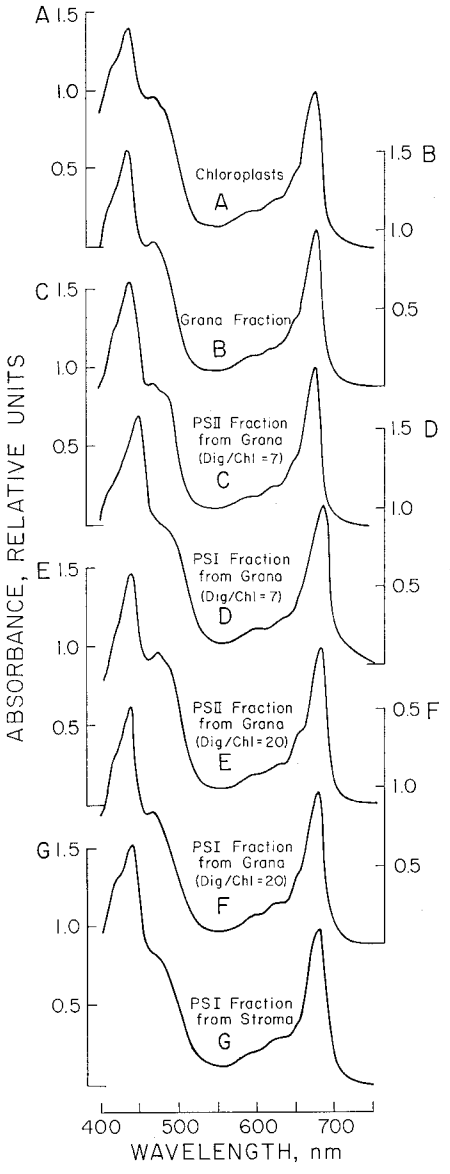

Fig. 1. Room temperature (20 °C) absorption spectra of suspensions containing 5 μg Chl/ml. The Chl *a* peak in the red region was adjusted to an arbitrary value of 1.0. A, Chloroplasts; B, Grana; C, Grana PS II (Dig/Chl = 7); D, Grana PS I (Dig/Chl = 7); E, Grana PS II (Dig/Chl = 20); F, Grana PS I (Dig/Chl = 20); G, PS I from stroma lamellae. Dig = digitonin; Chl = chlorophyll; PS I = photosystem I. PS II = photosystem II. There was no significant effect of digitonin on curve G (see text).

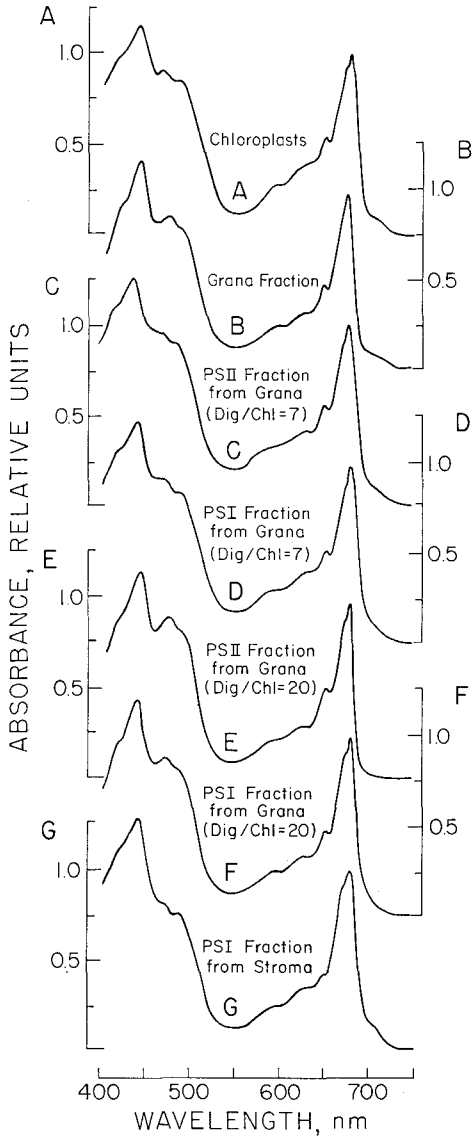

Fig. 2: Low temperature (77 K) absorption spectra of suspensions (pathlength, 0.08 mm; [Chl], 5 μg/ml). The Chl *a* peak in the red region was adjusted to an arbitrary value of 1.0. A, Chloroplasts; B, Grana; C, Grana PS II (Dig/Chl = 7); D, Grana PS I (Dig/Chl = 7); E, Grana PS II (Dig/Chl = 20); F, Grana PS I (Dig/Chl = 20); G, PS I from stroma lamellae. Peak and shoulder locations are shown in Table 1. Symbols as in Fig. 1; there was no significant effect of digitonin on curve G.

(I (= P 700 complex), II c (= LHPP) and III (= free chlorophyll)), two minor pigmented bands designated as II a and II b. Fig. 6 shows the absorption spectra at 77 K of two pigmented zones I (complex I) and II c (see below). Complex I contains mainly Chl *a* with a red wavelength maximum located at 679 nm, some carotenoids (shoulders at 460 and 490 nm) and almost no detectable Chl *b*. This complex is equivalent to P 700 – Chlorophyll *a* – protein complex described earlier (see THORNBER, 1975).

