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ANALYSES OF ABSORPTION AND FLUORESCENCE SPECTRA OF WATER-SOLUBLE CHLOROPHYLL PROTEINS, PIGMENT SYSTEM II PARTICLES AND CHLOROPHYLL *a* IN DIETHYLETHER SOLUTION BY THE CURVE-FITTING METHOD

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Summary

Absorption and fluorescence spectra in the red region of water-soluble chlorophyll proteins, Lepidium CP661, CP663 and Brassica CP673, pigment System II particles of spinach chloroplasts and chlorophyll *a* in diethylether solution at 25°C were analyzed by the curve-fitting method (French, C.S., Brown, J.S. and Lawrence, M.C. (1972) Plant Physiol. 49, 421—429). It was found that each of the chlorophyll forms of the chlorophyll proteins and the pigment System II particles had a corresponding fluorescence band with the Stokes shift ranging from 0.6 to 4.0 nm.

The absorption spectrum of chlorophyll *a* in diethylether solution was analyzed to one major band with a peak at 660.5 nm and some minor bands, while the fluorescence spectrum was analyzed to one major band with a peak at 664.9 nm and some minor bands. A mirror image was clearly demonstrated between the resolved spectra of absorption and fluorescence. The absorption spectrum of Lepidium CP661 was composed of a chlorophyll *b* form with a peak at 652.8 nm and two chlorophyll *a* forms with peaks at 662.6 and 671.9 nm. The fluorescence spectrum was analyzed to five component bands. Three of them with peaks at 654.8, 664.6 and 674.6 nm were attributed to emissions of the three chlorophyll forms with the Stokes shift of 2.0—2.7 nm. The absorption spectrum of Brassica CP673 had a chlorophyll *b* form with a peak at 653.7 nm and four chlorophyll *a* forms with peaks at 662.7, 671.3, 676.9 and 684.2 nm. The fluorescence spectrum was resolved into seven component bands. Four of them with peaks at 666.7, 673.1, 677.5 and 686.2 nm corresponded to the four chlorophyll *a* forms with the Stokes shift of 0.6—4.0 nm. The absorption spectrum of the pigment System II particles

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had a chlorophyll *b* form with a peak at 652.4 nm and three chlorophyll *a* forms with peaks at 662.9, 672.1 and 681.6 nm. The fluorescence spectrum was analyzed to four major component bands with peaks at 674.1, 682.8, 692.0 and 706.7 nm and some minor bands. The former two bands corresponded to the chlorophyll *a* forms with peaks at 672.1 and 681.6 nm with the Stokes shift of 2.0 and 1.2 nm, respectively.

Absorption spectra at 25°C and at −196°C of the water-soluble chlorophyll proteins were compared by the curve-fitting method. The component bands at −196°C were blue-shifted by 0.8—4.1 nm and narrower in half widths as compared to those at 25°C.

Introduction

The absorption spectrum of chlorophyll *a* in polar organic solvents shows a single and apparently symmetrical band in the red region [1], while the absorption band of chlorophyll *a* in the thylakoid membranes of chloroplasts is broad, asymmetric and red-shifted [2]. These findings suggest that the absorption spectrum of chlorophyll *a* in the membranes is produced by an overlap of some absorption bands with different degrees of band-shift to longer wavelengths. A concept "chlorophyll form" has been introduced in order to distinguish the chlorophyll molecules with different peak wavelengths [3]. It has been suggested that the chlorophyll forms are produced by different natures of interaction of chlorophyll *a* with other membrane constituents.

Butler and Hopkins [4] reported six forms of chlorophyll *a* and two forms of chlorophyll *b* in spinach chloroplasts by computing the fourth derivative of the absorption spectrum at −196°C. By using a curve-fitting method, French et al. [5] showed the occurrence of four major forms of chlorophyll *a* and two forms of chlorophyll *b* in a variety of green plant chloroplasts. These chlorophyll forms are called Cb640, Cb650, Ca662, Ca670, Ca677 and Ca684 according to their peak wavelengths at −196°C. A biological function of the chlorophyll forms is to funnel excitation energy to the reaction center chlorophylls. A computer calculation by Seely [6] on model arrays of chlorophylls showed that the occurrence of spectral varieties of chlorophyll *a* increased the rate of excitation transfer to the reaction centers by a factor of four. However, the molecular basis of the interaction of chlorophyll *a* to other substances that determines the chlorophyll forms has not yet been fully understood.

