RESULTS

Isolation and Characterization of Phycobilisomes

Isolation procedure

The outline of phycobilisome isolation method is presented in Fig. (1).

The modification of the procedure of Gray and Gantt (1975) as described in "Materials and Methods" allowed the rapid isolation of intact phycobilisomes from cyanobacterium, *Spirulina platensis*. Ultracentrifugation on sucrose step gradients of extracts prepared in 0.75 M K-phosphate buffer pH 7.0 yielded one major deep blue band containing chlorophyll free phycobilisome in 1.0 M sucrose region. Comparative study of phycobilisome, isolated by both conventional method as well as cationic detergent procedure, were done.

Phycobilisomes were identified spectrophotometrically and by its morphological appearance in electron microscope. The extent of release of phycobilisome from the thylakoid membranes was measured by absorption of phycocyanin at 615 nm in the soluble fraction of the homogenate (S₃₅) (Fig.2). Results indicate that phycobilisomes released by CTAB treatment were
**Triton X-100 Method**

Cells collected by vacuum filtration through buchner funnel

Suspended in 0.75 M K-PO₄ buffer (pH 7.0) 1:10 (w/v)

Cell disrupted by ultrasonicator for 2 minutes

Incubated with 2 percent Triton X-100 for 45-60 minutes at 20°C

**CTAB Method**

Culture were incubated with 0.01 percent CTAB for 40 seconds at 20°C

Harvested by vacuum filtration through buchner funnel

Suspended into 0.75 M K-PO₄ buffer (pH 7.0) 1:10 (w/v)

Cell disrupted by ultrasonicator for 30-40 seconds

**FURTHER STEPS WERE IDENTICAL**

Centrifuged at 35,000 g for 30 minutes; supernatant fraction separated (S₃₅)

Fraction S₃₅ was layered on sucrose gradient

Phycobilisome recovered from 1 M sucrose layer

Fig. 1. Comparative Outline of the Isolation Procedure of Phycobilisomes using Triton X-100 and CTAB detergent.
Fig. 2. Absorption spectra of CTAB and Triton X-100 treated homogenates ($S_{35}$) suspended in 0.75 M K-phosphate (pH 7.0) —— Triton X-100; ———— CTAB. Absorption at 615 nm is the index for the release of phycobilisome and at 436 and 678 nm, the presence of chlorophyll a.
almost free from chlorophyll a as no absorption was detected at 440 and 675 nm. However, small amount of chlorophyll a was invariably observed in Triton X-100 treated homogenates, S_{35}. Phycobilisomes were further purified on sucrose density gradient centrifugation and their structural and functional characterization were done.

In assessing the pigmented zones of the isolated gradient by absorption it was found, as previously reported (Gantt and Lipschultz, 1972; Gray and Gnatt, 1975; Koller, et al., 1977) that chlorophyll and carotenoids were on the top of the 0.25 M sucrose layer. A mixture of loose phycobiliproteins was also present in this layer and in 0.5 M sucrose layer. Intact phycobilisomes were present in the 1.0 M sucrose layer. In many preparations about 90 percent of the total phycobiliproteins were present in the PBS layer.

The absorption spectrum of purified phycobilisome of *Spirulina platensis* in 0.75 M K-Po₄ buffer pH 7.0 is shown in Fig. (3). Phycocyanin represents the major peak with absorption maximum at 618 nm and a shoulder at 650 nm shows the presence of allophycocyanin. Since the absorbance at 440 and 675 nm was almost negligible it indicates the absence of chlorophyll. Isolation of phycobilisomes by either of two methods resulted in
Fig. 3. Absorption spectra of purified phycobilisomes after sucrose density step gradient. Sample suspended in 0.75 M K-phosphate buffer (pH 7.0) — , Triton X-100; - - - - - - CTAB.
similar absorption spectra without contamination of the chlorophyll. Absorption at 260-275 nm is due to the protein part of the biliprotein and absorption at 353 nm is due to the bilin part of the biliproteins.

