THE EFFECTS OF pH ON THE SELF-ASSOCIATION OF PHYCOBILIPROTEIN FROM ANACYSTIS NIDULANS R_2

by

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INTRODUCTION :

Phycobilisomes are aggregates of light-harvesting proteins (phycobiliproteins) attached to the stroma side of the thylakoid membranes of the cyanobacteria and red algae. These light-harvesting proteins are replaced by chlorophyll binding proteins in higher plants. These phycobiliproteins are globular and soluble in water and are of three major groups, namely Phycoerythrin (λ_{max}~565nm), Phycocyanin (λ_{max}~620nm) and Allophycocyanin (λ_{max}~650nm). These proteins are comprised of linear tetrapyrrole chromophore covalently bound to the apoprotein. The red phycobiliprotein generally termed phycoerythrins (PE) occur mainly in red algae (R). The blue phycocyanins (PC) are predominantly found in blue-green algae (C). Accordinng to their distribution and occurrence among these organisms, the phycobiliproteins have been referred to as R-PE, R-PE, C-PE, R-PC, and C-PC. Several colorless proteins are also found within the phycobilisome and serve to link the phycobiliproteins to each other in an ordered fashion and also to attach the phycobilisome to the thylakoid membrane. The above classes of phycobiliproteins comprise upto 60% of the total soluble proteins within the cell (Bogorad, 1975).

Phycobiliproteins : Subunit composition and Spectral properties :-

All cyanobacteria are found to contain both PC (Phycocyanin) and AP (Allophycocyanin) while a few, in addition, contain phycoerythrin or phycoerythrocyanin. These phycobiliproteins collectively absorb light in the green, orange, and red regions of the spectrum,

allowing the organisms to conduct photosynthesis at water depths where only long wavelength light penetrates. The difference in spectral properties of these proteins are mainly due to the environment of the chromophore conferred by the apoprotein rather than due to the spectral properties of the chromophore itself. This is very clear in the spectral difference between PC and APC, while both contain phycocyanobilin chromophore. The aggregation states of biliproteins which might induce new chromophore-chromophore or chromophore-protein interactions, strongly influences their spectral properties (Mac Coll and Brens, 1981). The participation of the colorless linker proteins also determines the spectral properties of the individual phycobilisome components (Lundell et. al., 1981). Their respective positions in the energy transfer sequence within the phycobilisome might be due to the distinctive spectral properties of the phycocyanin aggregates containing different linker polypeptides (Yu et. al., 1981).

The quaternary structure and the nature of higher aggregates have been investigated more thoroughly than the secondary and tertiary structures. They are decisive for the biological function of the phycobiliproteins. The protein portion of the phycobiliproteins consists of two dissimilar polypeptides, designated α and β, which occur in 1:1 ratio in all phycobiliproteins. In some pigments, a third subunit designated has been found, as in B-Phycoerythrin and R-Phycoerythrin (Glazer, 1980). The building block for the phycobiliproteins is the monomer $(\alpha\beta)$, with trimer $(\alpha\beta)_3$ and hexamer $(\alpha\beta)_6$ being the most common

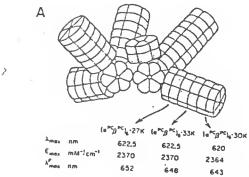
aggregation states. Glazer (1982) has proposed that interaction of a colorless linker protein with monomers of C-PC mediates the assembly into hexamers, via trimers. It has been previously (Davis et. al., 1984) suggested that the monomer-hexamer interconversion is a rapid equilibrium process and that the linker proteins serve to stabilize the hexamer, once formed. This explains why Neufield and Riggs (1969) could isolate monomer and hexamer peaks by gel filtration of pure C-PC. The apparent molecular weights vary between 9200 and 20,500 for the α-subunits and between 16,000 and 23,500 for the β-chain, with the α-subunits of the cryptophytan biliprotein at the lower limits (Boussiba and Richmond, 1979). The γ chains are considerably heavier. The subunits are classified by definition according to their molecular weights (α<β). The β-subunit always contains more chromophore molecules than the α-subunit. It has been shown that (Cohen-Bazire et.al., 1977) with APC, which has subunits carrying one chromophore each, might have similar molecular weights. Recently (Reginald and Gabriel, 1987) the structural gene for the PC and subunits from the unicellular cyanobacterium Anacystis nidulans R_2 have been cloned and sequenced. The deduced aminoacid sequence was 77% identical to that reported for Agmenellum quadruplicatum PR6. The calculated molecular weights are 17,000 for the α-subunit plus the chromophores and 19,000 for the β-subunit plus the chromophores.