The fluorescence spectrum at room temperature of chlorophyll *a* in the chloroplasts shows a maximum at about 683 nm and a broad band shoulder around 730 nm [7]. The fluorescence spectrum at liquid nitrogen temperature has three peaks at 684, 695 and about 730 nm [8]. The analysis of the low temperature fluorescence spectrum by the fourth derivative method has clearly demonstrated the occurrence of the two former bands [9,10]. These findings suggest that the fluorescence spectrum of chloroplasts is also composed of more than one bands with different peak wavelengths. In the present study, we analyzed by the curve-fitting method the fluorescence spectra of chlorophyll *a* diethylether solution, the water-soluble chlorophyll proteins and the pigment System II particles of spinach chloroplasts in comparison with the absorption spectra. It was found that each chlorophyll form in the absorption

spectra had a corresponding component band of fluorescence in the analyzed spectra.

Materials and Methods

Chlorophyll *a* was prepared according to the method of Sato and Murata [11] from a blue-green alga, *Anabaena variabilis* strain M-3, obtained from the Institute of Applied Microbiology, University of Tokyo. No contaminants such as other pigments, lipids and quinones were detected in the thin layer chromatography of Hager and Meyer-Bertenrath [12]. Water-soluble chlorophyll proteins, Lepidium CP661 and CP663, were prepared according to Murata and Murata [13] from the leaves of *Lepidium virginicum* cultivated from the seed collected near Mono Lake, California, and from those collected in the campus of Toho University, Izumicho, Funabashi, Japan, respectively. Another water-soluble chlorophyll protein, Brassica CP673, was prepared according to Murata and Murata [13] from the leaves of *Brassica nigra* collected in the campus of Stanford University, California. The pigment System II particles were prepared from spinach chloroplasts using digitonin according to the method of Ohki and Takamiya [14]. The chlorophyll *a* to chlorophyll *b* ratio of the preparation was 1.9.

For the measurements of absorption and fluorescence spectra, chlorophyll *a* was dissolved in freshly distilled diethylether. The chlorophyll proteins and the pigment System II particles were suspended in 0.1 M phosphate buffer, pH 7.0. The absorption spectra at 25°C were measured with a spectrophotometer (Shimadzu UV-200 or Shimadzu Double 40R), and digitally recorded. The light path length of the cuvette was 1 cm. Concentrations of samples were controlled so that absorbance did not exceed 0.4. For measurements of the absorption spectra of the chlorophyll proteins at −196°C, the samples dissolved in 0.1 M phosphate buffer were mixed with an equal volume of glycerol and then chilled. The spectra were measured with a spectrophotometer (Hitachi 356) and digitally recorded. Light pass length of the cuvette was 0.1 cm. Concentrations of samples were controlled so that absorbance did not exceed 0.4.

In the measurements of fluorescence spectra at 25°C, 3 ml of sample was put into a four-sided transparent cuvette with size of 1 cm × 1 cm × 4 cm. Concentrations of samples were controlled so as to give absorbance lower than 0.05 at the red absorption maximum. Excitation light was obtained from a 250 W incandescent lamp combined with optical filters (Hoya HA-50 and Corning 9782) and either of interference filters with transmission peak at 469 nm with half width of 13 nm, at 438 nm of 10 nm and at 431 nm of 14 nm (Japan Vacuum Optics Co.). Fluorescence emitted at the right angle to the excitation light was analyzed with a Bausch and Lomb 500 mm grating monochromator and detected with a photomultiplier (Hamamatsu TV R666). Half width of the slit of the monochromator was set at 2 nm. The signal from the photomultiplier was amplified and recorded on a strip chart servo recorder (Riken Denshi SP-H4) and also digitally printed out. The variation in transmission of the monochromator and sensitivity of the photomultiplier with wavelength was automatically corrected by changing the gain of amplifier

with wavelength to give a fluorescence spectrum expressed in terms of number of quanta per unit wavelength, which, however, was converted to that expressed in terms of number of quanta per unit wavenumber for calculation of the curve-fitting method.