The absorption spectrum of the phycobilisome at liquid nitrogen temperature is shown in Fig. (4). For identification of individual components which may overlap their spectra at room temperature, liquid nitrogen temperature spectrum was measured which gives sharper bands and more easily distinguishable components. The absorbance spectrum at 77 K showed peaks at 625 nm and 653 nm indicating the presence of phycocyanin and allophycocyanin respectively. The significance of 600 nm peak appearing at 77 K will be described in discussion.

The functional property of phycobilisome cluster were examined using fluorescence spectrophotometry. Fluorescence spectra of phycobilisome isolated by either method are shown in Fig. (5). The fluorescence emission maximum was observed at 678 nm at 20°C when excited at 580 nm, and 682 nm at liquid nitrogen temperature (Fig. 6) thus indicating that energy absorbed primarily by phycocyanin was effectively transferred to allophycocyanin. The emission wavelength of intact phycobilisome was independent of the exciting wavelength.
Fig. 4. Absorption spectrum of phycobilisomes at 77°K. PBS is suspended in 0.75 M K-phosphate buffer, pH 7.0 and mixed with equal volume of glycerol.
Fig. 5. Fluorescence spectra of intact phycobilisomes at 20°C. PBS were excited at 580 nm. ——— Triton X-100; ——— CTAB.
Fluorescence spectrum of intact phycobilisomes at 77°K. PBS was suspended in 0.75 M K-phosphate buffer and mixed with equal volume of glycerol.
The fluorescence excitation spectrum of phycobilisomes at 20°C is shown in Fig. (7). The excitation spectrum was scanned from 500 to 700 nm with the emission wavelength fixed at 678 nm. The fluorescence excitation spectrum of phycobilisomes exhibited a shoulder at approximately 600 nm and a peak at 625 nm and another shoulder at 650 nm. The excitation spectrum also matched with the absorption peaks with minor difference.

The excitation spectrum also confirmed the presence of phycocyanin and allophycocyanin in the purified phycobilisome. It also clearly demonstrated that the isolated phycobilisome was functionally active and capable of transferring energy between phycobiliproteins.

The structure of intact phycobilisomes isolated from white-light-grown *Spirulina platensis* was examined by electron microscopy. After fixation with glutaraldehyde and negative staining with uranyl acetate, the phycobilisomes were found to have a hemidiscoidal structure. They are composed of aggregates of disc-shaped morphological subunits as shown in Fig. (8). The centre of the phycobilisome consists of three discs forming a triangular core. Several arms extend outwards from the core, each arm is composed of a stack of discs similar in appearance to the discs in the core. The core discs appear in face view and
Fig. 7. Fluorescence excitation spectrum of phycobilisomes at 20°C (0.75 M K-phosphate buffer, pH 7.0). Excitation spectrum were measured with the emission at 678 nm.
the arm discs are seen in the edge view. The phycobilisomes isolated from white light grown cells were composed of six arms and four discs in each arm. A model of intact phycobilisome is presented in Fig. (9).

The constituent polypeptides of phycobilisomes were examined by SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis pattern of the PBS isolated by Triton X-100 and CTAB methods is shown in Fig. (10). Lanes 1 and 3 show the polypeptide pattern of phycobilisomes prepared by CTAB and Triton X-100 respectively. Lane 2 shows the standard molecular weight marker protein. Their molecular weights are given in figure legend. The result showed the similar polypeptide composition isolated by either method. Five high molecular weight polypeptides were resolved by the SDS-polyacrylamide gels. While lower molecular weight polypeptides 15 to 20 kD were visible without Coomassie Brilliant Blue stain. These polypeptides are chromophore containing which constitute 80 to 85 percent of the phycobilisome particles. These coloured polypeptides were not individually resolved on gels shown here due to overloading required to show clearly the uncoloured polypeptides and very little difference in the molecular weights of these biliproteins. However, they were determined separately from isolated phycobiliproteins. Molecular weight of high
Fig. 8. Electron micrograph of phycobilisomes from *Spirulina platensis*.

