Biochimica et Biophysica Acta, 724 (1983) 323–332
Elsevier

BBA 41353

EFFECTS OF CHAOTROPIC ELECTROLYTES ON THE STRUCTURE AND ELECTRONIC EXCITATION COUPLING OF GLUTARALDEHYDE- AND DIIMIDO ESTER-CROSS-LINKED PHYCOBILISOMES

GEORGE C. PAPAGEORGIOU and THOULA LAGOYANNI

Department of Biology, Nuclear Research Center Demokritos, Aghia Paraskevi, Athens (Greece)

(Received April 26th, 1983)

Key words: Phycobilisome; Glutaraldehyde; Diimido ester; Cross-linking; Chaotropic electrolyte; (Nostoc muscorum)

The tendency of isolated intact phycobilisomes to disperse into multimeric phycobiliprotein subunits in dilute phosphate buffer is diminished, or even eliminated, by means of covalent polypeptide cross-linking with glutaraldehyde, or dimethyl suberimidate. Employing sensitized fluorescence spectrophotometry in order to detect transitions of the type phycobilisome → phycobiliprotein multimers, and second-derivative absorption spectrophotometry to detect transitions of the type phycobiliprotein multimers → monomers, we investigated the molecular basis of phycobilisome stabilization by these two cross-linkers. A network of intrahexamer and interhexamer covalent cross-links prevents the dissociation of glutaraldehyde-treated phycobilisomes into monomers, even under the strong chaotropic effect of KNO_3 and KSCN solutions, but fails to protect against polypeptide unfolding in concentrated urea solution. Dimethyl suberimidate, on the other hand, introduces only intramonomer cross-links, and thus it does not prevent dissociation to monomers in the presence of KNO_3 and KSCN. Increased hydrophobicity of phycobiliprotein subunits, as a result of the alkylation of $-NH_2$ by the diimido ester, or the dialdehyde, is an additional structure-stabilizing factor.

Introduction

Phycobilisomes, the light-harvesting organelles of cyanobacteria and red algae, are highly organized structures of several hundred polypeptides locked together by noncovalent forces that originate predominantly from hydrophobic interactions (reviewed in Refs. 1–4). Gantt [1,2] succeeded in extracting intact phycobilisomes by performing the isolation in 0.75 M phosphate buffer, pH 6.8–7.0. These phycobilisomes are characterized by a tight electronic coupling of the bilin chromophores, as suggested by the fact that they emit almost exclusively allophycocyanin fluorescence, irrespective of which phycobiliprotein is excited.

Dissociation of phycobilisomes into smaller subunits influences the electronic energy levels and the electronic coupling of the bilin chromophores, and it can be detected by optical spectroscopic techniques. Dissociation into multimeric phycobiliproteins (trimers and hexamers) interrupts the excitation transfer sequence phycoerythrin → phycocyanin → allophycocyanin of the native phycobilisome, so that upon excitation each phycobiliprotein fluoresces individually [5]. Further dissociation of multimers into monomers (actually heterodimers $\alpha\beta$) affects the visible absorption characteristics of phycobiliproteins, as it can be detected by means of absorption spectroscopy [6–11], difference absorption spectroscopy [12,13], derivative absorption spectroscopy [14,15] and circular dichroism [16,17]. In addition, it lowers the yield [10] and the polarization [18,19] of phycobiliprotein fluorescence, and may influence the rate of intramonomer excitation transfers between

0005-2728/83/$03.00 © 1983 Elsevier Science Publishers B.V.

chromophores [20,21]. Finally, the drastic alteration of the protein environment of chromophores, and the change of chromophore conformation, which attend the dissociation and denaturation of monomer polypeptides, are reported by a suppression of the visible absorption bands of phycobiliproteins, and a simultaneous enhancement of the near-ultraviolet absorption [9,22].

Treatment of phycobilisomes, either in situ or in vitro, with protein cross-linking compounds, such as glutaraldehyde [15,23–27] and diimido esters [28,29], is known to stabilize the supramolecular associations of phycobiliproteins, and the electronic energy coupling between bilin chromophores. Two possible factors contribute to the stabilization: firstly, the network of interpolypeptide and intrapolypeptide covalent cross-links; and secondly, the enhanced subunit hydrophobicity of phycobiliproteins whose $-NH_2$ groups have been alkylated by the cross-linking compounds [15]. Our objective, in the present work, was to examine the kinds of established covalent cross-links, and to investigate the molecular basis of phycobilisome stabilization following treatment with glutaraldehyde, or dimethyl suberimidate.