To analyze the absorption and the fluorescence spectra, the curve-fitting method of French et al. [5] was modified so as to employ a three-step calculation as in the following:

Step 1: Initial conditions for the number of component bands and the values of the peak wavelengths were determined in different ways depending on the spectra. In the analyses of the absorption spectra at the liquid nitrogen temperature of Lepidium CP661, Brassica CP673 and the pigment System II particles and of the absorption and fluorescence spectra at room temperature of chlorophyll *a* in diethylether solution, Lepidium CP661 and CP663, the number of component bands and the peak wavelengths for the initial conditions were obtained applying the fourth derivative method of Butler and Hopkins [4] to these spectra. The initial values for the other parameters, half width, peak height and proportion of Gaussian and Lorentzian distribution curves were rather arbitrarily determined. In the absorption spectra at room temperature of Brassica CP673 and the pigment System II particles, the analyzed results of absorption spectra at liquid nitrogen temperature of these materials were used for the initial conditions. In the analyses of the fluorescence spectra at room temperature of Brassica CP673 and the pigment System II particles, the initial conditions were determined according to the analyzed results of the absorption spectra at room temperature of these materials, but the peak positions were shifted to longer wavelengths by 1—3 nm.

When a good fitting was not obtained between the observed and the analyzed spectra, the next calculation was performed by adding another appropriate band. This procedure was repeated until the spectra reached a satisfactory fitting to each other. It was found, however, that the resulting values varied slightly depending on the initial values put for the parameters in the calculation. Thus, the analyzed result in this step of calculation was not adopted for the final one, but was put forward to the next step.

Step 2: Two calculations were performed based on the result of step 1. In one calculation initial values for peak wavelengths 2 nm shorter than in the result of step 1 were used, while in the other calculation the values from those 2 nm longer were used. For other parameters, the values obtained in step 1 were used. In this step of the calculation, the lowest limits were set for changes in values for the parameters at each repeat of the calculation. The lowest limit was 5 cm^{-1} for changes in peak wavenumber and 2.5 cm^{-1} for changes in half width. The two calculations gave similar values for the parameters in most cases. Without setting the lowest limit, they produced relatively different results.

Step 3: The mean values in the results of two calculations in step 2 were used as initial values for the parameters in this calculation. The obtained values in the calculation were adopted as final results, and were printed out and also recorded on a chart.

The computer program RESOLV for the curve-fitting method was offered by courtesy of Dr. C.S. French and re-written to accommodate it to the com-

puter and plotter system used in the present study. The calculations were performed by Hitachi HITAC 8800/8700 computer system at the Computer Center, University of Tokyo.

Results

Chlorophyll a in diethylether solution

The absorption spectrum of chlorophyll *a* in diethylether solution had a major peak at 660 nm and a broad minor peak at 615 nm in the red region. The fluorescence spectrum had a major peak at 665 nm and a minor peak at 722 nm. Table I and Fig. 1 show analyzed results of the absorption and the fluorescence spectra. The absorption spectrum was composed of one major band with a peak at 660.5 nm and two minor bands with peaks at 615.0 nm

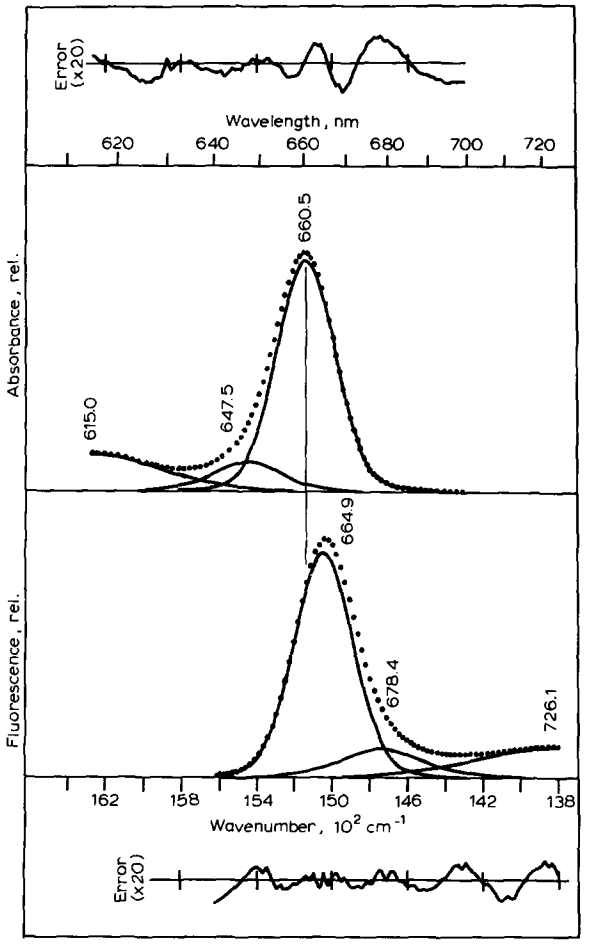

Fig. 1. Analyzed results of absorption and fluorescence spectra of chlorophyll *a* in diethylether solution at 25°C. Fluorescence was excited at 431 nm. ·····, observed spectra. ———, analyzed component bands.