Fig. 9. Phycobilisome model with the same fundamental phycobiliprotein arrangement. Stacked rods of phycobiliproteins are peripherally attached to a central allophycocyanin core.
molecular weight polypeptides were 70, 38, 30, 26 and 24 kD, which accounted for about 10-15 percent of the total weight of the particles. No difference between polypeptide migration during SDS electrophoresis was noted between boiled and non-boiled samples. However, fading of the phycobilisome was evident with boiled and non-boiled samples indicated that phycobiliproteins are sufficiently solubilized in 1 percent SDS and do not require high temperature for dissociation.

The second derivative absorption spectrum of phycobilisomes at pH 7.0 is shown in Fig. (11). Second derivative analysis of absorption spectrum showed two major components at 618 nm and 650 nm, indicating the presence of phycocyanin and allophycocyanin. A very small component at 593 nm was also observed which is due to the biliviolinoid chromophore.

The fluorescence excitation polarization spectra of intact phycobilisome (Fig. 12) measuring the emission through APC at 678 nm showed that there was essentially complete depolarization of the exciting light. The degree of polarization of APC increased because further transfer could not occur in phycobilisomes detached from the photosynthetic lamellae. A comparison of phycobilisomes with the isolated phycobiliproteins,
Fig. 10. SDS-polyacrylamide gel electrophoresis of the phycobilisomes. Lane 1, sample isolated by CTAB; lane 2, standard markers, Albumin, bovine, 68,000 d; Albumin, Egg, 45,000 d; Pepsin, 34,700 d; Trypsinogen, 24,000 d; B-lactoglobulin, Bovine milk, 18,400 d; Lysozyme, 14,300 d; lane 3, sample isolated by Triton X-100.
Fig. 11. Absorption spectra and second derivative absorption spectra of intact phycobilisomes. Second derivative spectrum was determined at intervals of 5 nm.
Fluorescence excitation polarization spectrum of phycobilisomes, emission monitored at 678 nm at 20°C. Phycobilisomes were suspended in 0.75 M K-phosphate buffer, pH 7.0.
at the same concentrations, shows that the polarization value of the free pigments is much higher (Fig. 22). This is in agreement with in vitro aggregation studies on PC (Goedheer and Birnie, 1965; Vernotte, 1971). These studies showed that higher aggregation state exhibited the lowest polarized fluorescence. The low value of fluorescence in the intact phycobilisomes has been interpreted to represent efficient energy migration in the phycobilisomes.

The circular dichroism spectra for the intact phycobilisome suspended in 0.75 M K-Phosphate buffer, pH 7.0 is shown in Fig. (13). The position of the bands in the circular dichroism spectrum of phycobilisome agree reasonably well with those of the absorption bands. Intact phycobilisome showed two positive CD bands, at 655 nm attributed to allophycocyanin and a peak at 626 nm arising from phycocyanin, one negative broad band at 345 nm was present due to chromophore of the biliproteins. Comparision of the absorption and CD spectra of phycobilisome obtained on the same protein solution reveals that spectra correspond quite closely in shape and the relative magnitude in the range of 500 to 700 nm.

The CD spectra of dissociated phycobilisome is also shown in Fig. (13). The difference in the intact and dissociated
Fig. 13. Circular dichroism spectra of phycobilisomes at 20°C. 
- - - - , intact phycobilisome (in 0.75 M K-phosphate, pH 7.0); --- , dissociated phycobilisome (in 10 mM K-phosphate pH 7.0). Samples were analysed at an absorbance of 0.8 cm at max.
phycobilisome were observed by CD spectra. A new band at 568 nm appeared upon dissociation of phycobilisome. The loss of 655 nm CD band of circular dichroism was observed.