Phycobilisome Structure :

From the absorption and fluorescence emmision maxima of the

phycobiliproteins one can predict that the most likely pathway for energy transfer in vivo would be PE->PC->APC->Chlorophyll a. It has been shown that a chromophore-containing linker polypeptide is associated with the thylakoid membrane and phycobilisome core (Gingrich et. al., 1982). This suggests that this polypeptide has a dual function of attaching the phycobilisome to the membrane and transferring excitation energy to chlorophyll a. The transfer of energy among the phycobiliproteins and from phycobilisome to chlorophyll a has shown to be very efficient (>97%) (Glazer et. al., 1985). The most commonly occurring structure of phycobilisomes is called hemidiscoidal, found both in red algae and cyanobacteria (Bryrant et. al., 1979). The domain structure of the rods and the core of A. nidulans phycobilisome and the possible pathway of energy transfer is shown below:

$$(\alpha\beta)_6 \cdot 30\ \mathrm{K} \rightarrow (\alpha\beta)_6 \cdot 33\ \mathrm{K} \rightarrow (\alpha\beta)_6 \cdot 27\ \mathrm{K}\ [87].$$



(Taken from Glazer (1984))

The usual hemidiscoidal model has an APC-containing core composed of three cylinders arranged so that their longitudinal axes are parallel and their ends form a triangle. There are six rod-like structure which contain PC and PE are attached to this core. PC is near

to the core and PE is far from the core. The axes of these rods are perpendicular to the longitudinal axis of the core. A slightly different type of phycobilisome is found in Synechococcus species 6301, which has only two core elements instead of three (Barbara and Linda, 1986). A. nidulans R_2 phycobilisome is very similar to Synechococcus 6301, but it is genetically better characterized. The A. nidulans phycobilisome has a central core which consists of two cylindrical, Allophycocyanin-containing complexes, to which are attached six rods. The rods contain Phycocyanin biliproteins and four non-pigmented linker polypeptides (33,000, 30,000, 27,000, and 9,000). The core has one small (~8,000) non-pigmented polypeptide, two types of Allophycocyanin biliproteins, and a large (~75,000) polypeptide which has been postulated to "anchor" the phycobilisome to PS II (Photosystem II). This anchor protein contains a covalently linked bilin (Lundell et. al., 1981).

Linker Polepeptides :

The ordered interaction of biliproteins to form phycobilisomes is mediated by a group of polypeptides called linker polypeptides, whose presence in phycobilisomes was first established by Tandeau de Marsac and Cohen-Bazire (1977). Certain of these linker polypeptides, as previously cited carry bilins, such as the larger core membrane linker polypeptide. The biliproteins are all acidic and hydrophilic whereas the linker polypeptides are basic and very hydrophobic (Lundell et. al., 1981).

Phycobilisome polypeptides fall into four classes based on their

molecular weights: Group I, 70,000-120,000, Group II, 25,000-35,000, Group III, 16,000-22,000, and Group IV, 9000-12,000. Group I polypeptides are core component involved in membrane attachment. This polypeptide undergoes rapid partial proteolytic degradation in vitro during the preparation of phycobilisomes, unless special precautions are taken to inhibit proteases (Ruscowski and Zilinskas 1982). Members of group II are linker polypeptides which function in the rod substructure assembly and in attachment of rods to the core. Group III polypeptides are the subunits of the biliproteins. Group IV polypeptides are linker polypeptides of the core components. In addition phycobilisomes contain polypeptides of 40,000-45,000 in less than one copy per phycobilisome (Zilinskas, 1982). Recently it has been shown that 75,000, 33,000, 30,000 and 27,000 phycobilisome subunits are concanavalin A-reactive and probably are glycoproteins (Reithman et. al., 1987).

Structure of phycobilisomes as related to its energy transfer function:

It is clear from the structure of phycobilisome cited in the previous section that 27,000 polypeptide discs are proximal to the core and 33,000, 30,000 complex are distal to the core. In this context, it is interesting to note the spectroscopic properties of these various complexes with phycocyanin. The fluorescence emission maxima for the phycocyanin complexes with 30,000 and 33,000 polypeptides are at 643 nm and 648 nm, respectively, while that of the complex with the 27,000 polypeptide is at 652 nm (Glazer, 1984).

So proceeding towards the core, the absorption and fluorescence emission of each disc are shifted to the red with respect to those of the adjoining distal disc. This modulation ensures that the flow of energy within the rod substructure is significantly biased towards the core. The energy difference between phycocyanin and allophycocyanin is sufficient for the core complexes to act as efficient traps. The main principle in the design of phycobilisome is seen to be the minimization of random walk within each component of the structure and between these components. This is achieved by special features of the macromolecular assembly, bilin environments and spacings to produce effecient directional intramolecular and intermolecular energy transfer pathways.

Previous studies of phycocyanin aggregation:

A number of studies have been done on the phycocyanin aggregation over more than fifty years. Phycocyanin purified by ion-exchange chromatography has been shown to exist in an equilibrium between monomer, trimer, and hexamer. The particular species present and their proportions have been shown to vary with pH, ionic strength, and organismal source of the protein. It has been observed with C-Phycocyanin in general that hexamer predominates near the isoelectric point of C-PC, ~pH 5. 0 whereas the monomer and trimer predominates at alkaline pH (Berns DS, 1971). In numerous studies by Berns and coworkers, (1971) phycocyanin-containing components of 3S, 6S, 11S, and 19S were detected. These were interpreted to represent the monomer, trimer,

hexamer and dodecamer of C-PC and the presence of even larger aggregates were reported. The procedures used for the purification of the phycocyanins used for these studies (Kao, 1973) indicates that such preparations must have contained linker polypeptides in undetermined amounts and that the presence of these components contributed to the observed polydispersity.