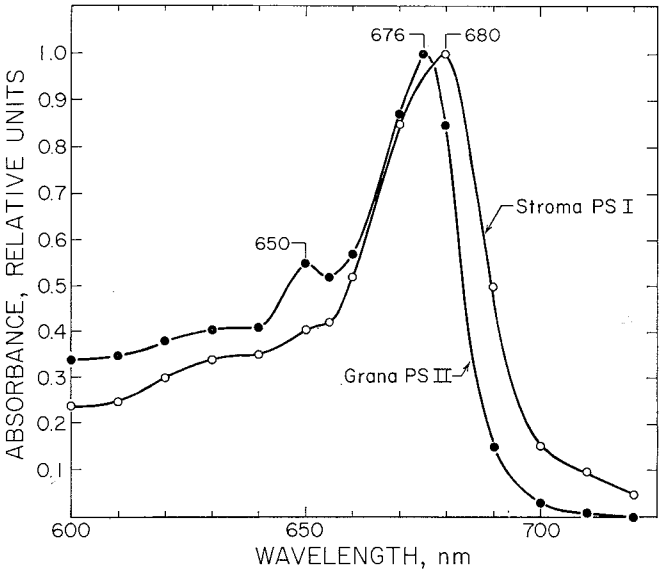

Fig. 3: 77 K absorption spectra of suspension of PS I from stroma lamellae and Grana PS II (Dig/Chl) = 20; [Chl], 5 μg/ml; pathlength, 0.08 mm). Chl *a* peak in the red region was adjusted to an arbitrary value of 1.0. Open circles, PS I from stroma lamellae, Solid dots, Grana PS II. Symbols as in Fig. 1. The 676 and 680 nm peaks are due to chlorophyll *a*, and 650 nm peak due to chlorophyll *b*.

Complex II c possesses Chl *a* and *b* almost in equal amount and more carotenoids than complex I. Its absorption maxima in the red region are at 673 and 650 nm. This complex corresponds to the light-harvesting chlorophyll *a/b* – protein complex (LHPP). First derivative absorption spectra of complex I and II c are shown in Fig. 7. A comparison of complex I and PS I from stroma lamellae and grana PS I particles shows that the major Chl *a* forms at 670, 679 and 685 nm are present in complex I; moreover, this complex is enriched in 692 and 705 nm Chl *a* forms. In complex II c, Chl *a* forms at longer wavelengths than 690 nm are not detected. Absorption spectra of complex II a did not show any detectable Chl *b* (data not shown).

Table 1: Some spectral characteristics, at 77 K, of Grana, Grana PS II, Grana PS I and PS I from stroma lamellae (S = Shoulder, P = Peak; main maxima are underlined; Shoulder λs are not well defined [Chl = Chlorophyll; Car = Carotenoid] (in nm).

Sample	Absorption (nm)	Excitation of Fluorescence (nm)
Grana	416 (S; Chl); 440 (P; Chl *a*); 472 (P; Chl *b* + Car); 485 (S; Chl *b* + Car); 595 (S; Chl); 630 (S; Chl); 650 (P; Chl *b*); 670 (S; Chl *a*); 677 (P; Chl *a*); 710 (S; Chl *a*)	–; 440 (P; Chl *a*); 472 (P; Chl *b* + Car) 490 (S; Chl *b* + Car); 595 (S; Chl); 625 (S; Chl); 650 (P; Chl *b*); 670 (S; Chl *a*); 677 (P; Chl *a*); 710 (S; Chl *a*)
PS I from stroma lamellae	418 (S; Chl); 441 (P; Chl *a*); 470 (S; Chl *b* (?) + Car); 486 (S; Chl *b* (?) + Car); 595 (S; Chl); 630 (S; Chl); 650 (S; Chl *b* (?) + Chl *a*); 672 (S; Chl *a*); 680 (P; Chl *a*); 710 (S; Chl *a*)	–; 441 (P; Chl *a*); 465 (S; Chl *b* (?) + Car); 486 (S; Chl *b* (?) + Car); 595 (S; Chl *a*); 652 (P; Chl *b* (?) + Chl *a*); 675 (S; Chl *a*); 680 (P; Chl *a*); 710 (S; Chl *a*)
Grana PS I (Dig/Chl = 20)	418 (S; Chl); 441 (P; Chl *a*); 472 (S; Chl *b* (?) + Car); 486 (S; Chl *b* (?) + Car); 595 (S; Chl); 625 (S; Chl); 649 (P; Chl *b* (?) + Chl *a*); 672 (S; Chl *a*); 678 (P; Chl *a*); 710 (S; Chl *a*)	–; 441 (P; Chl *a*); 470 (S; Chl *b* (?) + Car); 492 (S; Car); 595 (S; Chl); 625 (S; Chl); 650 (P; Chl *b* (?) + Chl *a*); 668 (S; not clear; Chl *a*); 678 (P; Chl *a*); 710 (S; Chl *a*)
Grana PS II (Dig/Chl = 20)	415 (S; Chl); 439 (P; Chl *a*); 472 (P; Chl *b* + Car); 492 (S; Chl *b* + Car); 595 (S; Chl); 625 (S; Chl); 650 (Chl *b*); 670 (S; Chl *a*); 676 (P; Chl *a*); 710 (absent)	–; 440 (P; Chl *a*); 472 (P; Chl *b* + Car); 492 (S; Chl *b* + Car); 595 (S; Chl *a*); 625 (S; Chl); 650 (Chl *b*); 670 (not clear S; Chl *a*); 676 (P; Chl *a*); 712 (S; very low; Chl *a*)