TABLE I

ANALYZED RESULTS OF ABSORPTION AND FLUORESCENCE SPECTRA OF CHLOROPHYLL *a* IN DIETHYLETHER SOLUTION AT 25°C

Fluorescence was excited at 431 nm.

Spectra	λ_{max} (nm)	$\Delta\lambda_{1/2}$ (nm)	Gaussian (%)	Stokes shift (nm)
Absorption	615.0	32.0	84.9	
	647.5	17.8	72.6	
	660.5 *	15.7	91.6	
Fluorescence	664.9 *	16.2	88.2	4.4
	678.4	23.7	50.1	
	726.1	59.2	99.8	

* Represents corresponding bands in Stokes shift.

and 647.5 nm. The 647.5 nm band was necessary for a good fitting of the composed to the observed spectrum, as suggested by French et al. [15]. The fluorescence spectrum was composed of one major band with a peak at 664.9 nm and two minor bands with peaks at 678.4 nm and 726.1 nm. In the absorption and the fluorescence spectra, the major bands were attributed mainly to Gaussian curves; the contribution of the Gaussian curve was 91.6% in the major band of the absorption spectrum and 88.2% in the major band of the fluorescence spectrum. The Stokes shift between the major component bands of the absorption and the fluorescence spectra was 4.4 nm. The mirror image between the analyzed spectra of absorption and fluorescence was notable. The minor fluorescence bands at 726.1 nm and 678.4 nm might correspond to the minor absorption bands at 615.0 nm and 647.5 nm, respectively.

Lepidium CP661 and Lepidium CP663

The water-soluble chlorophyll protein, Lepidium CP661, has a molecular weight of 80 000, and a chlorophyll *a* to *b* ratio of 1.0 [16]. This protein, when purified, forms crystals [16].

In Lepidium CP661, the red absorption peak appeared at 661 nm, and the fluorescence peak appeared at 672 nm at 25°C. Table II and Fig. 2 show analyzed results of the absorption and the fluorescence spectra. The absorption spectrum was resolved into three major component bands with peaks at 652.8, 662.6 and 671.9 nm, and two minor bands with peaks at 630.8 and 644.0 nm. The 652.8 nm band was attributed to the chlorophyll *b* form, Cb650, and the 662.6 and 671.9 nm bands were to the chlorophyll *a* forms, Ca662 and Ca670, according to French et al. [5].

The fluorescence spectrum was resolved into five component bands with peaks at 654.8, 664.6, 674.6, 686.5 and 707.7 nm. It is noted that every major absorption band had a corresponding fluorescence band with the Stokes shift of 2.0—2.7 nm (Table II and Fig. 2). The fluorescence band at 686.5 nm, having no corresponding absorption band, is likely to be a mixture of side bands of shorter wavelength-side component bands, as the 678.4 nm band in the diethylether solution of chlorophyll *a* in Fig. 1.

The absorption spectrum of Lepidium CP661 at −196°C was also analyzed

TABLE II

ANALYZED RESULTS OF ABSORPTION AND FLUORESCENCE SPECTRA OF LEPIDIUM CP661

Fluorescence was excited at 469 nm.

Spectra	λ_{max} (nm)	$\Delta\lambda_{1/2}$ (nm)	Gaussian (%)	Stokes shift (nm)	Blue shift (nm)
Absorption (25°C)	630.8	24.6	91.4		
	644.0	13.1	100.0		
	652.8 *	13.2	95.3		
	662.6 **	14.7	89.8		
	671.9 ***	16.8	69.3		
Fluorescence (25°C)	654.8 *	14.3	75.7	2.0	
	664.6 **	15.1	98.4	2.0	
	674.6 ***	15.8	95.0	2.7	
	686.5	19.9	97.7		
	707.7	32.6	98.3		
Absorption (−196°C)	630.5	24.6	100.0		
	645.4	12.9	100.0		
	651.7 *	12.0	100.0		1.1
	661.4 **	10.2	100.0		1.2
	669.3 ***	9.9	96.9		2.6

*,**,*** Represent corresponding bands in Stokes shift and blue shift.