Materials and Methods

Sterile cultures of *Nostoc muscorum* were grown for 6–8 days as described in Ref. 30. Intact phycobilisomes were isolated according to the method of Gantt et al. [31], as modified by Zilinskas and Glick [32].

Purified glutaraldehyde (Sigma grade I, 25% (v/v); $A_{235}/A_{280} = 0.25$, hence predominantly monomeric [33]) was used for cross-linking. Its molarity, determined according to the method of Hesse [34], was found to be 3.24 M. The reagent was neutralized with 1 M KOH, diluted to 0.1 M with 0.75 M potassium phosphate buffer (pH 6.9) and mixed with the phycobilisome suspension (0.4–0.5 mg phycobiliprotein in 0.75 M potassium phosphate, pH 6.9, and approx. 0.20–0.25 M sucrose) at 10 μmol glutaraldehyde/mg phycobiliprotein. After 18–20 h incubation at room temperature and in darkness, the mixture was centrifuged at $12000 \times g$ for 30 min. The pellet, consisting of intact phycobilisome aggregates, was rinsed with phosphate buffer and stored at 0–4°C.

To avoid hydrolysis of the imidate group, it is necessary to perform diimido ester cross-linking of proteins in strongly alkaline solutions [35]. Since, however, above pH 8.5–9.0 the phycobilisomes dissociate [29], we performed the cross-linking reaction at the compromise pH range of 8.0–8.2 as follows: To 5 ml phycobilisome suspension in 0.75 M potassium phosphate buffer (pH 6.9) containing 1.5 mg phycobiliprotein, 1.3 ml triethanolamine phosphate buffer (0.75 M potassium phosphate, 1 M triethanolamine hydrochloride, pH 10) were added, bringing the pH of the mixture to 8.2. The reaction mixture was cooled in ice bath and crystals of diimido ester hydrochloride (dimethyl adipimidate or dimethyl suberimidate; obtained from Pierce) were added until a ratio of 20 μmol cross-linker/mg phycobiliprotein was achieved. At this level, the molar ratio of imidate/NH_2 is between approx. 90 and 110, and more than 90% of the $-NH_2$ groups are amidinated [15]. During the addition of the diimido ester, the reaction mixture was stirred continuously, and the pH was maintained between 8.0 and 8.2, being adjusted with dilute KOH when necessary. After a 30 min incubation at 0°C, the reaction mixture was centrifuged at $12000 \times g$ for 30 min. The pellet, consisting of aggregated intact phycobilisomes, was washed once with phosphate buffer and stored at 0–4°C until use.

SDS-urea polyacrylamide gel electrophoresis was performed according to Technical Bulletin No. NWS-877 of Sigma Chemical Co. Samples were prepared by dissolving 1 mg protein in 1 ml medium, which contained 6 M urea, 1% (w/v) SDS, 1% (v/v) 2-mercaptoethanol and 0.1 M potassium phosphate (pH 6.9) and then incubating the mixture at 37°C for 2 h. The gels (10% (w/v) acrylamide in the main gel, 5% (w/v) acrylamide in the stacking portion, with 0.28% (w/v) *N*,*N*-bismethylene acrylamide) were preelectrophoresed for 2 h at 4 mA/gel, and then a 10 μl aliquot was applied to each gel, and the electrophoresis was continued for 20–22 h at the same current density. Cross-linked hemoglobin (16, 32, 48 and 64 kDa; Sigma Chemical Co.) was used for correlating the electrophoretic mobility to molecular size. Staining and destaining were performed according to the method of Chua [36]. The gels were scanned in a Joyce-Loebl chromoscan, employing a cutoff filter transmitting above 620 nm.