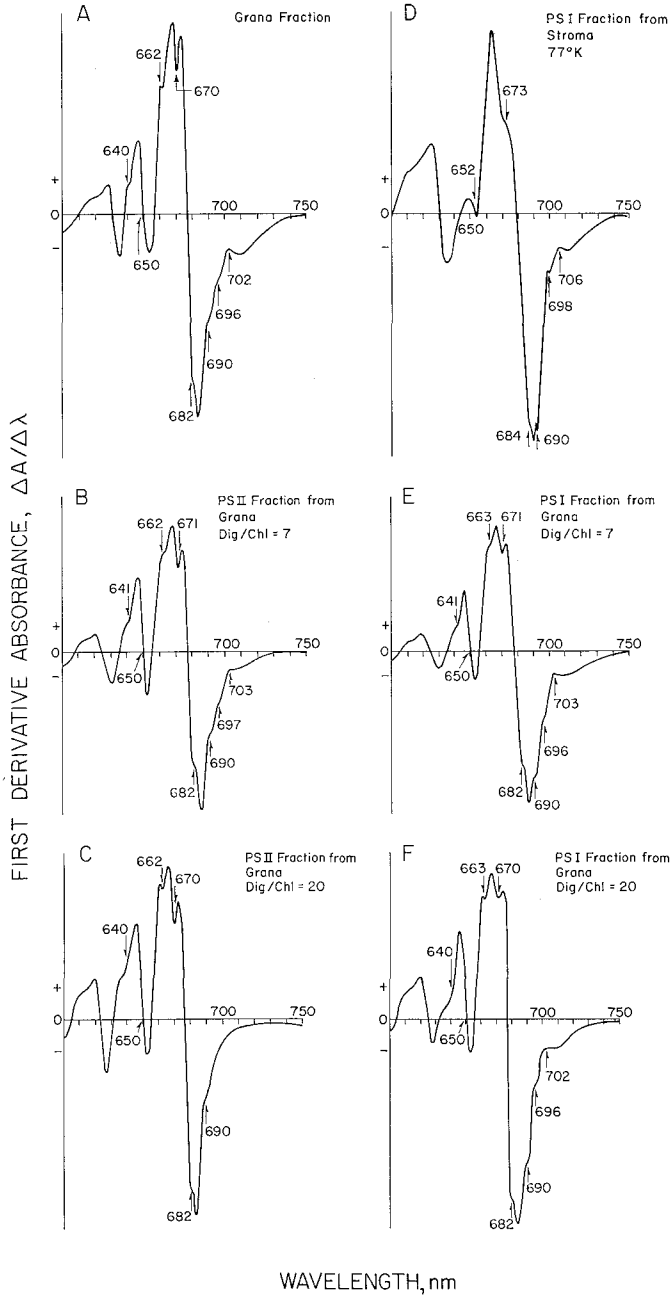

Fig. 4: First derivative absorption spectra ($\Delta A/\Delta\lambda$), at 77 K, as a function of wavelength in the 600–750 nm region. A, Grana fraction, B, Grana PS II (Dig/Chl = 7); C, Grana PS II (Dig/Chl = 20); D, PS I from stroma lamellae; E, Grana PS I (Dig/Chl = 7); F, Grana PS I (Dig/Chl = 20). Symbols as in Fig. 1. (See Table 2 for summary.)

Table 2: Suggested location of Peaks (P) and «Shoulders» (S) in 77 K derivative absorption spectra for different fractions (in nm).

Samples	Chl b		Chl a							
Grana	S 640	P 650	P 662	P 670	P max 677	S 682	S 690	S 696	P 702	S 710
Grana PS II (Dig/Chl = 20)	S 640	P 650	P 662	P 670	P max 677	S 682	S 690	–	–	–
Grana PS I (Dig/Chl = 20)	S 640	P 650	P 663	P 670	P max 677	S 682	S 690	S 696	P 702	S 709
PS I from stroma lamellae	–	P 652	–	S 673	P max 679	S 684	P 690	P 698	P 706	S 712

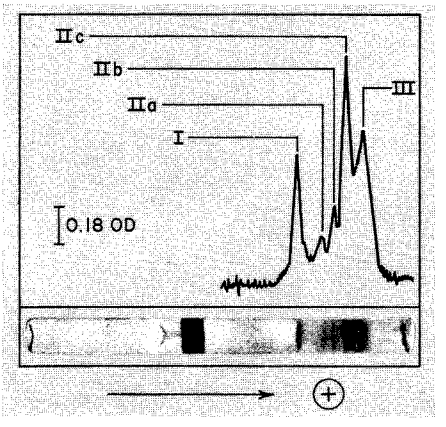

Fig. 5: Densitometric scans of electrophoretic gels of Sodium dodecyl sulfate (SDS) – pigment protein complexes obtained from wheat thylakoids. I = CPI (P700-Chl *a* complex); II a and II b = minor Chl *a* – protein complexes from photosystem II; II c = light harvesting pigment protein complex (LHPP) and III = free chlorophyll.

2. *Action Spectra of Chlorophyll a Fluorescence*

Action spectra of Chl *a* fluorescence, presented here, should parallel percent absorption spectra if all absorbing pigments transfer energy with 100 % efficiency to the Chl *a* forms fluorescing at 740 nm. As noted in the METHODS, our action spectra could be compared directly with the absorption spectra since very dilute suspensions were used. (For action spectra of Chl *a* fluorescence in different mixtures of acetone and water, see DAS and GOVINDJEE [1975].)