High salt concentration, low pH, and high biliprotein concentrations have been shown to maintain the phycobilisome structure by favoring interactions between biliproteins and linkers (Davis et. al., 1986). The nature of these interactions and the rates of biliproteins self association and dissociation has not yet been investigated. The main objectives of this research is four fold: 1. To prepare fractions containing different relative concentrations of C-PC, Allo-PC and linker polypeptides from the cyanobacterium Anacystis nidulans R_2. 2. To use non-denaturing gel electrophoresis to examine the self-assembly of biliproteins in response to pH. 3. To analyze the peptide composition of the pigmented bands from non-denaturing gels by Lithium dodecyl sulfate polyacrylamide gel electrophoresis and study the role of the linker proteins in maintaining the phycobilisome structure. 4. To use Gel filtration column chromatography to study the effect of salt concentration on phycobilisome association.

MATERIALS AND METHODS:

1. Cell cultivation:

Cells of A. nidulans R_2 were grown in shaking culture at 33^0C under constant illumination ($85 W/m^2$) from cool white fluorescent lights (Guikema and Sherman, 1982). Cultures were bubbled vigorously with air and reached late log phase in 2-3 days. After harvesting by centrifugation, the cells were used immediately or were stored as pellets at -70^0C.

2. Isolation of phycocyanin parent material:

Cell pellets were resuspended in a high salt medium (SPCM) containing 0.6 M sucrose, 80 mM KH_2PO_4, 0.55 M K_2HPO_4, 0.3 M Sodium citrate and 10 mM $MgCl_2$ pH 7.5 (Gantt, 1981). Mechanical breakage was done by using glass beads according to the method of Cramer et al (1983) and the unresolved cells were removed by centrifugation (5,000 g for 5 min). A pellet containing the thylakoid membranes was obtained by centrifugation (35,000 rpm for 30 min in a T-865 fixed-angle rotor). The blue supernatant was used to prepare four fractions as described in Fig. 1. All the buffers employed during cell breakage and biliprotein isolation contained 10 μg/ml Phenyl methyl sulfonyl fluoride, 1 mM Benzamidine, and 1 mM Norleucine to inhibit the protease activity. All PC fractions were dialyzed against dialysis buffer containing 10 mM Tricine pH 7.5 plus the protease inhibitors.

3. Non-denaturing electrophoresis:

Electrophoresis in the absence of detergents was performed. Poly acrylamide gels (7.5%) with no stacking gel were prepared by using five types of buffer systems. This allowed visualization of PC migration patterns at different pH values. The differnt types of buffer systems used were: 1. Acetate buffer pH 4.7, 2. Acetate buffer pH 5.5, 3. L-histidine and Citric acid monohydrate pH 6.2, 4. Imidazole and HEPES buffer pH 7.4, and 5. Tris buffer pH 8.8. The stock solutions for all the buffer systems were 2 M and the concentration of the buffer in the gel was 0. 075 M. In each case samples containing 25-60 μg PC (3-4 mg/ml protein), 0.075 M buffer, and 6% sucrose were applied and the gels were run in the cold (4-6^0 C) for 4-6 hours at 2.5 W constant power. The absorption profiles of samples and excised gel pieces after electrophoresis were obtained using an Aminco/SLM DW2-C recording spectrophotometer in dual beam mode. The % PC in each of the pigmented bands were calculated by scanning the gels using Kontes densitometer run at 2 cm/inch. The area under each peak was estimated by cutting and weighing, and was taken to represent the relative amount of PC in that band.

4. Lithium dodecyl sulfate polyacrylamide gel electrophoresis: (LDS-PAGE)

The excised gel pieces were also analyzed for the peptide composition by using LDS-PAGE. Also the peptide composition of the four fractions as designated in Fig. 1 was examined by LDS-PAGE. In

both cases 12.5 % polyacrylamide gel, having a 5% stacking gel were prepared using two buffer systems. The buffer system of Laemmli (1972) was employed, with 0.2% Lithium dodecyl sulfate in the running buffer. LDS (2%), 2-Mercaptoethanol (10%), and sucrose (6%) were added to the samples containing 7-30 μg PC (1.5-4.5 mg/ml protein). Sample mixtures were heated at 70^0 C for 25-30 min. The gels were run in the cold (4-6° C) for 4-5 hours at 1.5 W constant power. In the case of excised gel pieces, they were washed several times with distilled water, dried, then2% LDS, 2-Mercaptoethanol (10%), and very little stacking gel buffer (4-8μl) to bring the pH of the gel piece to pH 6. 8, were added. In all cases the gels were stained with 0.2% Silver nitrate (Morrissey, 1981).

5. Gel filtration column chromatography:

Samples were run in the presence and absence of 3 M KCl on Gel filtration column chromatography using the HPLC (High Pressure Liquid Chromatography) detector and pumps. Two buffers were used: 1. Dialysis buffer and 2. Dialysis buffer + 3 M KCl. Chromatography was carried at room temperature. The flow rate was 1 ml/min and the eluent was monitored with a detector at 620 nm. Standards like Myoglobin and Catalase were run for comparision.

6. Protein determination and PC concentration:

Protein concentration in the four fractions were determined by modified Lowry method (Markwell et. al., 1981)). The Phycocyanin concentrations were estimated spectrophotometrically after dialysis,

using molar extinction coefficients, estimated as an average values available in literature (Cohen-Bazire et. al., 1977; Bryant et al, 1976; Glazer and Hixon, 1975). The protein concentration and PC concentration was almost same in fraction A and B, but the protein concentration in fraction A′ and B′ were higher than the respective PC concentration due to the presence other contaminating proteins like Ribulose Bisphosphate Carboxylase.