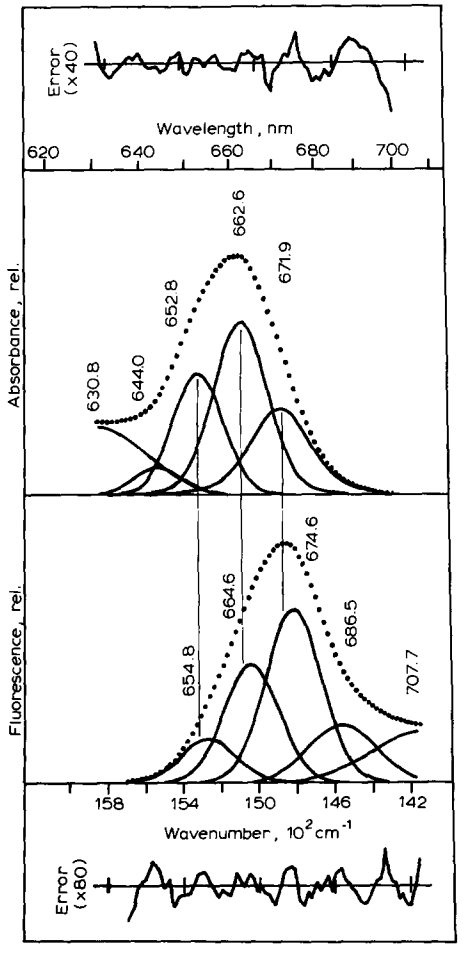

Fig. 2. Analyzed results of absorption and fluorescence spectra of Lepidium CP661 at 25°C. Fluorescence was excited at 469 nm. · · · · · ·, observed spectra. ———, analyzed component bands.

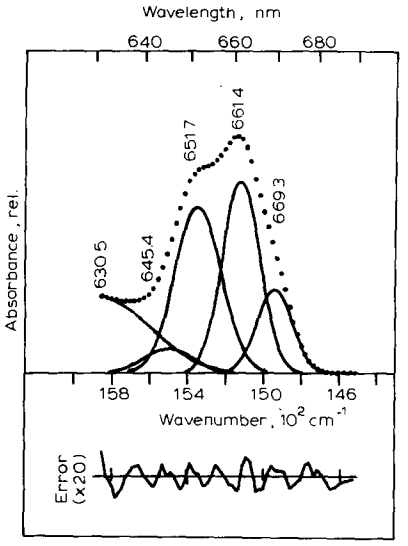

Fig. 3. Analyzed results of absorption spectrum of Lepidium CP661 at —196°C. · · · · · ·, observed spectrum. ———, analyzed component bands.

TABLE III

ANALYZED RESULTS OF ABSORPTION AND FLUORESCENCE SPECTRA OF BRASSICA CP673

Fluorescence was excited at 469 nm.

Spectra	λ_{max} (nm)	$\Delta\lambda_{1/2}$ (nm)	Gaussian (%)	Stokes shift (nm)	Blue shift (nm)
Absorption (25°C)	629.9	16.3	95.0		
	643.1	14.5	100.0		
	653.7 [a]	11.9	99.6		
	662.7 [b]	11.7	100.0		
	671.3 [c]	11.8	100.0		
	676.9 [d]	10.1	99.8		
	684.2 [e]	10.7	95.8		
	691.0	15.6	58.2		
Fluorescence (25°C)	659.5 *	13.5 *	46.6 *		
	666.7 [b]	11.0	82.2	4.0	
	673.1 [c]	11.4	98.4	1.8	
	677.5 [d]	11.4	96.6	0.6	
	686.2 [e]	13.0	91.2	2.0	
	694.1	22.0	82.4		
	717.3	41.9	100.0		
Absorption (—196°C)	629.0	11.8	86.6		
	638.8	12.1	89.4		
	649.6 [a]	11.1	89.7		4.1
	660.7 [b]	12.8	100.0		2.0
	670.5 [c]	11.3	99.9		0.8
	675.3 [d]	8.4	98.1		1.6
	682.7 [e]	9.5	79.7		1.5

* The values were not definite, since the component band was very weak.

[a,b,c,d,e] Represent corresponding bands in Stokes shift and blue shift.

(Table II and Fig. 3). The spectrum in the red region was composed of three major bands with peaks at 651.7, 661.4 and 669.3 nm. The component bands in the absorption spectrum were shifted by 1.1—2.6 nm to shorter wavelengths and narrower in half widths than those at 25°C (Table II).