Figure 8 shows the 77 K action spectra of Chl *a* fluorescence from grana, from particles enriched in PS I and PS II from grana, and PS I from stroma lamellae. The

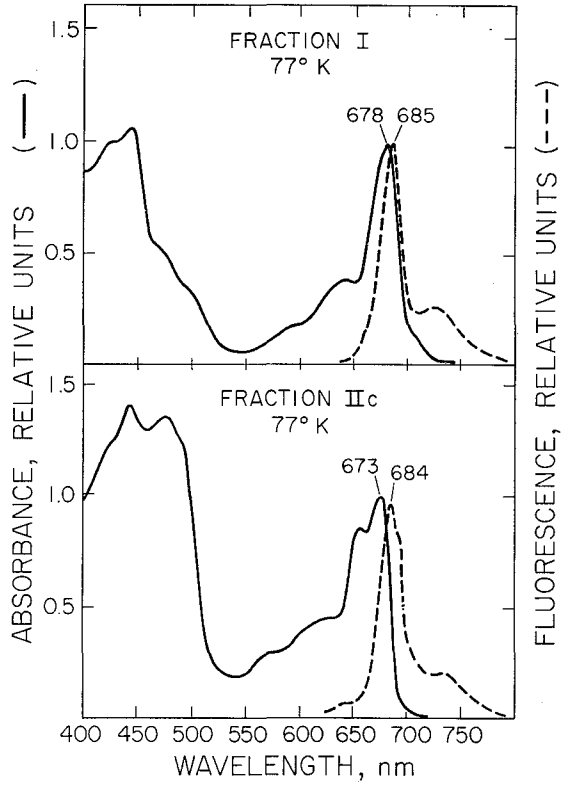

Fig. 6: 77 K absorption (solid) and fluorescence emission (dashes) spectra of preparations enriched in fraction CPI and II c (see the legend of Fig. 5).

major shoulders and peaks are listed in Table 1 and the ratios of fluorescence excited by some key wavelengths in Table 3. The action (excitation) spectrum of grana (Fig. 8, B) shows 4 major bands: at 677 nm (due to Chl *a*), at 650 nm (due to Chl *b*), at 476 nm (due to Chl *b*), and at 440 nm (due to the Soret band of Chl *a*). Carotenoids contribute to Chl *a* emission for all exciting wavelengths in the blue region, but they have considerably higher contributions at 460 nm, and 490–500 nm (see SATOH and BUTLER [1978]; cf. GOVINDJEE, 1960); a shoulder at 708 nm is due to a long wavelength form of Chl *a*. PS II fraction from grana (Fig. 8, C) also shows this shoulder, but the latter disappears when the digitonin/Chl ratio is increased to 20, and we obtain a more-enriched PS II (Fig. 8, D).

The major spectral differences between PS I from stroma lamellae and grana PS II (obtained with a digitonin/Chl ratio of 20) are shown in Fig. 9. In contrast to PS I-enriched particles, PS II-enriched particles show a higher Chl *b*/Chl *a* ratio, lower excitation in the long wavelength region (680–720 nm) and a shorter

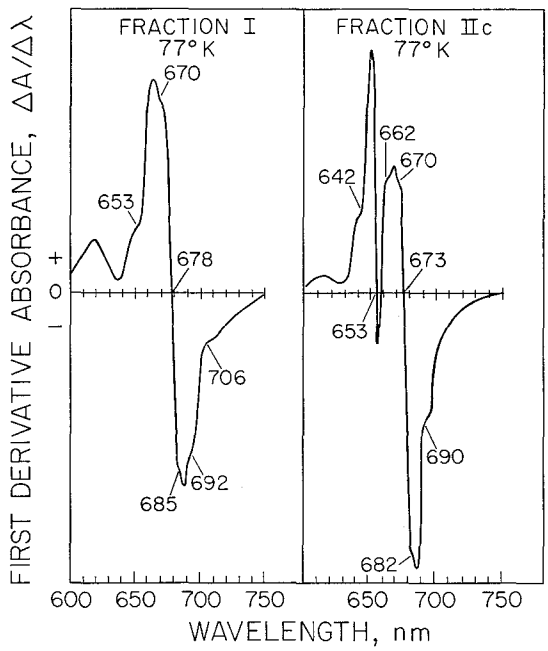

Fig. 7: First derivative absorption spectra ($\Delta A/\Delta\lambda$), of CPI and II c, at 77 K, as a function of wavelength in the 600–750 nm region. (See the legend of Fig. 5.)

Table 3. The ratio of maxima at 77 K in fluorescence excitation spectra. (Fluorescence measured at 740 nm.)

Samples	Ratios				
	676–680*) / 650	676–680 / 710	676–680 / 490	440 / 490	440 / 480
Grana	2.08	11.1	2.38	2.02	1.70
PS I from stroma lamellae	2.38	9.09	2.56	2.38	2.21
Grana PS II (Dig/Chl = 7)	1.81	14.2	2.04	1.77	1.67
Grana PS II (Dig/Chl = 20)	2.0	25.0	2.22	1.88	1.63
Grana PS I (Dig/Chl = 7)	1.92	11.1	2.38	1.95	1.76
Grana PS I (Dig/Chl = 20)	2.13	11.1	2.27	1.93	1.78

*) Wavelength of excitation, in nm; the aproximate assignments are: 677–678, Chl *a;* 650, Chl *b* + Chl *a*; 710, longwave form of Chl *a*; 490, Car + Chl *b*; 480, Chl + Car. (Chl = Chlorophyll; Car = carotenoid)

