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# ABSORPTION AND FLUORESCENCE SPECTRA OF CHLOROPHYLL-PROTEINS ISOLATED FROM *EUGLENA GRACILIS* *

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## Summary

A spectroscopic study of chlorophyll-protein complexes isolated from *Euglena gracilis* membranes was carried out to gain information about the state of chlorophyll in vivo and energy transfer in photosynthesis. The membranes were dissociated by Triton X-100 and separated into fractions by sucrose gradient centrifugation and hydroxyapatite chromatography. Four different types of chlorophyll-protein complexes were distinguished from each other and from detergent-solubilized chlorophyll in these fractions by examination of their absorption, fluorescence excitation (400—500 nm) and emission spectra at low temperature. These types were: (1). A mixture of antenna chlorophyll *a*- and chlorophyll *ab*-proteins with an absorption maximum at 669 and emission at 682 nm; (2) a *P*-700-chlorophyll *a*-protein (chlorophyll : *P*-700 = 30 : 1), termed CPI with an absorption maximum at 676 nm and emission maxima at 698 and 718 nm; (3) a second chlorophyll *a*-protein (CPI-2) less enriched in *P*-700, with an absorption maximum at 676 nm and emission maxima at 680, 722 and 731 nm; (4) a third chlorophyll *a*-protein ($CPa_1$) with no *P*-700, absorption maxima at 670 and 683 nm, and an unusually sharp emission maximum at 687 nm. Treatment of $CPa_1$ with sodium dodecyl sulfate drastically altered its spectroscopic properties indicating that at least some chlorophyll-proteins isolated with this detergent are partially denatured. The results suggest that the complex absorption spectra of chlorophyll in vivo are caused by varying proportions of different chlorophyll-protein complexes, each with different groups of chlorophyll molecules bound to it and making up a unique entity in terms of electronic transitions.

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Abbreviations: Chl, chlorophyll; CP, chlorophyll-protein complex; SDS, sodium dodecyl sulfate.

## Introduction

Absorption spectra of chlorophyll in photosynthetic membranes are complex and suggest that the pigment occurs in several different states or forms in vivo [1]. Resonance Raman spectroscopic studies have demonstrated the existence of about five discrete pools of Chl *a* in many different plant species and indicate that most if not all of the chlorophyll is bound to protein [2,3]. The separation and description of various Chl-proteins have recently been reviewed by Thornber et al. [4]. Most of these studies have involved the dissociation of chloroplast membranes by a detergent (usually SDS) followed by polyacrylamide gel electrophoresis. Under appropriate conditions nearly all of the chlorophyll remains attached to various polypeptides, again indicating that most of the chlorophyll in photosynthetic membranes is bound to protein.

Because the electronic absorption spectra of Chl *a* in the various complexes isolated with SDS are similar [4], other detergent procedures for dissociating intrinsic chloroplast membrane proteins have been investigated. Thus far two complexes with distinct and different absorption spectra in the red regions have been isolated, CP$a_1$, from *Euglena* [5] and a Chl *ab*-protein from spinach [6], by use of Triton X-100 followed by hydroxyapatite chromatography or sucrose density gradient centrifugation.

In the following experiments Triton was used to dissociate *Euglena* chloroplasts, and the absorption, fluorescence excitation (400—500 nm), and emission spectra of four different Chl-protein complexes were compared; also some effects of detergent action were studied. *Euglena* was selected for these experiments in part because the long wavelength-absorbing forms of Chl *a* are particularly prominent in spectra of this unicellular alga [7,8], and the unique Chl-protein, CP$a_1$, mentioned above can be isolated from this species in addition to Photosystem I reaction center (*P*-700) and antenna or light-harvesting chlorophyll complexes which have also been separated from other plants [9,10]. Although CP$a_1$ comprises less than 1% of the total chlorophyll in *Euglena*, the fact that it can now be isolated by sucrose density gradient centrifugation as well as by hydroxyapatite chromatography suggests that it is not an artifact of the isolation procedure. This Chl *a* complex contains no Chl *b* or *P*-700 and could possibly function near the reaction center of Photosystem II.

## Materials and Methods

*Euglena gracilus* (University of Texas Culture Col. No. 752) was cultured in 1 l batches of Cramer and Myers [11] medium. Cultures were shaken over four, cool-white, fluorescent lamps at about 22°C for several days until the cell density appeared to be at the end of the logarithmic growth phase. Air-enriched 3% $CO_2$ was passed through the flasks.