The water-soluble chlorophyll protein Lepidium CP663 has a molecular weight of 80 000 and a chlorophyll *a* to *b* ratio of 1.6 [16]. This protein, when purified, forms crystals [16]. Lepidium CP663 had a red absorption peak at 663 nm and a fluorescence peak at 673 nm at 25°C. The absorption and the fluorescence spectra at 25°C were also analyzed, and essentially the same results as in CP661 were obtained. However, relative amounts of the component bands were different. This fact corresponded to the different chlorophyll *a* to chlorophyll *b* ratios in CP663 and CP661. It was also found that each of the three component bands in the absorption spectrum had a corresponding fluorescence band also in CP663.

Brassica CP673

The water-soluble chlorophyll protein, Brassica CP673, has a molecular weight of about 90 000 [17] and a chlorophyll *a* to *b* ratio of 8.0 [13]. Brassica CP673 had the red absorption peak at 673 nm and the fluorescence peak at 684 nm at 25°C. Table III and Fig. 4 show analyzed results of the absorption and the fluorescence spectra at 25°C. The absorption spectrum was resolved into component bands with peaks at 629.9, 643.1, 653.7, 662.7, 671.3, 676.9, 684.2 and 691.0 nm. The 653.7 nm band was attributed to the chlorophyll *b* form, Cb650, and the 662.7, 671.3, 676.9 and 684.2 nm bands were to the chlorophyll *a* forms, Ca662, Ca670, Ca677 and Ca684, respectively. The 629.9 and 643.1 nm bands were attributed to side bands. The origin of the small 691.0 nm band was unclear, since it disappeared at −196°C.

The fluorescence spectrum was resolved into seven component bands with peaks at 659.5, 666.7, 673.1, 677.5, 686.2, 694.1 and 717.3 nm. Every major absorption band except for the 653.7 nm band had a corresponding component band of fluorescence with the Stokes shift of 0.6—4.0 nm. The fluorescence component band of chlorophyll *b* at 659.5 nm was so weak that the calculation did not give definite values for this band. Although the correspondence apparently existed between the component band of absorption at 691.0 nm and the component band of fluorescence at 694.1 nm, it was probably accidental. The fluorescence band, which had a broader half width than the short wavelength-side component bands, might be a mixture of side bands of the short wavelength-side fluorescence bands.

The absorption spectrum at −196°C was resolved into five major component bands with peaks at 649.6, 660.7, 670.5, 675.3 and 682.7 nm (Table III). Absorption of the chlorophyll forms Cb650, Ca670, Ca677 and Ca684 at −196°C were shifted to shorter wavelengths by 0.8—4.1 nm, and narrower in half widths than those at 25°C. Ca662, however, was broader; this might have been produced by uncertainty of the calculation.

Pigment system II particles

The pigment System II particles of spinach chloroplasts had the red absorption peak at 679 nm and the fluorescence peak at 683 nm at 25°C. Table IV