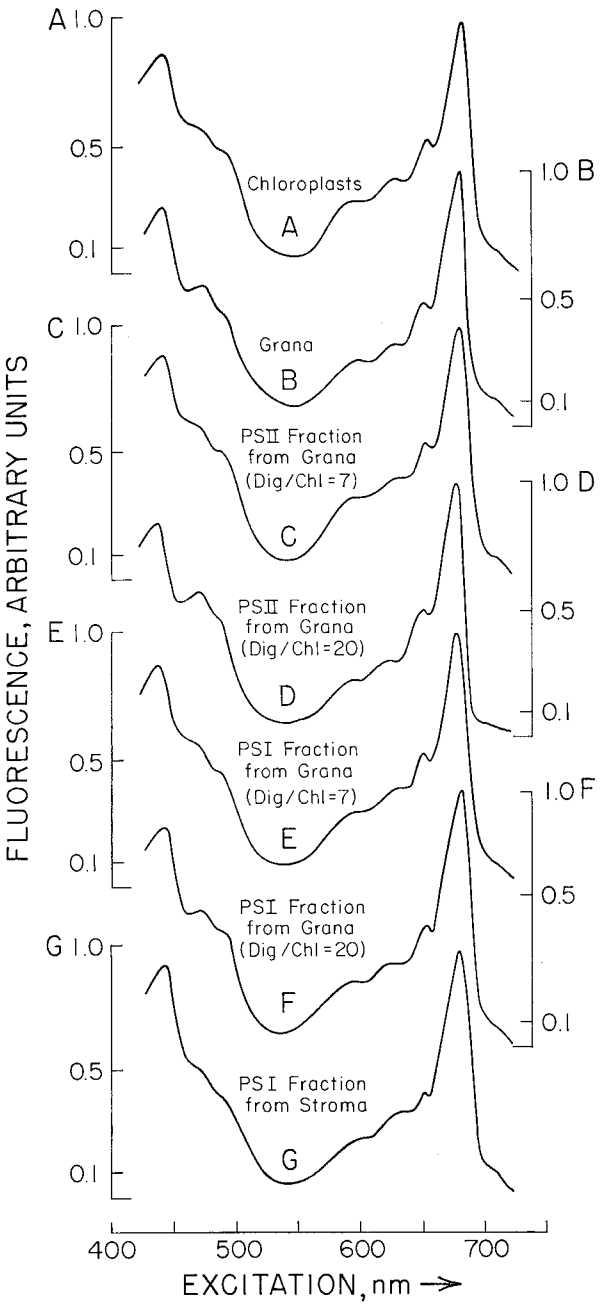

Fig. 8: Action spectra of chlorophyll *a* fluorescence at 77 K (wavelength of observation, 740 nm). [Chl], 5 μg/ml. Ordinate, fluorescence intensity divided by the number of incident quanta; the excitation peaks in the red region were arbitrarily adjusted to a value of 1.0. A, Chloroplasts; B, Grana; C, Grana PS II (Dig/Chl = 7); D, Grana PS II (Dig/Chl = 20); E, Grana PS I (Dig/Chl = 7); F = Grana PS I (Dig/Chl = 20); G, PS I from stroma lamellae. Symbols as in Fig. 1. (See Table 1 for location of peaks and shoulders.)

wavelength (676 nm) of the peak maximum. In PS I, the Chl *b* peak at 650 nm is drastically reduced, the maximum of excitation peak is at 680 nm and the shoulder at 708 nm is clear. The above results conform exactly to the results on the absorption spectra. Grana PS I again had some similarity to grana PS II and could be due to either some contamination of PS II or true differences between the two PS I from grana and stroma lamellae.

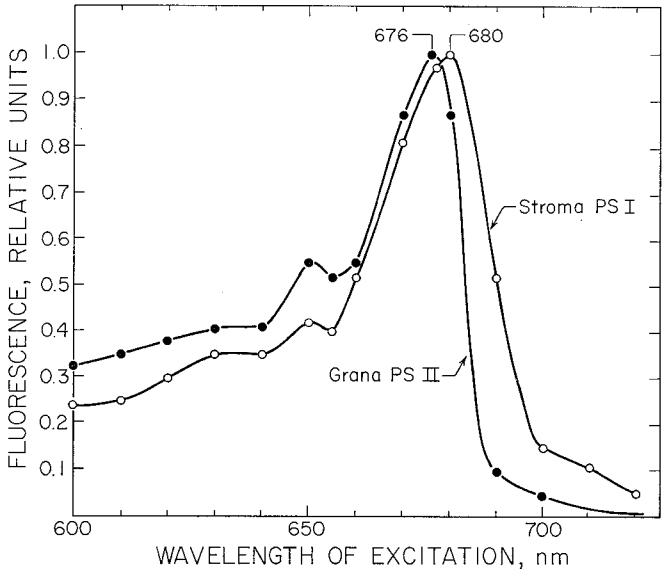

Fig. 9: Action spectra of chlorophyll *a* fluorescence at 77 K in the 600–700 nm range. Open circles, PS I from stroma lamellae; solid dots, Grana PS II (Dig/Chl = 20). Ordinate, fluorescence intensity at 740 nm divided by the number of incident quanta; excitation peaks were adjusted to a value of 1.0. The 676 and 680 nm peaks are due to Chl *a*, and a band at 650 nm is due to Chl *b*.

As in the absorption spectra (Fig. 2), using the digitonin/Chl ratio of 20, in contrast to a ratio of 7, action spectra provided a more enriched PS II spectrum for grana PS II (cf. Fig. 8, D with 8, C). There was a lower excitation at 690 nm as well as a slightly lower excitation in the carotenoid region with the higher digitonin treatment. Changing the ratio from 7 to 20 had a much smaller effect on grana PS I – there was only a slight reduction in the carotenoid region without any effect in the 690 nm region (cf. Fig. 8, F with 8, E). Almost parallel effects of changing the digitonin/Chl ratio on absorbance (Fig. 2) and action spectra of fluorescence (Fig. 8) indicate almost no change in the efficiency of excitation energy transfer.