The cells were harvested by centrifugation, resuspended in 0.4 M sucrose, 0.1 M Tris-HCl (pH 8) and forced through a French pressure cell. The cell fragments were centrifuged at 10 000 × *g* for 10 min, resuspended in 100 mM sorbitol, 5 mM EDTA, brought quickly to pH 6 with 0.1 N HCl, and centrifuged again. This cation-depleted sediment was resuspended in sorbitol and centrifuged again after an aliquot was withdrawn for chlorophyll determination

[12]. Each final pellet, containing about 20 mg chlorophyll, was stored in a freezer (−20°C).

One of the fractionation procedures was essentially the same as that developed by Burke et al. [10] for pea chloroplasts except that *Euglena* fragments, homogenized in 0.5% Triton X-100 (Triton/chlorophyll: 10 : 1, w/w), were incubated for 15 min (instead of 30 min) and centrifuged at 27 000 × *g* for 10 min (instead of 41 000 × *g* for 30 min). 8 ml of this supernatant were layered over a 23 ml gradient of 0.1—1 M sucrose in 0.5% Triton and 2 ml of a 2 M sucrose cushion. The tubes were centrifuged in a Sorval vertical rotor (TV850) at 178 000 × $g_{av}$ for 4.5 h. The resulting darker-pigmented layers were removed with a pipette and dialysed overnight against 2 mM sodium phosphate (pH 7.6).

The other fractionation procedure was described in Refs. 5 and 13. *Euglena* pellets were dissociated in 1% Triton, 50 mM Tris-HCl (pH 8) (Triton/chlorophyll: 75 : 1, w/w) and fractionated by hydroxyapatite chromatography. With green algae and higher plants, CPI was adsorbed to the column and remained bound during successive washings with 10 mM phosphate and 1% Triton. However, as observed eariler [14], CPI from *Euglena* was released by 10 mM phosphate. A second green band (CPI-2) could be eluted by continued washing with 10 mM phosphate. After washing with 1% Triton until the eluate was nearly colorless, a final green fraction, CP$a_1$, could be eluted in 200 mM phosphate.

The first 10 mM phosphate eluate contained not only most of the *P*-700-Chl *a*-protein, but also some Triton-solubilized chlorophyll and carotenoid from the tail of the original sample. To purify the CPI further, the sample was dialysed and diluted with 50 mM Tris-HCl (pH 8) to lower the phosphate concentration before applying to a fresh hydroxyapatite column. All of the pigment became bound at the top of the column, and most of the chlorophyll and carotenoid was subsequently removed by washing with 1% Triton. The CPI which remained bound through washing with 50 mM Tris-HCl (pH 8) or 200 mM phosphate was eluted completely by 100 mM phosphate in 0.05% Triton, (pH 7.5—8.0).

*Spectrophotometry.* Absorption spectra were measured with a Cary 17 spectrophotometer equipped with a scattering transmission attachment. The base of an aluminum sample holder was suspended in a Dewar flask containing liquid $N_2$ and placed in the compartment of the spectrophotometer. The temperature of the sample was approx. 89 K. The optical pathlength of the sample was 2 mm and the half-bandwidth of the measuring beam at 680 nm was approx. 1 nm.

Fluorescence excitation and emission spectra were measured with a Perkin-Elmer MPF-3L fluorimeter equipped with a red-sensitive Hamamatsu R446 photomultiplier and a Dewar flask in the sample compartment. The sample in a glass EPR tube (4 mm) was immersed in liquid $N_2$ during measurement. To minimize artifactual results caused by self-absorption, all samples were diluted to the point where further dilution changed only the amount of fluorescence and not the shape of the spectrum. Consequently, the chlorophyll concentration in different samples varied between 2 and 10 μM depending upon the fluorescence yield. The instrument was connected on-line to a Hewlett-Packard

computer system for processing of the data. Emission spectra were corrected for the spectral response of the instrument. Artifactual peaks in the excitation spectra caused by the xenon arc emission lines near 470 nm were removed by dividing each sample spectrum by an excitation spectrum of Rhodamin b recorded with the same slitwidth [15].