Emission spectra of the time course dissociation of phycobilisome is shown in Fig. (14). Excitation of phycocyanin and allophycocyanin at 580 nm during dissociation in 200 mM K-Phosphate buffer showed a drop in 678 nm fluorescence emission which was followed by a shift in the emission to shorter wavelength. Time course dissociation study indicated that dissociation of all the phycobilisome pigment is inversely related to ionic strength and within ten minutes the emission peak shifted from 678 nm to 663 nm which indicates the physical release of allophycocyanin-B molecule from the phycobilisome aggregate. After 60 minute of treatment the peak shifted to 648 nm and finally to 642 nm, the emission peak of phycocyanin.

The effect of urea on the phycobilisomes were studied and the results are shown in Fig. (15). The ratio of the absorbance of the visible to near ultraviolet absorption bands was $> 4$ for native. The ratio decreases with the increase in the molarity of the urea. The intensity of absorbance of visible bands declined steadily and a corresponding hyperchromacity observed in the UV bands.
Fig. 14. Fluorescence emission spectra of phycobilisome as a function of time in 200 mM K-phosphate buffer, pH 7.0. For measurement of spectra, PBS suspended in 0.75 M K-phosphate buffer were suspended in 0.2 M K-phosphate buffer. The spectrum is measured at 20 minutes interval. Line 1 denotes 0 time.
Fig. 15. Absorption spectra of phycobilisomes in 0.75 M K-phosphate (pH 7.0) at different molarity of urea. ---, control; --- --- 2 M urea; --- --- --- 4 M urea; --- --- --- 6 M urea.
Effect of cationic detergent on the fluorescence emission spectra of the phycobilisomes was studied. The Fig. (16) shows the treatment of phycobilisomes at neutral pH with 1 percent CTAB. The emission spectra were measured at different time intervals. The fluorescence emission spectra of phycobilisomes treated with 1 percent CTAB showed a gradual shift towards shorter wavelength. The peak shift was also followed by increase in the fluorescence intensity.