3. Excitation Energy Transfer: Quantum Yield Action Spectra

The efficiency of excitation energy transfer from a donor D may be calculated from the action spectra of the acceptor fluorescence and the percent absorption spectra as follows:

$$T = \frac{F_A{}^A/A_A}{F_D{}^A/A_D} \times 100$$

where, T is the percent efficiency of excitation energy transfer from a donor D to an acceptor A, $F_A{}^A$ is the fluorescence intensity of the acceptor fluorescence, when excited by absorption in the acceptor molecule, A_A is fractional (percent) absorption

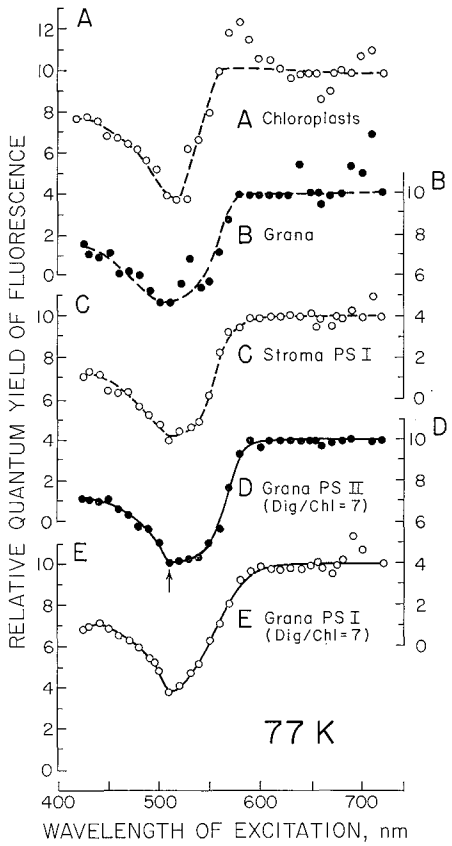

Fig. 10: Relative quantum yield of chlorophyll *a* fluorescence at 740 nm as a function of wavelength of exciting light, at 77 K. A, Chloroplasts, B, Grana; C, PS I from stroma lamellae; D, Grana PS II (Dig/Chl = 7); E, Grana PS I (Dig/Chl = 7). Symbols as in Fig. 1; details in the 600–700 nm are not drawn (see Fig. 11 for further details on more enriched PS I and PS II preparations).

(not OD or absorbance, unless very dilute samples are used when % absorption parallels OD), F_D^A is fluorescence intensity of acceptor fluorescence when excited by absorption in the donor molecule, and A_D is fractional absorption by the donor molecule. When several pigments absorb at a wavelength of excitation, the fractional distribution of quanta among the various pigments should be known (see RABINOWITCH, 1951, p. 718).

The quantum yield action spectra of Chl *a* fluorescence are calculated as F_λ^A/A_λ where λ is the wavelength of excitation, and plotted as a function of excitation wavelength. Such spectra provide information on the efficiency of excitation energy transfer. Fig. 10 shows a family of such spectra in relative units; here, larger errors are possible in regions where absorption and fluorescence are low and where the cuves are too steep to read precisely. In spite of these possible problems, all the quantum yield action spectra are approximately flat in the 580–720 nm region (for a discussion of differences, see later). Contrary to room temperature curves of chloroplasts and grana (not shown; see DAS and GOVINDJEE, 1973), there is no «red drop» confirming that at 77 K none of the Chl a species are non or weakly fluorescent and the efficiency of excitation energy transfer from one Chl *a* form to another is about 100 % in all cases. The almost flat quantum yield action spectra in the 600 to 700 nm region in all cases proves, without further calculations, that the efficiency of excitation energy transfer from Chl *b* to Chl *a* is also almost 100 % in all cases where Chl *b* is present.

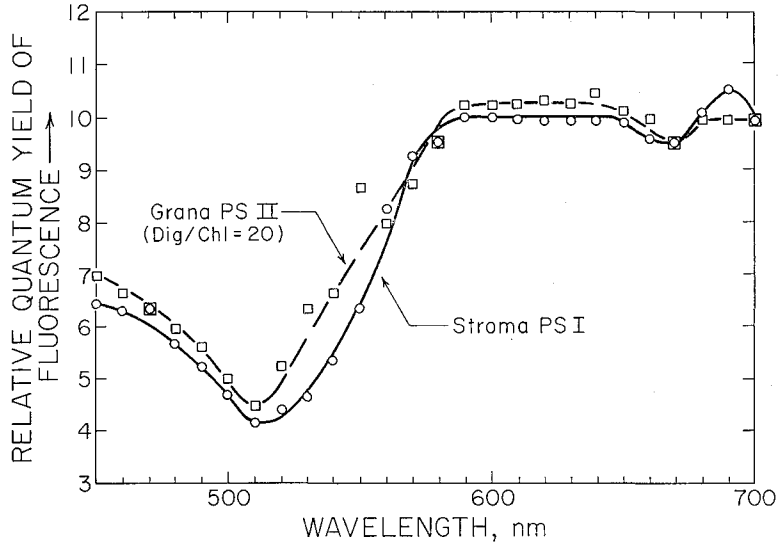

Fig. 11: Relative quantum yield of chlorophyll *a* fluorescence at 740 nm as a function of wavelength of exciting light, at 77 K. □–□, Grana PS II (Dig/Chl = 20); ○–○, PS I from stroma lamellae. Symbols as in Fig. 1. Note a small dip at 670 nm, and a small peak at 690 nm.

Minor differences (slightly lower values in the 660–670 nm region, and higher values in the 690–710 nm region), however, are, perhaps, real. In order to asses the reality of these changes an average of all available data was made; this average curve also showed these differences. A small positive band at 690–700 nm band is explained as follows: in PS I, the fluorescence at 740 nm is primarily from a Chl *a* 690–720 nm (see DAS and GOVINDJEE, 1967) and the small positive band is produced due to a slightly reduced (still 95–98 %) efficiency of energy transfer from other Chl *a* species to it. The negative band at 660–670 nm be due to a further reduced (still 90 %) energy transfer of ~ 90 % from a Chl *a* form (absorbing in this region) to Chl *a* fluorescing at 740 nm. The large dip at 510 nm will be discussed below.