It was observed earlier [6,15] that detergent-solubilized Chl *a* fluoresces maximally at 670 nm with a high yield. Excitation spectra for this emission show a sharp decrease beyond 440 nm. However, chloroplast particles and Chl-protein complexes fluoresce at longer wavelengths with a relatively lower yield, and excitation spectra for their emission do not show such a sharp decrease beyond 440 nm. Therefore it is possible to excite the Chl-proteins preferentially at 450 nm where their relative absorption is greater than that of free chlorophyll. Because several of the fractions studied here contained both Chl-proteins and free chlorophyll, emission spectra from 436 and 450 nm exciting light were compared to give some estimation of the relative proportions of the two chlorophyll states.

The concentration of *P*-700 was determined by measuring the extent of a light-induced absorbance change at 698 nm against a 725 nm isosbestic reference wavelength with a Perkin-Elmer, 356 Two-Wavelength Spectrophotometer. A Corning No. 4-96, blue filter was placed in the actinic beam and a Schott RG-2 red cut-off filter screened the photomultiplier. Sufficient sodium ascorbate was added to the sample to reduce the light-oxidized $P\text{-}700^+$ with a half-time of about 10 s [16]. An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used to calculate the amount of *P*-700 [17].

The cytochrome content was measured with the same instrument in the split-beam mode. The difference in absorption between ferricyanide-oxidized and ascorbate-reduced samples at 554 nm indicated the amount of *c*-type cytochrome. A small amount of sodium dithionite added to the cuvette containing ascorbate reduced any *b*-type cytochrome which was observed at 563 nm. An extinction coefficient of 20 $mM^{-1} \cdot cm^{-1}$ was used to calculate the amounts of both cytochromes [18].

## Results

### *Sucrose gradient band fractions*

After centrifugation in a sucrose gradient, the partially dissociated *Euglena* membranes were distributed in three dark green bands and a brown layer between the two upper layers. All bands were superimposed on a yellow-green background coloration. The approximate pigment concentrations of these bands are given in Table I. Absorption, fluorescence excitation (400—500 nm) and emission spectra of the original 0.5% Triton extract and of the three green gradient fractions are shown in Fig. 1.

*Band 1.* This fraction which contained the lightest of the particles dissociated by 0.5% Triton was analogous to the band from peas shown by Burke at al. [10] to contain antenna or light-harvesting Chl-protein complexes. We repeated their experiments using spinach and also observed this highly fluorescent fraction which contained most of the Chl *b* [6]. Although the *Euglena* used here had relatively little Chl *b*, most of it remained in the upper band as

TABLE I

THE PIGMENT CONCENTRATIONS OF *EUGLENA* PARTICLE FRACTIONS AFTER SEPARATION IN A SUCROSE DENSITY GRADIENT

For Chl *a*/*b* the procedure of Mackinney [12] for the determination of chlorophylls in 80% acetone/water mixtures gives only an approximate value for Chl *b* when its concentration is low.

| Sample | Chlorophyll (μM) | Chl *a*/*b* | Chl *a*/P700 | Cytochrome (μM) | |
|---|---|---|---|---|---|
| | | | | *c* | *b* |
| Initial 0.5% | | | | | |
| Triton extract | 230 | 10 | >500 | — | — |
| Gradient band | | | | | |
| 1 | 130 | 7 | — | — | — |
| b | 10 | — | — | 0.45 | 0.90 |
| 2 | 11 | ∞ | 50 | — | — |
| 3 | 14 | ∞ | 108 | — | — |

expected (Table I).

The absorption spectrum of this upper gradient band (Fig. 1B) is similar to that of the original Triton extract (Fig. 1A) with major Chl *a* maxima at 437 and 669 nm and carotenoid maxima at 464 and 497 nm. A small maximum in the excitation spectrum near 466 nm shown by the gradient band may reflect the enrichment of Chl *b* or possibly a carotenoid together with excitation energy transfer to Chl *a* emitting at 682 nm. The higher fluorescence yield (Table II) of gradient band 1 compared to the total 0.5% Triton extract is expected from an antenna complex depleted in reaction centers. The small blue shift in the emission maximum of the upper gradient band compared to the original extract indicated that the true emission of this complex may be close to 680 nm (see also Ref. 19).

*Band b.* A cytochrome *f*-$b_6$ complex can be dissociated from most chloroplast membranes by Triton action [20]. After centrifugation through a sucrose gradient, this complex from spinach chloroplasts remained within the upper, darker green band; but with *Euglena* it was evidently heavier than the antenna complex and migrated farther down the gradient.