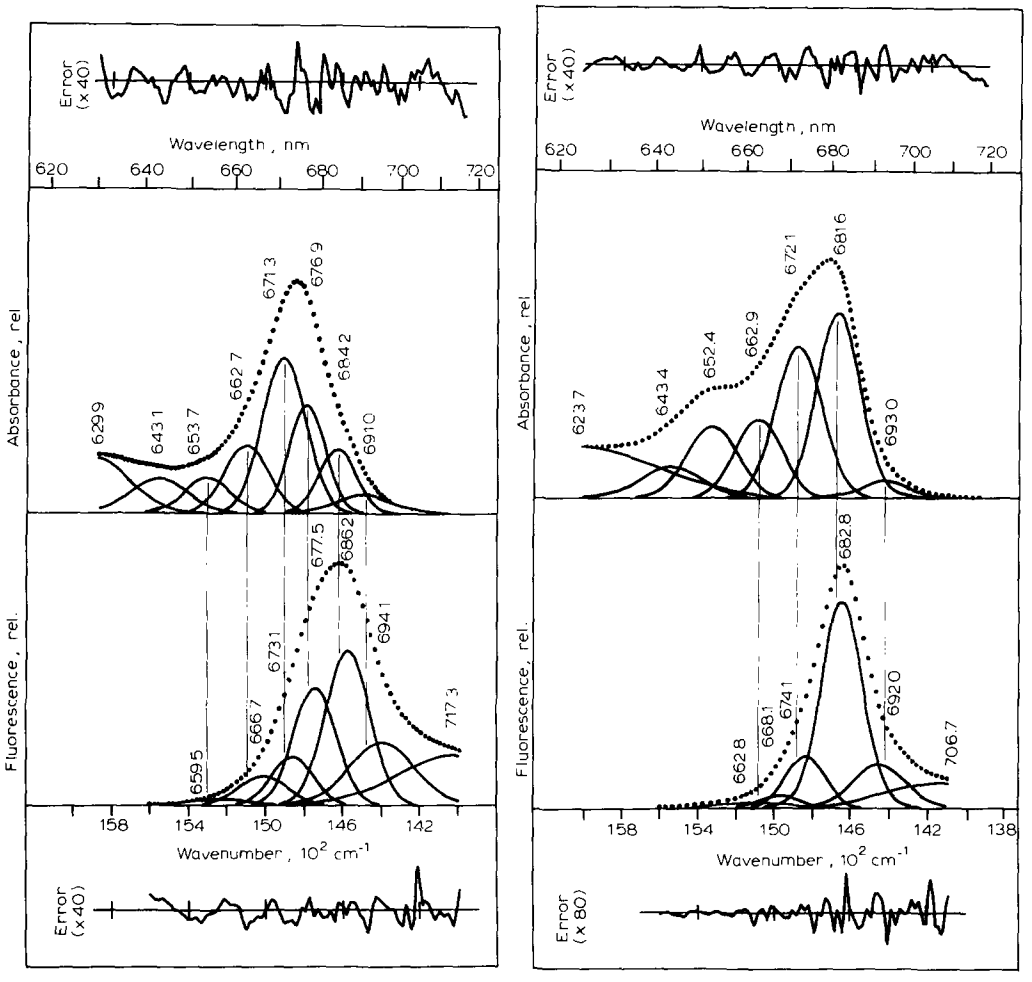

Fig. 4. Analyzed results of absorption and fluorescence spectra of Brassica CP673 at 25°C. Fluorescence was excited at 469 nm. · · · · · ·, observed spectra. ———, analyzed component bands.

Fig. 5. Analyzed results of absorption and fluorescence spectra of pigment system II particles of spinach chloroplasts at 25°C. Fluorescence was excited at 469 nm. · · · · · ·, observed spectra. ———, analyzed component bands.

and Fig. 5 show analyzed results of absorption and fluorescence spectra of the pigment System II particles. The absorption spectrum in the red region was resolved into four major component bands with peaks at 652.4, 662.9, 672.1 and 681.6 nm and two minor bands with peaks at 643.4 and 693.0 nm.

The fluorescence spectrum was resolved into four major component bands with peaks at 674.1, 682.8, 692.0 and 706.7 nm, and two minor bands with peaks at 662.8 nm and 668.1 nm. The component bands of absorption with peaks at 672.1 and 681.6 nm, had corresponding component bands of fluorescence with the Stokes shift of 2.0 and 1.2 nm, respectively. The fluorescence band at 692.0 nm was attributed partly to the side band of the 682.8 nm

TABLE IV

ANALYZED RESULTS OF ABSORPTION AND FLUORESCENCE SPECTRA OF PIGMENT SYSTEM II PARTICLES OF SPINACH CHLOROPLASTS AT 25°C

Fluorescence was excited at 438 nm.

Spectra	λ_{max} (nm)	$\Delta\lambda_{1/2}$ (nm)	Gaussian (%)	Stokes shift (nm)
Absorption	623.7	38.5	100.0	
	643.4	15.2	79.6	
	652.4	13.4	100.0	
	662.9	12.7	99.9	
	672.1 [a]	12.9	97.9	
	681.6 [b]	12.2	90.5	
	693.0	14.1	21.2	
Fluorescence	662.8 *	21.9 *	70.7 *	
	668.1 *	12.0 *	65.5 *	
	674.1 [a]	11.2	86.5	2.0
	682.8 [b]	11.8	89.2	1.2
	692.0 **	15.4 **	68.8 **	
	706.7	32.9	99.5	

* The values were not definite, since the component bands were very weak.

** This band was attributed to the side band of 682.8 nm fluorescence band and the emission from 693.0 nm absorption band.

[a,b] Represent corresponding bands in Stokes shift.

fluorescence band as in the case of the fluorescence band at 678.4 nm of chlorophyll *a* in diethylether solution (Fig. 1) and partly to the emission from the absorption band at 693.0 nm.