Characterization of PC

Phycobilisome may be kept structurally and functionally intact in high phosphate concentration (0.75 M) but they begin to dissociate at low phosphate concentration. However, the dissociation of phycobilisomes was comparatively moderate when phycobilisomes concentration was as high as $A_{620}^{\text{PC}}$ 50. To obtain PC and APC subparticles (subcomplexes) separately, this dissociate was subjected to hydroxylapatite gel fractionation (see Materials and Methods). Most of the APC remained in the column while the eluate in 10 mM phosphate contained a pool of PC which was further fractionated on the linear sucrose density gradient (0.2 to 0.5 M) in 0.3 M Phosphate buffer. The absorbance property of the pooled phycocyanin is shown in Fig. (17a). The phycocyanin (Pool) showed absorption maxima at 618
Fig. 16. Fluorescence emission spectra of phycobilisome in 0.75 M K-phosphate buffer, pH 7.0, treated with 1% CTAB as function of time. Curve 0 indicates without CTAB. Rest of the spectra were taken after 10 minutes interval.
The emission spectra of the phycocyanin (Pool) is also shown in Fig. (17b). The emission spectra of the phycocyanin when excited at 580 nm showed a peak at 644 nm. Sub-particles of the phycocyanins fractionated on linear sucrose density gradient is shown in Fig. (18). For convenience the lowest band is designated as PC I, middle as PC II and the band which is at the top of the gradient is designated as PC III. The absorption spectra of these subparticles at 20°C are shown in Fig. (19a). PC I and PC II showed absorption maxima at 618 and 617 nm respectively. PC III showed a little difference in absorption property compared to PC I and PC II. PC III showed a shoulder at 600 nm. The shoulder at 600 nm indicates the presence of a different chromophore in the phycocyanins. The fluorescence emission spectra of these PC subparticles are shown in Fig. (19b). The fastest migrating bands, PC I, showed emission peak at 652 nm. PC II and PC III showed fluorescence emission maxima at 644 and 640 nm respectively. These subparticles were further characterized by measuring absorption spectra at liquid nitrogen temperature. At liquid nitrogen temperature individual chromophores were resolved into sharper bands. At liquid nitrogen temperature all the subparticles showed absorption maxima at 630 and 600 nm (Fig. 20a). The low temperature absorbance spectrum is more useful in characterizing the phycocyanin whereas at room temperature the individual
Fig. 17a. Absorption spectra of phycocyanin and allophycocyanin (pooled) in 10 mM K-phosphate buffer, pH 7.0.
Fig. 17b. Fluorescence emission spectra of pooled phycocyanin and allophycocyanin in 10 mM K-phosphate buffer, pH 7.0.
Fig. 18. Linear sucrose density gradient sedimentation profile of phycocyanin sub-particles.
Fig. 19a. Absorption spectra of PC sub-particles in 0.3 M K-phosphate buffer, pH 7.0, at 20°C. - - - - - - PC I; --- --- PC II; ----- PC III.
Fig. 19b. Fluorescence emission spectra of PC sub-particles in 0.3 M K-phosphate buffer, pH 7.0 at 20°C ($\lambda_{ex}$ 580 nm).
- - - PC I; - - - - - PC II; --- PC III.
chromophore's absorption properties are masked by another chromophore. For identification of existence of chromophore having absorbance at 600 nm, fluorescence emission spectra of phycocyanin at liquid nitrogen temperature were carried out. The fluorescence emission spectra of PC subparticles at liquid nitrogen temperature is shown in Fig. (20b). All the subparticles showed emission maxima at approximately 646 nm and another peak at 604 nm. Fluorescence excitation spectra of all the PC subparticles were measured at emission fixed at 647 nm (Fig. 21). All the subparticles showed similar excitation spectra. The fluorescence-excitation polarization spectra of all the subparticles showed that the polarization is not constant throughout their absorption band (Fig. 22). The value of polarization of PC III was higher than PC I and PC II. CD spectra of PC II and PC III are shown in Fig. (23). PC II showed one positive CD band at 624 nm while PC III showed shift in the CD band towards shorter wavelength. PC III showed one characteristic positive CD peak at 568 nm. Polypeptide compositions of the different subparticles were analyzed by using SDS-polyacrylamide gel electrophoresis. The pattern of the SDS-polyacrylamide gel electrophoresis is shown in (Fig. 24). The PC I subparticles is associated with two linker polypeptides of molecular weight 30 and 26 kD. The PC II and PC III were free from other linker polypeptides.
Fig. 20a. Absorption spectra of PC sub-particles at liquid nitrogen temperature. (i) PC I; (ii) PC II; (iii) PC III.
Fig. 20b. Fluorescence emission spectra of PC sub-particles at 77 K (excitation wavelength 580 nm). (i) PC I (ii) PC II (iii) PC III.
Fig. 21. Fluorescence excitation spectra of PC sub-particles (em 678 nm). (i) PC I (ii) PC II (iii) PC III.
Fig. 22. Fluorescence excitation polarization spectra of PC sub-particles in 0.3 M phosphate buffer, pH 7.0. Emission measured at their emission $\lambda_{\text{max}}$. 

$\lambda_{\text{max}}$: maximum wavelength.
Fig. 23. Circular dichroism spectra of PC II and PC III sub-particles (0.3 M K-phosphate buffer, pH 7.0) at 20°C. Samples were analysed at an absorbance of 1 cm⁻¹ at λmax. —— PC II; ——— PC III.
Fig. 24. SDS-polyacrylamide gel electrophoresis pattern of separated PC sub-particles. Lane 1, Phycobilisome; lane 2, PC I; lane 3, PC II; lane 4, PC III.
Characterization of APC