RESULTS:

The flow-chart in Figure 1 shows the method used to get four biliprotein containing fractions from a thylakoid membrane fraction of Anacystis nidulans R_2. Rates of Photosystem I and Photosystem II electron transport were high if the thylakoids were isolated by using high salt (Pakrasi and Shérman, 1984). The biliproteins were removed from the thylakoids by diluting to 10% SPCM and centrifuged to yield a washed membrane fraction and a brilliant blue supernatant fraction. The supernatant contained very little chlorophyll as the contaminant. Ammonium sulfate fractionation was used to further purify the supernatant fraction. To one volume of the supernatant fraction two volumes of saturated ammonium sulfate solution was added. But this was done in two steps. To one volume of the supernatant 1/2 volume of the saturated ammonium sulfate solution was added which gave a 33% saturation. In this saturation almost all chlorophyll was pelleted. Then in the second step the rest 1.5 volume of the saturated ammonium sulfate solution was added which gave a 66% saturation. In this saturation most of the biliproteins pelleted. The two fractions designated 0-33% and 33-66% ammonium sulfate cuts were resuspended and dialyzed overnight in the dialysis buffer. These fractions were again dialyzed against 0.1 M acetate buffer pH 4.7. The difference in the pH resulted in a pellet which was removed by centrifugation. Fractions A′,A, B′and B resulted from the above treatment. These fractions were again dialyzed in dialysis buffer to remove the acid. Their absorption profiles are shown in Figure 2. Fraction A′ was contaminated with chlorophyll

Figure. 1: Isolation protocol of four biliprotein fractions from Anacystis nidulans R_2. Preparations were resuspended in dialysis buffer and dialyzed against the same buffer.

FIG.1

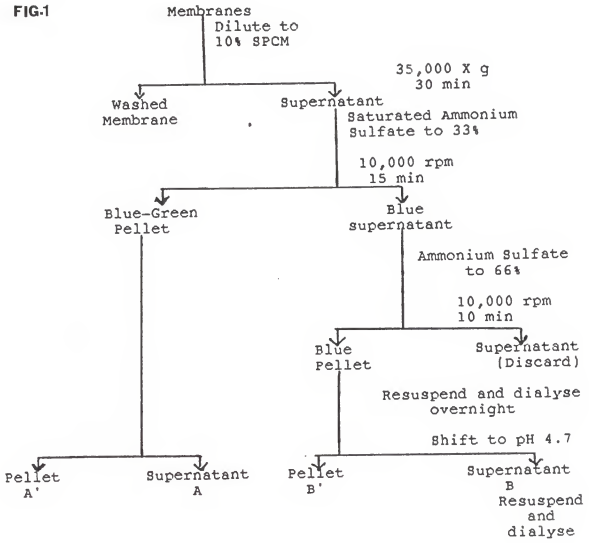

Membranes
Dilute to 10% SPCM
35,000 X g 30 min
Washed Membrane
Supernatant
Saturated Ammonium Sulfate to 33%
10,000 rpm 15 min
Blue-Green Pellet
Blue supernatant
Ammonium Sulfate to 66%
10,000 rpm 10 min
Blue Pellet
Supernatant (Discard)
Resuspend and dialyse overnight
Shift to pH 4.7
Pellet A'
Supernatant A
Pellet B'
Supernatant B
Resuspend and dialyse

Figure. 2: Absorption spectra of four fractions as designated in Fig. 1. Concentrated samples were diluted in the dialysis buffer and the absorption spectra were recorded. Full-scale deflections was about 1.0 A for fraction A′ and B′ and 0.7 for fractions A and B. The tracings were offset for easier comparisions.

FIG. 2

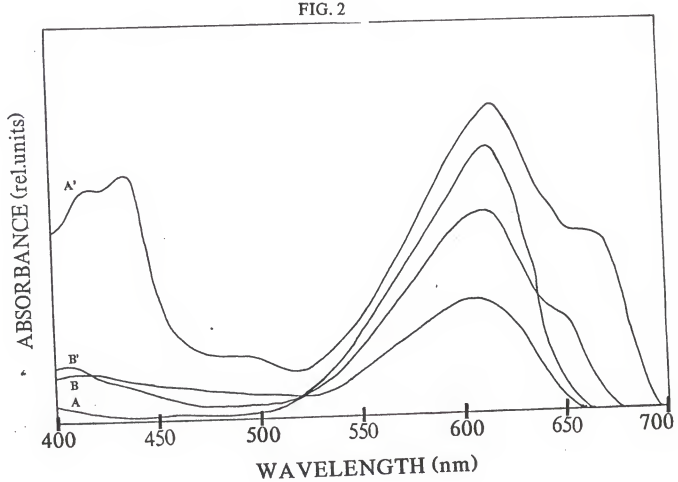
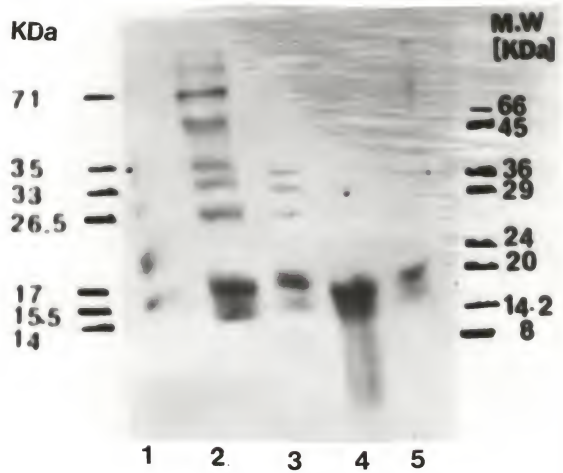