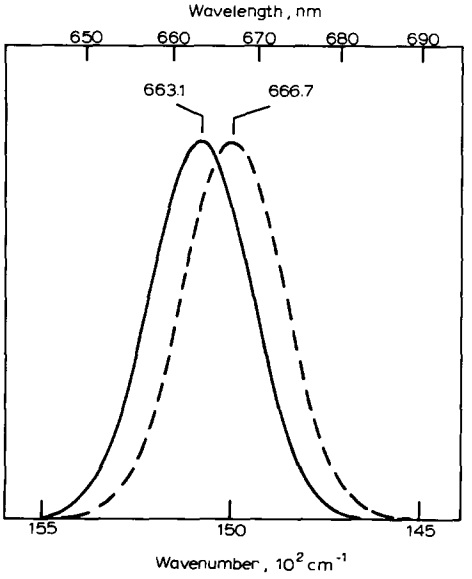

Fig. 6. Comparison of an absorption band with Gaussian shape and its fluorescence emission band calculated by the equation of Stepanov [18]. The absorption band with peak at 663.1 nm and with half width of 14.0 nm is used. The calculated fluorescence band has a peak at 666.7 nm and half width of 14.2 nm. ———, absorption band. - - - - - -, calculated fluorescence band.

Discussion

Photosynthesis of most plants proceeds at room temperature. Thus, it is preferable to use the absorption and the fluorescence spectra at room temperature rather than at liquid nitrogen temperature for the precise analysis in the curve-fitting method. However, difficulties were found in the analysis of room temperature spectra in the curve-fitting method. The wide half widths of component bands were greater than at −196° C. With the half widths, a simple application of the curve-fitting method did not give definite answers: the resulting values somewhat varied depending on the initial values put for the parameters in the calculation. Therefore, we employed the three-step calculation method to obtain the most probable values for the parameters.

It was found that the red absorption band of chlorophyll *a* in diethylether solution was analyzed to one major (660.5 nm) and one minor (647.5 nm) component bands with the relative contribution of 6 : 1. The main band of fluorescence was analyzed also to one major (664.9 nm) and one minor (678.4 nm) component bands with the relative contribution of 5 : 1. In both the absorption and the fluorescence spectra, the contribution of the minor band was very small. This gives a basis for using a curve-fitting method of French et al. [5] in analyzing the absorption and the fluorescence spectra of the chlorophyll proteins and the chloroplast fragments into component bands. It is assumed in this method that one component of chlorophyll has single and symmetrical absorption and fluorescence bands.

Each major absorption band of chlorophyll in the water-soluble chlorophyll proteins and in the pigment System II particles had a corresponding fluorescence band with the Stokes shift of 0.6—4.0 nm. Half widths of corresponding absorption and fluorescence bands were almost the same. The values for the Stokes shift were smaller than that between the major component bands of absorption and fluorescence of chlorophyll *a* in diethylether solution (4.4 nm). Assuming an absorption band of Gaussian shape with peak at 663.1 nm and with half width of 14.0 nm, a fluorescence band emitted from this absorption band can be estimated using the equation of Stepanov [18]. The calculated fluorescence band has a peak at 666.7 nm and a half width of 14.2 nm, thus giving the Stokes shift of 3.6 nm (Fig. 6). This is within the range of values for the Stokes shift between the corresponding absorption and fluorescence component bands of the water-soluble chlorophyll proteins and the pigment System II particles. This treatment verifies the correspondence between the component bands of absorption and fluorescence spectra.

Thornber [19] has suggested that chlorophyll molecules are bound to proteic moieties in the photosynthetic membranes. Lutz [20], studying the resonance Raman spectroscopy, indicated that chlorophyll molecules in the thylakoid membranes are bound to foreign molecules, probably proteins, rather than exist as oligomers or polymers. The water-soluble chlorophyll proteins Lepidium CP661 and CP663 have three common chlorophyll forms, Cb650, Ca662 and Ca670, and Brassica CP673 contained, in addition to these, two more forms, Ca677 and Ca684. Peak wavelengths of these forms are almost the same as those of chlorophyll forms in the chloroplasts [5] and the pigment System II particles in Table IV. These findings also suggest that the chlorophyll

forms in the chloroplasts are determined by different interactions of chlorophyll molecules to proteic moieties. It should be mentioned, however, that our recent study on the lipid-water suspension including chlorophyll *a* has shown that Ca670 can be produced also by the interaction of chlorophyll *a* to lipid or water [21].

As shown in Figs. 2, 4 and 5, even when chlorophyll *b* was excited in the water-soluble chlorophyll proteins and the pigment System II particles, the fluorescence was emitted mainly from chlorophyll *a*, especially from the chlorophyll *a* form with the longest peak wavelength. This suggests that the electronic excitation energy is efficiently transferred from chlorophyll *b* to chlorophyll *a* and from the chlorophyll *a* form of shorter wavelengths to that of longer wavelengths.

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