The allophycocyanins which were eluted from the hydroxylapatite column by using 0.3 M K-Phosphate buffer, pH 7.0 were fractionated on linear sucrose density gradient centrifugation. The fractionation pattern on linear sucrose density gradient is shown in Fig. (25). Two bands were resolved on the linear sucrose density gradient. The fastest migrating band was low in allophycocyanin content was designated as APC I. The slow migrating band was designated as APC II. The absorption spectra of both the subparticles APC I and APC II are shown in Fig. (26). Both the subparticles had similar absorption pattern. The absorption peaks were observed at 654 and 653 nm for APC I and APC II respectively. Shoulder at 615 nm was observed in both the subparticles. However, the ratio of A650/A615 were found to be different in the two species. Fluorescence emission spectra of the APC subparticles are shown in Fig. (27). Both APC I and APC II showed similar emission spectra. Fluorescence excitation polarization spectra of APC I and APC II are shown in Fig. (28). Fluorescence polarization was not constant across the visible absorption bands. Higher polarization was found at longer wavelengths. The polypeptide composition of APC I and APC II is shown in SDS electrophoretogram (Fig. 29). The figure clearly indicates the association of 70 kD high molecular weight linker
Fig. 25. Sucrose density gradient sedimentation profile of allophycocyanin sub-particles.
Fig. 26. Absorption spectra of APC sub-particles at 20°C (0.3 M K-phosphate, pH 7.0). (i) PC I (ii) PC II.
Fig. 27. Fluorescence emission spectra of APC sub-particles at 20°C (λ<sub>ex</sub> 580 nm). — APC I; — — — APC II.
Fig. 28. Fluorescence excitation polarization spectra of APC sub-particles in 0.3 M K-phosphate buffer, pH 7.0. Emission measured at 660 nm.
Fig. 29. SDS polyacrylamide gel electrophoresis of APC sub-particles. Lane 1. Phycobilisomes; lane 2, APCII; lane 3, APC I.
polypeptide with APC I. APC II was found free of any colourless polypeptide.

**Isolation and characterization of APC-B**

Another species of allophycocyanins was isolated by using chromatographic procedure. This species was present in extremely low amount and it had very different spectroscopic property. Phycobilisomes were isolated in large amount by pelleting the homogenate \( S_{35} \) at 50,000 g for 15 hrs (details are given in Materials and Methods). APC-B was isolated from hydroxylapatite using linear gradient of K-Phosphate buffer (0.01 to 0.25 M) and the elution profile is shown in Fig.(30).

The peak B was analysed by spectrophotometry and was identified as allophycocyanin-B. APC-B has different absorption property. It showed absorption peak at 673 nm and 621 nm (Fig. 31). Fluorescence emission spectra of APC-B showed maximum emission at 678 nm (see Fig. 32) which is the absorption maxima of the chlorophyll. Fluorescence excitation polarization spectra also indicate that polarization values are not constant throughout the absorption band (Fig. 31).
Fig. 30. Elution profile of allophycocyanin-B from hydroxylapatite column with increasing phosphate concentration. Absorbance at 280 nm was measured. 3.2 ml fractions were collected. Peak B indicates the APC-B fractions. Flow rate was 20 ml/hour.
Fig. 31. Absorption spectra of APC-B and their fluorescence excitation polarization spectra in 0.2 M phosphate buffer, pH 7.0.
Fig. 32. Fluorescence emission spectrum of APC-B in 0.2 M K-phosphate buffer, pH 7.0, at 20°C.
Characterization of PC subunits

Subunits of the PC were isolated from Bio-rex column by using acidic urea gradient 5 to 10 M. The elution profile of the isolated sub-units is shown in Fig. (33). Different peak area of $\alpha$ and $\beta$-subunits of phycocyanin indicates unequal ratio of $\alpha$ and $\beta$-subunits of phycocyanin. The absorption spectra of $\alpha$ and $\beta$-subunits and phycocyanin in 8 M urea is shown in Fig. (34). The absorption spectra of PC and the $\alpha$ and $\beta$-subunits in unfolded state is identical. The relative number of chromophore were derived from the absorbance ratio at 666 nm in the unfolded state. Upon refolding of the protein, the absorbance in the wavelength range (500-650 nm) increased and also the shape of the spectrum became more assymetrical. The shape of $\beta$-subunit was similar to native phycocyanin, but the $\alpha$-subunit differ from these two. The absorbance peak is split into two component about 30 nm apart at 595 nm and 568 nm (Fig. 35a ). Fluorescence emission spectra of $\alpha$ and $\beta$-subunits are shown in Fig.(35 b). The fluorescence excitation polarization of and subunits are shown in Fig. 36. It indicates that the polarization is not constant in the absorption range.