ABSORBANCE (rel.units)
A'
B'
B
A
400
450
500
550
600
650
700
WAVELENGTH (nm)

Figure. 3: Polypeptide profiles of fractions described in Fig. 1. The numbers on the left show the migration of molecular weight marker proteins run on the same gel. Numbers on the right give the relative molecular weights (in $M_r \times 10^{-3}$) of three biliproteins (14-17) and three linker proteins (26.5-35). Lane 1: Molecular weight markers; Lane 2: Fraction A'; Lane 3: Fraction A; Lane 4: Fraction B'; and Lane 5: Fraction B. For experimental details, see "Materials and Methods".

FIGURE.3



which had a peak at 673 nm. Probably this peak might contain Allophycocyanin B (Glazer, 1984). Most of the allophycocyanin was found in fraction A′ and B′ which peaked at 652 nm. It is likely that a shift in the pH enriches this biliprotein. The absorption profile for B was little bit shifted to blue when compared to A, suggesting the loss of allophycocyanin from B and probably a lowered organizational state due to decrease of linkers and dilution.

Figure 3 shows the polypeptide composition of the above mentioned four fractions. Fraction A′ and B′ contained many of the contaminating proteins like Ribulose bisphosphate carboxylase, suggesting a pH shift might precipitate these proteins. Three colored polypeptides corresponding to α and β subunits of C-PC and allo-PC at 14,000, 15,500, and 17,000 M_r were present in all four fractions, but I could not resolve the α subunit of allo-PC from that of C-PC. Some of the fractions (Fraction A′, A and B′) contained colorless polypeptides with M_r of 26,500, 33,000, and 35,000 which was similar to linker polypeptides described by Glazer (1984) for Synechoccocus 6301. But fraction B contained only a very small amount of the linker polypeptide 35,000. This difference suggested that these preparations can be used to study the role of these linker proteins in protein/protein interactions between biliproteins. The methods used for these studies were gel filtration column chromatography and gel electrophoresis.

Figure 4 shows a typical migration profile of fraction B on gel filtration chromatography in the presence and absence of 3 M KCl. A single symmetrical peak was observed in small zone. In the presence

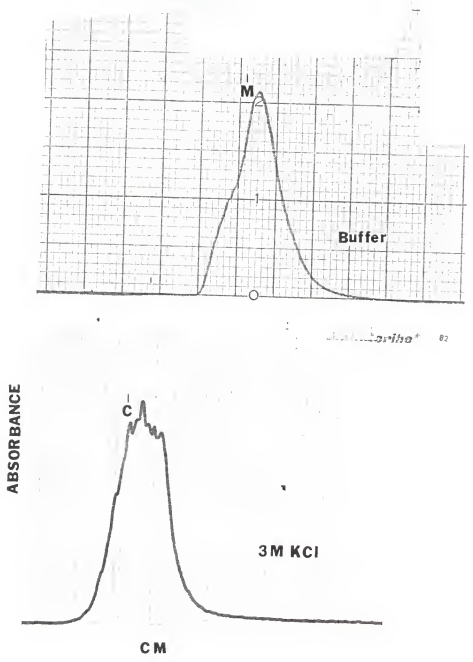


Figure 4: Gel filtration column chromatography profiles of fraction A run in the presence and absence of 3 M KCl. Fraction A (1.5-4.5 mg/ml, protein, 20-30 g PC) was run on the Sephadex G 200 column in the presence and absence of 3 M KCl. Standards like myoglobin and catalase were (designated as M and C in the Fig.) run on the same column for comparision.

of 3 M KCl, a single more rapidly moving zone was produced. This suggested that the association was the rapid-equlibrium type and was induced by high salt. The molecular weight was consistent with a hexamer (180,000 M_r) relative to that of catalase. The leading edge of the gel filtration experiment run in the presence of high salt, contained all the three linker proteins (Guikema, unpublished results). This suggests that association is tight with slow dissociation. As the concentration is varying during a chromatographic run when a small zone is applied, association constants cannot be estimated accurately. So broad zone experiments were used. But unfortunately the sample started sticking to the column and so the trailing edge never ended and the peak was not symmetrical. Probably the linker proteins were interacting with the gel matrix, because these linker proteins were recently shown to be glycoproteins (Riethman et. al., 1987). The alternate method I could try was to run gel electrophoresis in the presence of different salt concentrations. But this would be impractical, due to high current requirement to run the gel. So I switched over to the other alternative method, in which the self-association of PC was studied as a function of pH by using polyacrylamide gel electrophoresis.