*Band 2.* When the procedure of Burke et al. was followed with peas [10] or spinach [6], Photosystem I particles aggregated in the low-salt medium and sedimented to the bottom of the gradient. However, with *Euglena* a middle band which had the greatest enrichment of *P*-700 (Chl: *P*-700 = 50) became separated. Although the spectra of this fraction (Fig. 1C) show some of the characteristics of more purified CPI such as low carotenoid and relatively more long-wavelength absorption, the real emission of CPI is masked by the relatively higher yield fluorescence of solubilized chlorophyll. Excitation at 436 and 466 nm caused emission by solubilized Chls *a* and *b* at 674 and 652 nm, respectively. The amount of Chl *b* causing this emission was below the level of detection by the procedure in Ref. 12 and represented contamination by the free chlorophyll in the Triton extract.

*Band 3.* The emission spectrum of this fraction (Fig. 1D) had a narrow band at 687 nm which is characteristic of the Chl-protein, $CPa_1$ [9], that is described in more detail below (Fig. 3A). The chlorophyll to *P*-700 ratio of 108 indicated

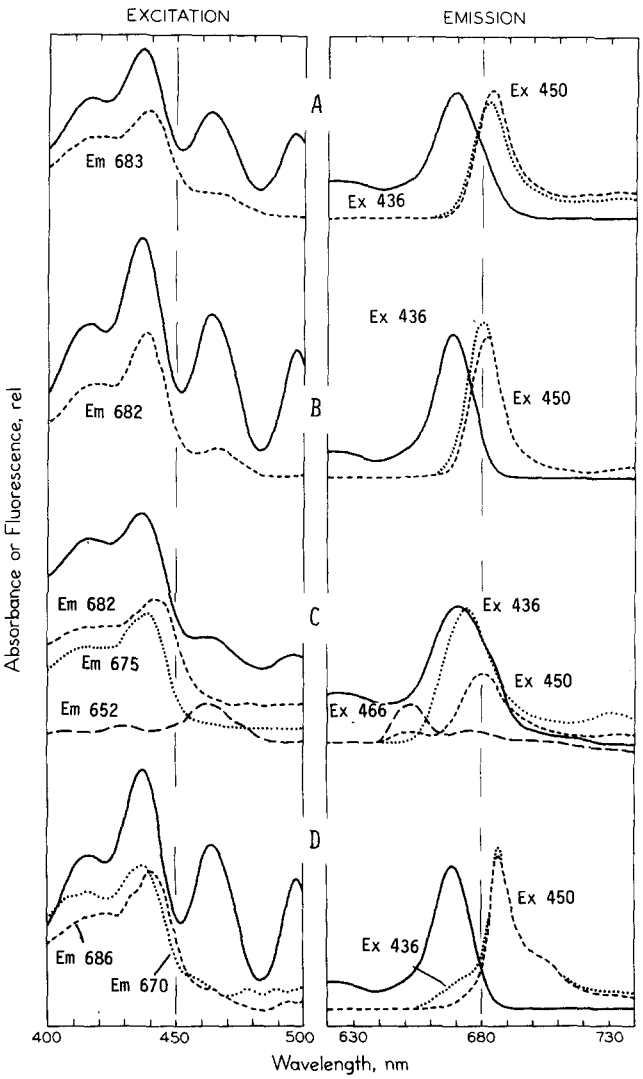


**Fig. 1. Absorption (———), fluorescence excitation and emission (- - - - -) spectra of (A) *Euglena* chloroplast membranes in 0.5% Triton X-100; (B) sucrose gradient band 1; (C) gradient band 2, and (D) gradient band 3 measured near 77 K. Emission wavelengths for excitation spectra and exciting wavelengths for emission spectra are shown adjacent to curves. For all fluorescence spectra, the bandwidth of the reference wavelength was 5 nm, and that of the measuring wavelength, 3 nm.**

that some CPI was also in this band. There was little or no Chl *b*, but carotenoid absorption was high. The small bands in the excitation spectrum between 450 and 500 nm indicate some possible excitation energy transfer between carotenoid and Chl *a* in CP$a_1$.