Characterization of APC Subunits

$\alpha$ and $\beta$-subunits of APC were isolated using similar method as described for PC subunits. The absorption spectra of $\alpha$ and $\beta$-subunits in 8 M Urea are shown in Fig. (37). Both $\alpha$
Fig. 33. Separation of phycocyanin sub-units by ion-exchange chromatography on Bio-Rex-70 columns. Absorbance at 280 nm was measured. Flow rate was 20 ml/hour.
Fig. 34. Absorption spectra of phycocyanin and its subunits in 8 M urea. --- PC; ------ α-subunit; --------- β-subunit.
Fig. 35a. Absorption spectra of \( \alpha \) and \( \beta \) subunits of PC in 50 mM K-phosphate buffer, pH 7.0 at 20°C. 
(i) \( \alpha \)-subunit; (ii) \( \beta \)-subunit.
Fig. 35b. Fluorescence emission spectra of and subunits of PC in 50 mM K-phosphate buffer, pH 7.0 at 20°C. Samples were excited at 580 nm. (i) -subunit; (ii) -subunit.
Fig. 36. Fluorescence excitation polarization spectra of PC subunits in 10 mM K-phosphate buffer, pH 7.0. Emission measured at 644 nm.
Fig. 37. Absorption spectra of allophycocyanin and its subunits in 8 M urea. --- APC; --- α-subunit; --- β-subunit.
and β-subunits of APC showed similar absorption pattern in 8 M Urea. Upon refolding of the subunits the difference in absorption were observed. The subunit of APC showed absorption peaks at 620 and at 650 nm (Fig. 38a). In contrast the subunit showed absorption maxima at 650 nm (Fig. 38b). The fluorescence emission spectra of α and β-subunits are shown in Fig. (39a,b). The α-subunits showed emission maxima at 642 nm and a shoulder at 655 nm. The β-subunit of APC showed emission maxima at 660 nm. The fluorescence excitation polarization spectra is shown in Fig. (40). It shows that polarization is not constant throughout the absorption range.

**Trypsin digestion study**

Quaternary interaction in the *Spirulina* phycobilisome is revealed by partial tryptic digestion. The digests were analysed at different time interval by SDS-polyacrylamide gel electrophoresis (Fig. 41). The 38 kD polypeptide is seen to be very susceptible to trypsin digestion and is almost completely digested within 1 minute. The 70 kD polypeptide is also susceptible to digestion but it gets digested slowly. It takes approximately one hour for complete digestion of 70 kD polypeptide. The fluorescence emission spectra of phycobilisome at various stages of incubation with a trypsin is shown in Fig. (42). The fluorescence emission spectra indicates drop in 678
Fig. 38. Absorption spectra of and subunits of APC in 50 mM K-phosphate buffer, pH 7.0 at 20°C. (a) α-subunit; (b) β-subunit.
Fig. 39. Fluorescence emission spectra of α and β subunits of APC in 50 mM K-phosphate buffer, pH 7.0 at 20°C. Samples were excited at 580 nm. (a) α-subunit; (b) β-subunit.
Fig. 40. Fluorescence excitation polarization spectra of α and β subunits of APC in 10 mM K-phosphate buffer, pH 7.0.
Emission monitored at 660 nm.
Fig. 41. Trypsin digestion of *S. platensis* phycobilisomes. Phycobilisomes (1.5 mg/ml) in 0.75 M K-phosphate, pH 7.0 were treated with 5% (w/w) trypsin. At the time indicated below, samples were withdrawn, TCA was added to 10% (w/v). Samples were run on SDS-polyacrylamide gels. Lane A, Phycobilisome control; lane B, after trypsin digestion time of 1, 2, 5, 10, 15, 30 and 60 minutes.
Fig. 42. Fluorescence emission spectra of phycobilisome treated with trypsin as a function of time. Phycobilisomes in 0.75 M K-phosphate was treated with 5% trypsin (w/w) and the fluorescence emission spectra were measured at 15 minutes interval. Line 1 denotes 0 time. Samples were excited at 580 nm.
nm fluorescence emission which is followed by a shift in the emission to shorter wavelength. It clearly indicates that with digestion of the phycobilisomes, the interaction among the phycobiliprotein is decreased. The complexes formed after 10 minutes of digestion were analysed on linear sucrose density gradient, which resulted in two complexes. The fastest migrating complexes were designated as complex I while the slow migrating complex as complex II. Both the components were analysed for spectroscopic properties. The absorption spectra of complex I indicated the presence of allophycocyanin with phycocyanin while complex II was exclusively phycocyanin (Fig. 43a). The fluorescence emission spectra of both the complexes are shown in Fig. (43b)). The complex I showed emission maxima of allophycocyanin while complex II showed phycocyanin emission.