The differences between PS I from stroma lamellae and grana PS II (obtained with a digitonin/Chl ratio of 20) are shown in Fig. 11. In PS II, the 690 nm band is absent (Fig. 9) confirming the absence of long wavelength form of Chl *a* (assigned to PS I) and pointing out the differences in the nature of 740 nm emission in PS II from that in PS I. The small dip at 670 nm in the quantum yield spectrum is still present. There are no large differences between the PS I and PS II regarding energy transfer in the 650 nm to 680 nm region. Both PS I and PS II spectra show, however, a large dip at 510 nm (Fig. 11) although PS II spectrum rises at shorter wavelengths than PS I spectrum in the 510 to 560 nm region, perhaps, suggesting some differences in the tail end of the absorption by carotenoids in PS I and PS II. The large dip at 510–520 nm region in all cases is due to a low efficiency of excitation energy transfer from carotenoids to Chl *a*.

Efficiency of excitation energy transfer from carotenoids to Chl *a* may be calculated by making some reasonable assumptions: efficiency of energy transfer from Chl *b* to Chl *a* is ~ 100 % as shown in the red region of the spectrum, and (2) the fractional absorption by Chl *a*, Chl *b*, and carotenoids can be estimated, as was done for the green alga *Chlorella* by EMERSON and LEWIS (1943) and GOVINDJEE

Table 4: Estimated fractional absorption of pigments in the carotenoid region in chloroplasts.

λ, nm	Chl *a*	Chl *b*	Carotenoids
440	0.65	0.05	0.30
450	0.30	0.14	0.55
460	0.05	0.20	0.76
470	0.02	0.35	0.65
480	0.01	0.45	0.55
490	0.02	0.35	0.63
500	0.04	0.21	0.75
510	0.10	0.17	0.72

(1960); this estimate is made by extracting and separating the pigments, obtaining their absorption spectra, shifting the latter to match their peaks *in vivo*, and then calculating the fractional absorption by each pigment (Table 4).

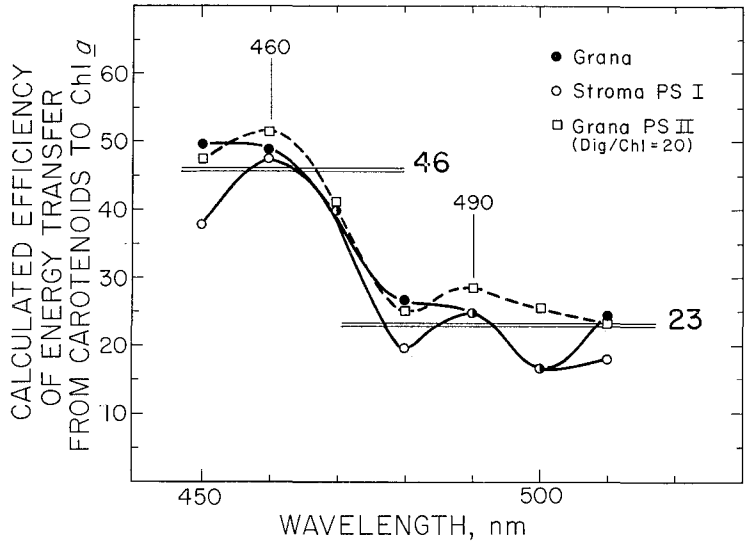

Fig. 12: Calculated efficiency of energy transfer from carotenoids to chlorophyll, *a*, at 77 K, as a function of excitation wavelength. ●, Grana; ○, PS I from stroma lamallae; □, Grana PS II (Dig/Chl = 20). Symbols as in Fig. 1. The bold numbers next to the double lines indicate average efficiencies of energy transfer in the regions indicated.

Since the amount of total carotenoids is about the same in both PS I and PS II, fractional absorption by carotenoids was estimated as a function of wavelength, and, using the following equation, the percent efficiency of energy transfer from carotenoids to Chl *a* was estimated: Φ/Φ_{680} (quantum yield of fluorescence at an exciting wavelength relative to that at 680 nm) = α_1 fractional absorption by Chl *a* + Chl *b*). «1» + α_2 (fractional absorption by carotenoids) · t, where *t* is efficiency of energy transfer from carotenoids to Chl *a* and «1» indicates that energy transfer from Chl *b* to Chl *a* is 100 %. These results are shown in Fig. 12. The main result is that in each case the efficiency of energy transfer was ~ 50 % (average of all) in the 460 nm region and ~25 % (average of all) in the 490 nm region. This suggests that there is a heterogeneity of carotenoids and that the forms having higher absorbance in the 490 nm region are relatively less efficient in energy transfer to Chl *a* than the others. If proven true this may have implications on the mechanism of energy transfer from carotenoids to Chl *a* but we don't know what it may be at this time [for a discussion of energy transfer in in chloroplasts from bundle sheath and mesophyll cells, see BAZZAZ and GOVINDJEE (1973)].