*Fractions from hydroxyapatite chromatography*

The first, dark green, 10 mM phosphate eluate which was contaminated with the tail of the sample typically had a chlorophyll to *P*-700 ratio of about 90 and spectra shown in Fig. 2A. As expected from the presence of free Chls *a*

TABLE II

THE RED ABSORPTION AND FLUORESCENCE EMISSION MAXIMA AND RELATIVE FLUORESCENCE YIELDS AT THE MAXIMA FROM EXCITATION AT 436 OR 450 nm OF *EUGLENA* MEMBRANES BEFORE AND AFTER TRITON TREATMENT AND OF VARIOUS FRACTIONS

Relative yield calculated from chlorophyll concentration, instrument sensitivity and curve height at the emission maximum.

| Sample | Absorption maximum | Emission maximum (excitation 436 nm) | Relative yield | Emission maximum (excitation 450 nm) | Relative yield |
|---|---|---|---|---|---|
| Chloroplast fragments [a] | 673,680 | 733 | 1 | 733 | — |
| 1% Triton extract [a] | 669 | 676 | 10 | 682 | 3 |
| 0.5% Triton extract | 669 | 683 | 4 | 684 | 2 |
| Gradient band 1 | 669 | 681 | 6 | 682 | 6 |
| Gradient band 2 | 671 | 674 | 2 | 681 | 1 |
| Gradient band 3 | 669 | 687 | 2 | 687 | 2 |
| CPI, Chl/*P*-700 = 90 | 669 | 674 | 6 | 679 | 2 |
| CPI-2, Chl/*P*-700 = 50 | 676 | 680,726 | 0.4 [b] | 722,731 | 0.2 [c] |
| CPI, Chl/*P*-700 = 30 | 675 | — | — | 698,718 | 0.1 [d] |
| $CPa_1$ | 670,683 | 687 | — | 687 | 3 |
| $CPa_1$ + SDS | 671 | 679 | 8 | 683 | 5 |
| $CPa_1$ heated | 669 | 683 | 2 | 685 | 1 |

[a] Spectra shown in Ref. 6.
[b] Measured at 726 nm.
[c] Measured at 731 nm.
[d] Measured at either 698 or 718 nm.

and *b*, the emissions at 674 and 650 nm were relatively high.

*CPI-2.* When the column was washed further with 10 mM phosphate, a light green band (CPI-2) was eluted which had a chlorophyll to *P*-700 ratio of about 50 and spectra shown in Fig. 2B. This ratio is about the same as that of gradient band 2, but comparison of the spectra of these two fractions (Figs. 1C and 2B) shows striking differences. Part of the difference is caused by the free or solubilized chlorophyll in the gradient band fraction; but excitation at 450 nm which is absorbed by the Chl-proteins preferentially, caused a high emission beyond 720 nm in the results of Fig. 2B (CPI-2) as opposed to the 681 nm maximum emission in Fig. 1C (band 2). Excitation of CPI-2 at 436 nm caused emission at 680 and 726 nm, whereas excitation at 450 nm caused less emission near 680 nm and a splitting of the long-wavelength fluorescence into two maxima at 722 and 731 nm. The fluorescence yield of this fraction was five times lower than that of the gradient band 2 (Table II) apparently because of less free chlorophyll in the former, but was slightly higher than the more purified CPI with its higher concentration of reaction centers.

*CPI.* By rechromatography of the first 10 mM phosphate eluate a CPI with a chlorophyll to *P*-700 ratio of about 30 was obtained. When this purified