Absorption ($\Delta\lambda = 1$ nm) and second-derivative absorption ($\Delta\lambda = 2$ nm) spectra were recorded with a Hitachi Model 557 dual-wavelength spectrophotometer. Fluorescence was measured with a Perkin-Elmer Model MPF-3L spectrofluorometer, operated in the ratio mode. The fluorescence spectra are displayed uncorrected for the variation of the photomultiplier sensitivity and of the transmittance of the analyzing monochromator with the wavelength. The correction factors, however, vary little with the wavelength in the spectral region studied. Phycobiliproteins were determined according to the method of Bennett and Bogorad [37], total protein as described by Lowry et al. [38] and primary amino groups as detailed by Böhlen et al. [39].

Results

One prerequisite for preserving intact phycobilisomes in suspension is a high concentration of phosphate anion (0.65 M or more) which as an antichaotropic agent generates sufficiently strong hydrophobic interactions among phycobiliprotein subunits [32]. When the concentration of phosphate is lowered, the antichaotropic effect is diminished and phycobilisomes dissociate with a concomitant loss of excitation coupling between phycobiliproteins [5]. As performed in the present work, phycobilisome dissociation in dilute phosphate yields primarily trimers, whereas under the influence of strongly chaotropic anions the main products are monomeric phycobiliproteins [10,11,40].

It has been shown that the treatment with glutaraldehyde or dimethyl suberimidate makes phycobilisomes resistant to dissociation in dilute phosphate buffer [15,29]. Here, we have examined whether they can also resist dissociation in the presence of the chaotropic anion SCN^-. Phycobilisomes were incubated for 1 h at room temperature and in darkness, either in 0.75 M potassium phosphate buffer (pH 6.9), or in a medium containing 0.375 M KSCN and 0.003 M potassium phosphate (pH 6.9). The two dissociation media were nearly isoionic ($I = 0.375$ and 0.383, respectively). Dissociation was assessed in terms of phycoerythrin-sensitized ($\lambda_{exc} = 540$ nm) and phycocyanin-sensitized ($\lambda_{exc} = 620$ nm) allophycocyanin fluorescence. Fig. 1 shows that with either excitation wavelength, control phycobilisomes and cross-linker-reacted phycobilisomes emit allophycocyanin fluorescence (approx. F_{660}), as long as they remain suspended in the concentrated phosphate medium. On the assumption that structural integrity is a prerequisite for the electronic energy coupling of phycobiliproteins, these phycobilisomes must be intact. In the SCN^--containing medium, however, only the glutaraldehyde-treated phycobilisomes retain the capability for sensitized allophycocyanin fluorescence (Fig. 1, lower spectra). Control (Fig. 1, upper spectra) as well as dimethyl suberimidate-

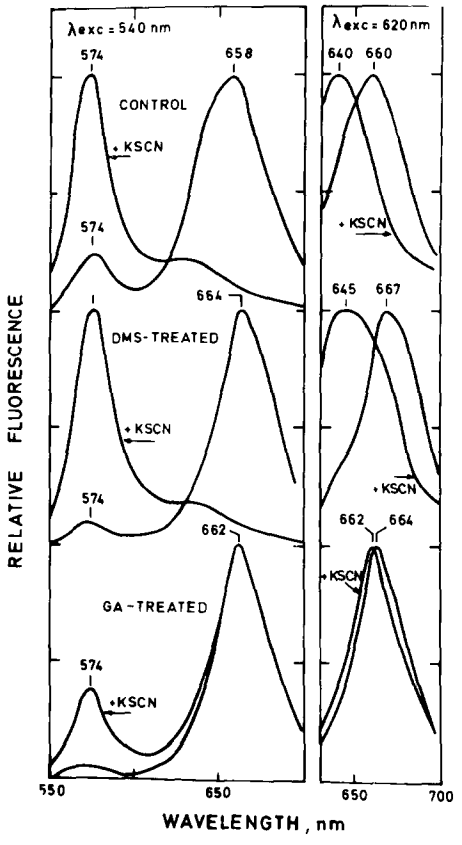


Fig. 1. Fluorescence spectra of control, dimethyl suberimidate (DMS)-treated and glutaraldehyde (GA)-treated phycobilisomes after 1 h incubation either in 0.75 M potassium phosphate buffer (pH 6.9) or in 0.375 M KSCN and 0.003 M potassium phosphate (pH 6.9). The KSCN-treated samples are indicated on the figure. Excitation, $\lambda = 540$ nm and $\lambda = 620$ nm; $\Delta\lambda = 2$ nm. Emission, $\Delta\lambda = 2$ nm. The spectra are normalized at the maximum. Samples, 40 μg phycobiliprotein/ml.