4. Emission Spectra of Different Chlorophyll-protein Complexes

BUTLER and KITAJIMA (1975) have associated F685 with LHPP, F696 with Chl a_{II} and F730 with Chl a_I (also see GOVINDJEE and YANG, 1966). However, the emission

band for isolated LHPP is in the 680–684 nm region, depending on the preparation, and for isolated Chl a_{II}, it is at 686 nm (KE and VERNON, 1967; SATOH and BUTLER, 1978; VERNON et al., 1971; WESSELS, 1977). RIJGERSBERG et al. (1979) pointed out that F680 should belong to LHPP, and thus both F685 and F696 to Chl a_{II}. F696 is clearly present in chloroplasts (GOVINDJEE and YANG, 1966) and in PS II-enriched

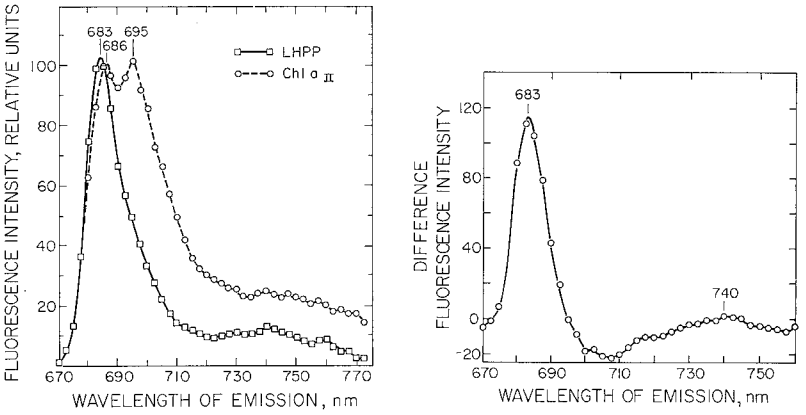

Fig. 13: (Left) Fluorescence emission spectra, at 77 K, of light-harvesting pigment protein complex (LHPP, squares) and Chl a_{II} – enriched preparations (circles). (Right): Difference emission spectrum of LHPP minus Chl a_{II} spectra.

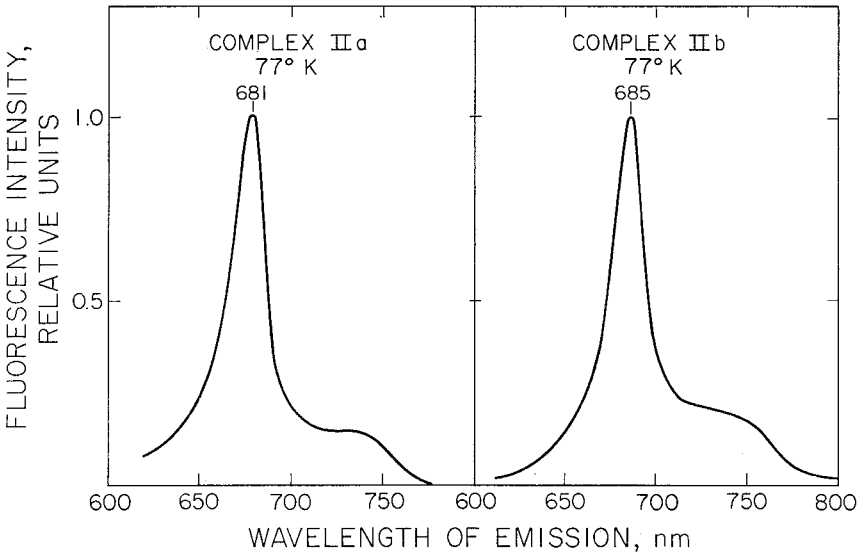

Fig. 14: Fluorescence emission spectra of gel pieces containing complex II a and II b, at 77 K (see the legend of Fig. 5).

particles (Gasanov and Govindjee, 1974; Mohanty et al., 1972). Figure 13 shows emission spectra of Chl a_{II}-enriched and LHPP-enriched samples at 77 K; the Chl a_{II} is clearly enriched in F696. The question is what happened to it in other preparations (Wessels, 1977). This could have been due to a cleaner preparation of PS II, but there are several possible reasons why one may have failed to observe correlation between F696 and Chl a_{II}: (1) if the band width of observation is too large, the two bands at 685 and 696 nm may appear as a single band at an intermediate location; (2) if the detergent solubilized chlorophylls, that emit at shorter wavelengths, present in the samples, mask the F696 band; and (3) there may be two separate Chl a complexes – one with F686 and the other with F696; some preparations may only contain one of them (Wessels, 1977), whereas others a mixture of the two bands (see Wessels and Borchert, 1975; this paper). Analysis of fluorescence emission spectra of chlorophyll-protein complexes, obtained by short term electrophoresis, and other methods, is consistent with the third interpretation.

The emission spectrum of P700 – chlorophyll *a* – protein complex (complex I) in Fig. 6 agrees quite well with that measured previously in the purified CPI at 77 K (Brown, 1976). The spectrum of LHPP (complex II c), in Fig. 6, also agrees reasonably well with the spectrum of purified light-harvesting chlorophyll *a/b* complex at 77 K (Satoh and Butler, 1978). The emission spectrum of the pigmented zone II b (Fig. 14) shows a maximum at 685 nm. These spectra are close to that obtained by several authors for Chl a_{II} and comparison of these spectra with those of Fig. 13 is not inconsistent with our assumption that two separate Chl a_{II} complexes – one with F685 (Fig. 14), and the other with F696 (see Fig. 13) may exist in chloroplast membrane. A proof, however, requires an isolation of a complex with only F696. The pigment zone II a with fluorescence maxima at 681 nm (Fig. 14) may be derived from LHPP (also see Remy et al., 1977; Dunkey and Anderson, 1979).

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Dr. Ralphreed Gasanov, Institute of Botany, Azerbaijan Academy of Sciences, Patamdarskoe 40, Baku 370073 (U.S.S.R.).

Professor Govindjee, Department of Botany, 289 Morrill Hall, University of Illinois, Urbana, Illinois 61801 (U.S.A.).