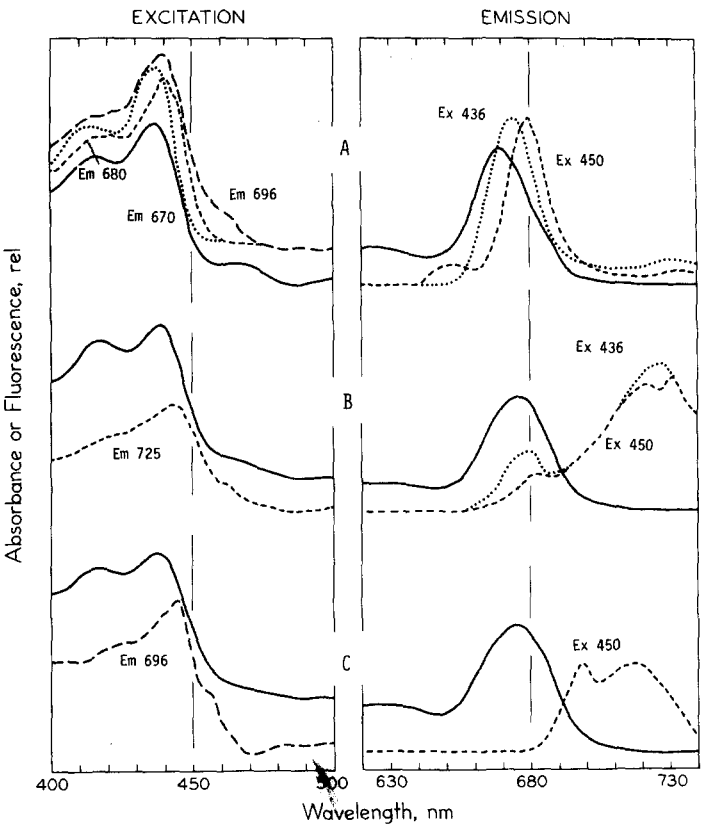


Fig. 2. Absorption (———), fluorescence excitation and emission (- - - - - -) spectra of (A) first 10 mM phosphate eluate from hydroxyapatite: (B) second 10 mM phosphate eluate (CPI-2), and (C) rechromatographed CPI. Measurement conditions same as for Fig. 1.

fraction was eluted from the column in 100 mM phosphate, 0.05% Triton, a spectrum measured within 1 h showed a relatively high emission at 675 nm from excitation at 436 nm. But after dialysis, the 675 nm maximum disappeared, and the spectrum shown in Fig. 2C was recorded. This emission spectrum shows a distinct band near 696 nm. The relative height of this maximum was previously noted to be correlated with *P*-700 concentration in CPI preparations from several plants [9,21]. Because of the very low fluorescence yield of CPI (Table II), this 696 nm emission would not be detectable from chloroplasts, and therefore should not be confused with the 695 nm emission of Photosystem II. A comparison of the excitation spectra in Fig. 2 suggests that a band near 455 nm may also be correlated with a high concentration of reaction centers. The fact that the lowest fluorescence yield was shown by samples with the highest *P*-700 concentration is in agreement with the concept that reaction centers act as energy traps (Table II).

*CPa*$_1$. Most of the remaining chlorophyll on the original column was removed by washing with 1% Triton; subsequently a yellow-green pigment complex, CP*a*$_1$, was eluted in 200 mM phosphate. The *c*- and *b*-type cytochromes were also concentrated in this final eluate. CP*a*$_1$ comprised less than 1% of the

original chlorophyll and exhibited the very unusual spectra shown in Fig. 3A. The sharp absorption peak at 683 nm and emission band at 687 nm are unique characteristics of $CPa_1$. The absorption and excitation spectra between 400 and 500 nm were very similar to those shown in Fig. 1D of the lowest gradient band. Considerably more carotenoid absorption at 463 and 497 nm was present in spectra of $CPa_1$ (Fig. 1D) than of CPI (Fig. 2); nor could this yellow pigment be removed by rechromatography on hydroxyapatite. The shape of the emission spectrum was independent of exciting wavelength between 400 and 500 nm.

Fig. 3B and C shows the effects of treatment of $CPa_1$ by 1% SDS or heating to 100°C for 1 min on the shapes of its absorption and emission spectra. Treatment with SDS (Fig. 3B) reduced the absorption at 683 nm, shifted the emission to shorter wavelengths, and increased the fluorescence yield. The extent of