Figure 5A, 5B, 5C, 5D and 5E shows the non-denaturing gel electrophoresis of fraction A run at five different pH using different buffer systems (refer to materials and methods). A heterogenous migration pattern was observed at low pH (pH 4.7, 5.5,6.2) suggesting biliprotein association. At high pH

Figure 5: Non-denaturing electrophoretic profiles of fraction A run at various pH. The conditions of electrophoresis were: Fig 5A: pH 4.7; 5B: pH 5.5; 5C: pH 6.2; 5D: pH 7.4; and 5E: pH 8.8. the sample was examined on a 7.5% polyacrylamide gel using different buffer system for various pH. Bands were visualized by pigment absorption. Three pigmented are labeled with arrows.For experimental details, see "Materials and Methods"

Figure·5

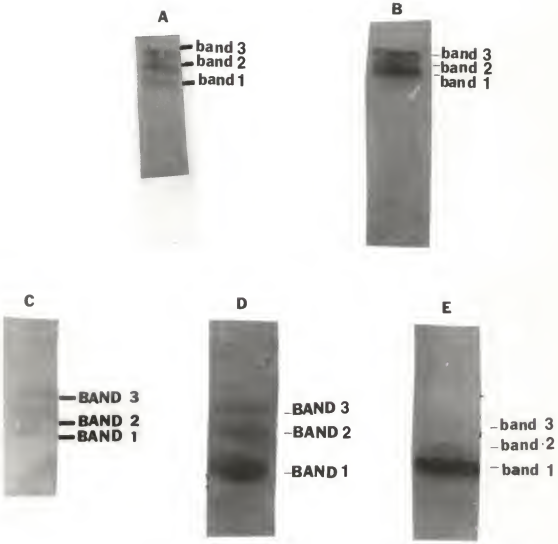

A
band 3
band 2
band 1
B
band 3
band 2
band 1
C
BAND 3
BAND 2
BAND 1
D
BAND 3
BAND 2
BAND 1
E
band 3
band 2
band 1

Figure 6: Absorption spectra of biliprotein bands eleectrophoretically resolved as designated in Fig.5. Gel pieces containing bands 1-3 were cut and placed in the sample beam of a split beam spectrophotometer. Full-scale deflection was about 0.4 A for bands 3 and 2 and 0.1 for band 1 but the tracings are offset for easier comparisions.The absorption peaks for PC ranged from 610-628 nm depending upon the pH. Fig. 6A: pH 4.7; 6B: pH 5.5; 6C: pH 6.2; 6D: pH 7.4 and 6E: pH 8.8.

FIG. 6a

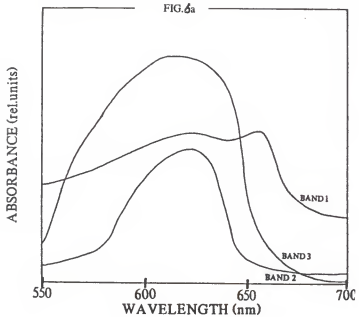

ABSORBANCE (rel.units)
BAND 1
BAND 3
BAND 2
550
600
650
700
WAVELENGTH (nm)

FIG. 6b

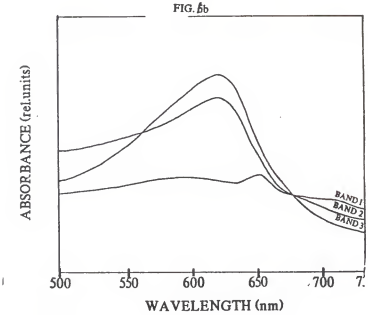

ABSORBANCE (rel.units)
BAND 1
BAND 2
BAND 3
500
550
600
650
700
WAVELENGTH (nm)

FIG. 6c

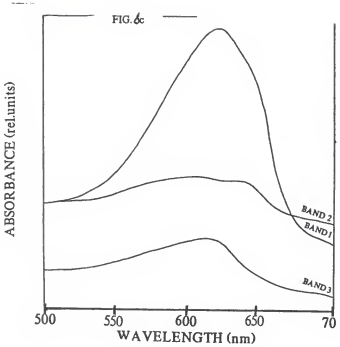

ABSORBANCE (rel.units)
BAND 2
BAND 1
BAND 3
500
550
600
650
70
WAVELENGTH (nm)

FIG. 6d

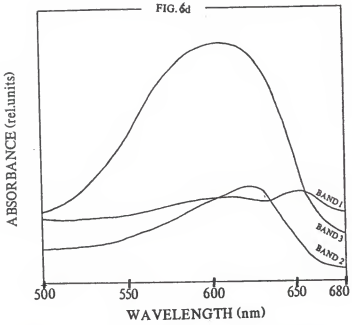

ABSORBANCE (rel.units)
BAND 1
BAND 3
BAND 2
500
550
600
650
680
WAVELENGTH (nm)

FIG. 6e

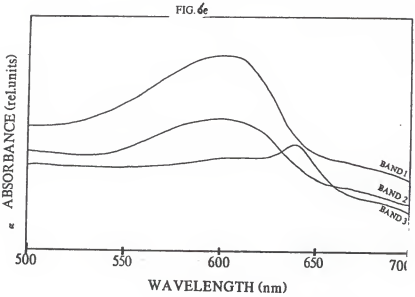

ABSORBANCE (rel.units)
BAND 1
BAND 2
BAND 3
500
550
600
650
WAVELENGTH (nm)