**Dynamics of Phycobilisomes**

After nitrogen starvation, cultures were harvested in parallel and used for phycobilisome isolation. Fig. (44) shows a sucrose density gradient profile of phycobilisome prepared from control cells and nitrogen starved cells.

Absorption spectra of the phycobilisome isolated from one day and three days starved cells are shown in Fig.(45a). There is no gross difference in the shape of the curve but the
Fig. 43a. Absorption spectra of Complex I and Complex II of phycobilisomes isolated after trypsin digestion. Phycobilisomes (1.5 mg/ml) in 0.75 M K-phosphate, pH 8.0 were treated with 5% trypsin (w/w) for 10 minutes and the reaction was stopped with soyabean trypsin inhibitor. The digest was fractionated as linear 5-10% sucrose density gradient in 0.75 M K-phosphate buffer, pH 7.0, into two coloured zones.
Fig. 43b. Fluorescence emission spectra of complex I and complex II of phycobilisomes isolated after trypsin digestion.
Fig. 44. A sucrose step gradient profiles after centrifugation of the soluble supernatant fractions from control cells and cells that were starved 2 days for nitrogen. Number on the left side represents the initial molar sucrose concentration.
quantitative change is most apparent at 620: 650 nm absorption ratio. Control phycobilisome have a 620:650 nm absorption ratio of about 2.4, while phycobilisome from nitrogen-starved cells displayed ratio of about 1.2 after three days of nitrogen starvation. Fluorescence emission spectra of phycobilisomes prepared from control cells and those from cells starved for three days is shown in Fig. (45b). When samples were excited at 580 nm, both phycobilisome exhibited spectra characteristic of emission from allophycocyanin-B. The phycobilisome from nitrogen starved cells for 2 days showed a shift in the emission maximum from 678 to 674 nm. Thus the partial loss of phycocyanin upon nitrogen starvation has little effect on the efficiency of energy transfer within the residual phycobilisome structure. The SDS-polyacrylamide gel profiles of phycobilisomes isolated from starved cell are shown in Fig. (46). Lanes 1, 2, 3 and 4 shows control, 1 day starved, 2 days starved and 3 days starved cells phycobilisome polypeptide pattern respectively. Only one colourless polypeptide (26 kD) disappeared during one day of nitrogen starvation. During later stage of nitrogen starvation, two other polypeptides (70 and 24 kD) disappeared.
Fig. 4Sa. Absorption spectra of phycobilisomes from control cells (-----) and cells starved 3 days for combined nitrogen (-- -- --) prior to phycobilisome isolation. Samples were in 0.75 M K-phosphate, pH 7.0.
Fig. 45b. Fluorescence emission spectra of phycobilisomes from control cells (——) and cells starved from 3 days for nitrogen (---) prior to phycobilisome isolation. Samples were in 0.75 M K-phosphate, pH 7.0.
Fig. 46. SDS-polyacrylamide gel electrophoresis from control and nitrogen starved cells. Lane 1- PBS Control; lane 2- 1 day starved; lane 3- 2 days starved; lane 4- 3 days starved.