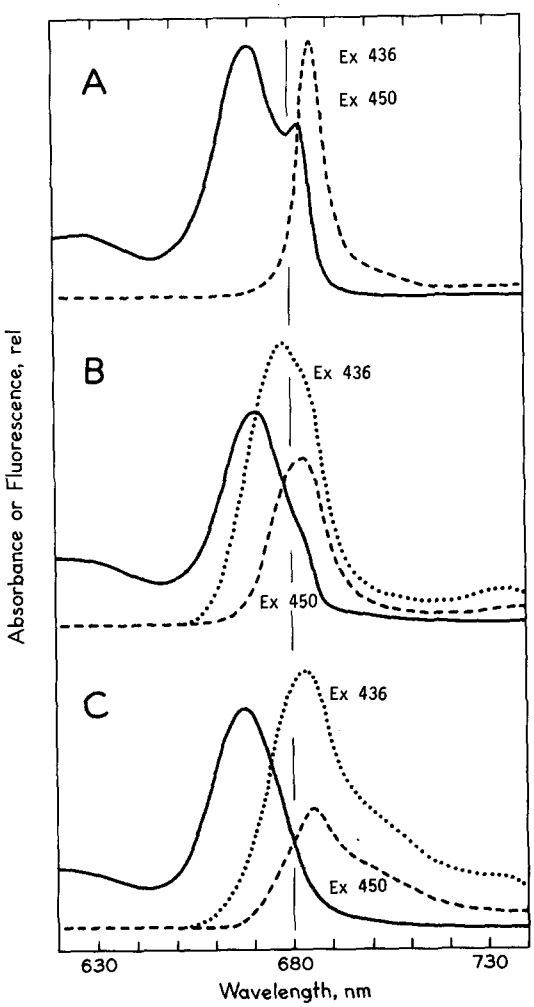


**Fig. 3. Absorption (———) and fluorescence emission (- - - - -) spectra of Chl-protein, $CPa_1$. (A) Untreated; (B) in 1% SDS, and (C) heated to 100°C for 1 min. Measurement conditions same as for Fig. 1.**

these effects was proportional to the SDS concentration and time of incubation at room temperature. The difference in emission spectra between excitation at 436 and 450 nm suggests that part of the chlorophyll was solubilized or disoriented by the detergent; the remainder still bound to protein, fluoresced at 683 nm. Spectra of the heated $CPa_1$ (Fig. 3C) show that both the 683 nm absorption and 687 nm emission bands have disappeared. Although the emission remaining at 683 nm from 436 nm excitation or 685 nm from 450 nm excitation was decreased in yield compared to the original 687 nm emission, longer wavelength emission around 700 nm was increased.

## Discussion

Within the seven isolated *Euglena* fractions, four different Chl-proteins can be distinguished by their spectroscopic characteristics.

*Light-harvesting chlorophyll complexes*

Antenna complexes were isolated in an upper band following sucrose density gradient centrifugation. In contrast to some higher plants [22], *Euglena* [23] forms less Chl *b* and more long wavelength-absorbing Chl *a* in response to light-limited growth conditions. It is possible that the upper gradient band contained a mixture of antenna Chl *a*- and Chl *ab*-protein complexes. Recently Machold and Meister [24] resolved two different antenna Chl-protein complexes from *Vicia faba*. Because the *Euglena* band-1 probably contained a mixture of Chl complexes, the spectra of this fraction (Fig. 2B) should not be considered to be definitive for *Euglena* antenna chlorophyll. It would be worthwhile to repeat our experiments with *Euglena* that contain more Chl *b* than those cells used here.

*P-700-Chl a-proteins*

Five of the seven fractions were enriched in the reaction center chlorophyll of Photosystem I, *P*-700. The lowest ratio of chlorophyll to *P*-700 (30) was obtained in the rechromatographed samples from hydroxyapatite. The emission spectrum of this purified CPI with a major peak near 696 nm (Fig. 2C) is similar to spectra of CPI preparations from other plants [9] which have been purified to nearly the same level. The 696 nm peak is not visible when the chlorophyll to *P*-700 ratio is much above 40, or if solubilized chlorophyll with its much higher fluorescence yield is present. As mentioned in Results, the shoulder near 455 nm on the excitation spectrum may be correlated with 696 nm emission. The longwavelength emission maximum (718 nm) and absorption beyond 680 nm were relatively higher in *Euglena* CPI preparations than in CPI from other plants. These observations will be discussed in more detail below.

The data are insufficient to decide whether the fraction labeled CPI-2 is composed of a mixture of CPI (Chl : *P*-700 = 30) and other Chl *a*-proteins without *P*-700, or if it originated from a different area of the thylakoid membrane. The first hypothesis is supported by recent work of Mullet et al. [25,26] who found three spectral populations of Chl *a* antennae in 'native' Photosystem I complexes and correlated nearly half of these antennae with polypeptides of

21 500—24 500 daltons. The molecular weight of the *P*-700-containing core was 68 000. Their conclusions were based in part on differences in long-wavelength emission.