TABLE I: Aggregation of PC as a function of pH

Band Name	Relative Area Under Peaks (% Total PC) pH$_c$				
	4.7	5.5	6.2	7.4	8.8
Band 1^f	7.5	12.5	24	66.8	75.5
Band 2	20.5	12.5	25	16.6	15
Band 3^s	72	75	50	16.6	10

^{c} Concentration of buffer is 0.075 M for all pH values.
^{s} Slow moving band
^{f} Fast moving band

a homogenous migration pattern was observed. The other noticable point is the migration rate of C-PC is slow relative to A-PC at low pH when compared to high pH, suggesting a shift in the relative charge. Figure 6A, 6B, 6C, 6D, and 6E shows the absorption spectrum of the pigmented bands from non-denaturing electrophoresis run at different pH. At low pH band 1 was predominant in A-PC and at high pH band 3 was mostly rich in A-PC. Band 1 at low pH was an aggregation of linker polypeptides plus the biliproteins. Band 2 at low pH was enriched in phycocyanin. The maximum absorbance wavelength for PC changed as the pH increased. It shifted a little towards the blue at high pH (pH 8. 8).

Table 1 shows the scanning results of native gels run at different pH. As we go up the pH from 4.7 to 8.8, the percentage PC in the slow moving band (band 3) decreases , suggesting aggregtion of phycocyanin at low pH and dissociation at high pH. It is possible that band 3 is hexamer, band 2 is trimer and band 1 is monomer of PC.

The polypeptide composition of these pigmented bands were studied by Lithium dodecyl polyacrylamide gel electrophoresis. Figure 7A, 7B, 7C, 7D and 7E shows the polypeptide profiles of the excised blue bands from non-denaturing electrophoresis run at different pH. The slowest moving band (band 3) contained all the three linkers 26,500, 33,000, and 35,000 dalton at low pH. But at pH 8.8, there was only very little amounts of the linkers 26.5- and 35-KDa and 33 KDa polypeptide was completely absent (Table II). Band 2 contained α and β -subunits of PC (14,000 and 17,000). Probably PC is associating at low pH and linker proteins stabilizes the

Figure 7: Polypeptide composition of pigmented bands as designated in Fig. 5 by LDS-PAGE. The bands were excised and washed several times with distilled water, dried, and equilibrated with diluted stacking gel buffer (pH 6.8), 2% LDS, 10% 2-mercaptoethanol, 6% sucrose. Then these bands were examined on a 12.5% polyacrylamide gels in the presence of LDS to determine the polypeptide composition. Numbers to the right gives the measured molecular weights (as $M_r \times 10^{-3}$) for linker proteins and biliproteins. In Fig. 7B, 7C, 7D, and 7E, Lane 1: Band 1, Lane 2: Band 2 and Lane 3: Band 3. In Fig. 7A, Lane 1: Band 1, Lane 2: Band 3 and Lane 3: Band 2.

FIGURE.7

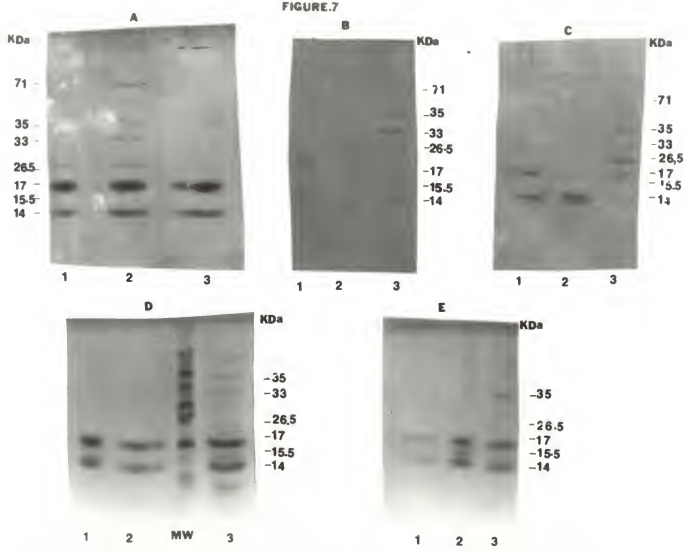

A
KDa
71
35
33
26.5
17
15.5
14
1 2 3
B
KDa
71
35
33
26.5
17
15.5
14
1 2 3
C
KDa
71
35
33
26,5
17
15.5
14
1 2 3
D
KDa
35
33
26,5
17
15.5
14
1 2 MW 3
E
KDa
35
26.5
17
15.5
14
1 2 3

TABLE II: Effect of pH on the concentration of linker proteins

Band Name	Relative Area under Peaks (%) pH 4.7	5.5	6.2	7.4	8.8
Linker 1	4	4.3	3.1	2.5	1.1
Linker 2	6	6	5.2	1.4	--
Linker 3	5	4.7	3	2	1.5
β-PC	50	57	56	78	70
α-PC	23	25	25	16	26
α-APC	--	--	--	--	--
β-APC	12	1.5	3	1	2

aggregative form once formed. This result is consistent with the one observed by Grossman (1983).