Differences in long-wavelength emission also distinguish two of the *P*-700-containing *Euglena* fractions; CPI-2 (Fig. 2B) has more emission centered at 731 nm than has CPI (Fig. 2C). In Ref. 7, low-temperature absorption and emission spectra of unfractionated *Euglena* particles before and after protease digestion were shown. Digestion caused loss of absorption at 695 and 705 nm as well as emission at 718 and 730 nm, clearly suggesting a correlation between these absorption and emission bands. A possible model to explain the CPI spectra would be to assume that fluorescence at 696 nm comes from $P\text{-}700^+$ absorbing near 690 nm [27] or from a few chlorophyll molecules located very close to it [21]; at 718 nm from those chlorophylls bound to the reaction center core that absorb at 695 nm, and at 730 nm from other antenna chlorophyll molecules bound to polypeptides which are different from but closely associated with the core polypeptides. These latter chlorophylls may be the same as C-705 described by Butler which fluoresces near 735 nm [28]. There have been several recent studies of this long-wavelength emission [29—31].

It has long been known that the longer wavelength-absorbing forms of Chl *a* in vivo are highly oriented [32,33]. In view of the above model one may visualize groups of chlorophyll molecules attached to different polypeptides in oriented arrangements that are the sources of long-wavelength absorption. That no Chl-proteins absorbing exclusively at longer wavelengths have been isolated, and that detergent action generally decreases long wavelength absorption may result from the disorienting effect of such treatments.

*$CPa_1$*

This Chl-protein was noted previously [5], and its low-temperature absorption spectrum was analysed. Attempts to find a similarly absorbing fraction from spinach chloroplasts by hydroxyapatite chromatography have not been successful, but recently Delepelaire and Chua [34] isolated a Chl-protein, CPIV, with an absorption shoulder at 682 nm from *Chlamydomonas* by lithium dodecyl sulfate polyacrylamide gel electrophoresis. On the basis of immunochemical techniques, a thylakoid membrane polypeptide homologous to the *Chlamydomonas reinhardtii* polypeptide in CPIV was identified in spinach, corn, barley, and *E. gracilis*. CPIV, like $CPa_1$, was also estimated to represent about 1% of the total chlorophyll. However, Chua recently analysed lyophilized $CPa_1$ preparations by gel electrophoresis and failed to observe an enrichment of CPIV. Therefore a relationship between the two complexes remains uncertain. Nevertheless, the suggestion [34] that a Chl-protein with 682 nm absorption may be involved in the primary photochemistry of Photosystem II deserves further attention.

A comparison of spectra in Fig. 3A and B shows the drastic effect of SDS on chlorophyll absorption and emission. A similar effect of SDS treatment on the absorption of a light-harvesting complex from spinach was also observed [6] in which a 676 nm absorption peak was reduced to a shoulder on the side of a broadened 670 nm maximum. These results emphasize that the chlorophylls attached to various polypeptides isolated by SDS-polyacrylamide

gel electrophoresis may not be in their native states.

The developing concept is that photosynthetic membranes possess Chl-protein complexes each of which contains more than one group of chlorophyll molecules having a common binding and unique electronic absorption characteristic. The absorption spectrum of an intact membrane will then represent the sum of the absorption properties of all such groups of chlorophyll molecules. It should be emphasized that this idea is different from the previous, more simplified hypothesis in which it was assumed that Chl *a* exists in vivo in four major and a few minor absorbing states [1]. The latter concept arose from the fact that numerous different absorption spectra of various plant species could be resolved by curve analyses into four major Gaussian components located at specific wavelengths and with similar bandwidths, but in different proportions [8]. To explain those results utilizing the newer knowledge of Chl-protein complexes, we may assume that each of four or five complexes has groups of chlorophyll molecules bound to different polypeptide subunits, and it is this binding and/or orientation of the chlorophyll that cause the different electronic absorption bands. If similar groups of chlorophylls with their corresponding absorption bands are attached to different protein complexes, excitation energy transfer from one complex to another within the membrane would be facilitated.

Because the Chl-proteins discussed here occurred in different gradient fractions and chromatographic elution patterns, it may be assumed that the proteins are different. However, further work is required to determine the purity of the proteins and their polypeptide composition. Also the fractions discussed above can account for at most 50—60% of the total chlorophyll, and other non-destructive techniques should be explored to isolate the remaining Chl-protein complexes.

Although *Euglena* chloroplasts behave photosynthetically like those of higher plants, and may even have evolved from endosymbiotic green algae [35], the large variability in their content of Chl *b* and long-wavelength absorption of their Chl *a* are distinguishing features [23]. The study reported here shows that *Euglena* Chl-proteins while functionally analogous to some of those of higher plants, require different or modified techniques for their isolation. These results indicate that the basic membrane structure and/or polypeptide composition of *Euglena* chloroplasts are also probably different.

## Acknowledgements

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