DISCUSSION:

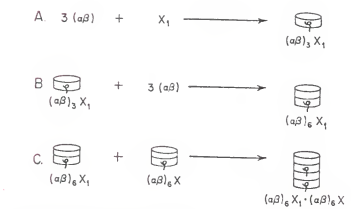
In this study, I have used polyacrylamide gel electrophoresis to study the structure of phycobiliproteins from Anacystis nidulans R_2. Low pH enhanced the association of peptides within fraction A. Previously it has been suggested (Davis et. al., 1986) by direct scanning gel filtration that high salt induced the biliprotein association. The polypeptide composition of fraction A was similar to the one observed by Grossman (1983) and also similar to the phycobilisome composition of a closely related strain of Synechococcus 6301. The distinct banding pattern suggests a tight pH induced association. AT pH 4.7 and 5.5 (near isoelectric pH of PC ~5.2) the charge repulsion will be minimum and and also a decrease in the solubility of non-polar groups in water. Both of these factors tend to result in the enhancement of the hydrophobic interactions needed for the formation of larger aggregates. At higher pH, the acidic and the basic side chain of all proteins are mostly in the charged state with the maximum effect of charge repulsion. So hydrophobic interaction between molecules are decreased and dissociation occurs.

The LDS-PAGE electrophoresis (Fig. 7) on the excised blue bands suggests that the linker polypetides 26,500, 33,000, and 35,000 has a role in stabilizing the rod substructure of phycobilisome. It is interesting to note that the phycocyanin fluorescence depolarization measurement of Goedheer (1957) indicates inductive energy transfer for phycocyanin in the pH 4-6 region. This is the region of greatest stability of the hexameric

structure, and the hexamer structure certainly would favor internal inductive energy transfer between subunits and subsequently fluorescence from differently oriented chromophores. At pH 7 and above, the depolarization decreases, which is consistent with my results. Probably one or two chromophoric tetrapyrroles are associated with each minimal unit. My observation confirms those of others (Hattori, 1965, and Scott and Berns, 1965), that the absorption spectrum changes with pH (628-610 nm). Although some changes in the spectrum appear to occur with dilution, it is by no means clear whether the major changes in the absorption are due to changes in the aggregation states or merely result from changes in the chromophore environment with pH. It is not surprising the change in the aggregation state of the protein or hydrogen ion concentration of the environment should affect the spectrum (Neufield and Riggs, 1969). It is reasonable to assume that the orientation of the chromophoric groups in relation to the protein moiety and neighbouring chromophores or subunits change slightly, but significantly when the quartenary structure is altered. A change in the environment might effect either the co-planar structure of the chromophores or the interaction of the chromophore with the protein moiety or the relation of chromophores to each other.

From Figure 7 and Table II it is reasonable to infer that the other components of the phycobilisome fulfill the organizing or linker functions that lead to the assembly of the biliproteins into rods and core and attachment of the phycobilisome to the

membrane. In vitro studies (Yu and Glazer, 1981) on the rod substructures of Synechococcus 6301 shows that the ratio of monomers to those of phycocyanin in an assembled rod would not be less than 1:6. This agrees with the values in Table II for low pH and differs for high pH, suggesting at low pH PC is assembled into rods by linkers and dissociates at higher pH. The mechanism of assembly might take place in two ways: A) Initiation of aggregation by a linker and a monomer of PC or B) an aggregation of PC monomers and then binding by a linker. A reasonable mechanism of assembly might be as follows: Phycocyanin monomers first assembles into trimers which may or may not incorporate a molecule of linker polypeptide depending on the concentration of the linkers. After a trimer is formed it proceeds to bind with another trimer to form a hexamer . Probably a trimer containing no linker will not bind to another unless the latter contains a linker polypeptide. The high resolution crystal structure of C-PC from Mastigocladus laminosus shows that trimeric discs of C-PC serves as the template for the formation of hexameric discs (Tilman Schirmer, 1985). The above arguement suggests that in the phycobilisome model presented previously, the rods are built up from hexameric double discs, $(\alpha\beta)_6$, which consists of two trimers $(\alpha\beta)_3$ stacked on top of each other. In other words , in the rods the discs are in face to face contact. Pathway of in vitro assembly of the rod substructures of cyanobacterial phycobilisomes studied by Glazer (1982) is shown below.



(Taken from Glazer (1981))

In vitro assembly experiments like Trypsin digestion might provide evidence for confirming the above proposed mechanism of assembly. In addition studies of the exchange of labeled proteins might give useful information about the interaction of these linker proteins in the assembly of phycobiliproteins

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THE EFFECTS OF pH ON THE SELF-ASSOCIATION OF PHYCOBILIPROTEIN FROM *ANACYSTIS NIDULANS* R_2

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ABSTRACT

The effects of pH and salt concentrations were studied by using polyacrylamide gel electrophoresis and gel filtration chromatography. Fractions containing different concentrations of phycocyanin, allophycocyanin, and linker polypeptides were prepared from Anacystis nidulans R_2. Three pigmented bands and three colorless polypeptides were found in some of those fractions. The effects of pH on the pigment-protein profiles in non-denaturing polyacrylamide gel electrophoresis showed a greater degree of biliprotein association at acid pH and dissociation at alkaline pH. The migration rates of biliproteins were monitered in gel filtration columns using an HPLC system. High salt concentrations enhanced biliprotein migration rates, suggesting increased association under those conditions. Lithium dodecyl sulphate polyacrylamide electrophoresis on the excised blue bands shows depletion of linker polypeptides (26,500, 33,000 and 35,000), suggesting these peptides organize or stabilize biliprotein aggregates.