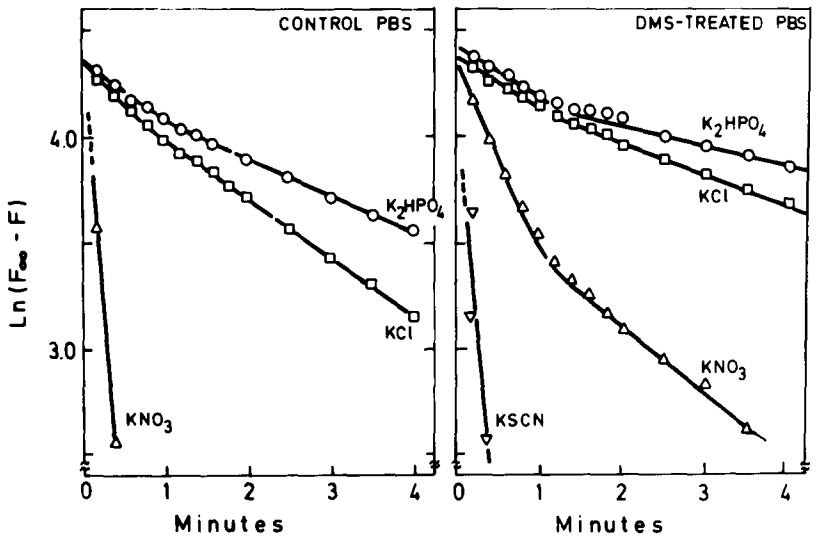


Fig. 2. Logarithmic plots of phycoerythrin fluorescence (F_{575}; $\Delta\lambda = 2$ nm) as a function of the incubation time of phycobilisomes (PBS) in buffered media containing isoionic concentrations ($I = 0.375$) of the indicated electrolytes. F_∞ and F, phycoerythrin fluorescence at the end of phycobilisome dissociation and at any given time, respectively. Excitation, $\lambda = 540$ nm, $\Delta\lambda = 2$ nm.

treated phycobilisomes (Fig. 1, middle spectra) dissociate, so that each phycobiliprotein fluoresces only when excited directly.

When all other conditions are identical, the anions of the suspension medium exert a dissociative effect on phycobilisomes, whose relative magnitude varies in accordance with the rank of the anion in the lyotropic series [32]. To determine whether this is valid also in the case of dimethyl suberimidate-treated phycobilisomes, we performed the experiment illustrated in Fig. 2. Phycobilisomes were incubated with the indicated electrolytes ($I = 0.375$) in media buffered at pH 6.9 with 0.003 M potassium phosphate, and the dissociation of phycoerythrin was followed in terms of its fluorescence intensity. This is proportional to the concentration of the released pigment because the measured samples were dilute (40 μg total phycobiliprotein/ml). In the case of control phycobilisomes, incubated in the presence of KH_2PO_4 and KCl, and in the case of diimido ester-treated phycobilisomes incubated in the presence of KH_2PO_4, KCl and KNO_3, the rise of phycoerythrin fluorescence appears to obey two consecutive first-order processes. Dissociation of phycoerythrin in the presence of KSCN was very fast, especially in the case of control phycobilisomes, with no evidence for a second first-order process.

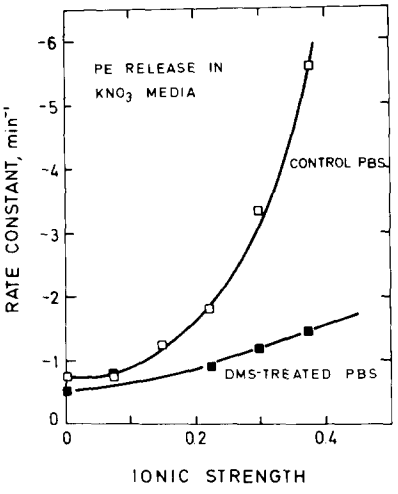


Fig. 3. The rate constant of phycoerythrin release from control and dimethyl suberimidate (DMS)-treated phycobilisomes (PBS), as a function of the ionic strength of KNO_3 in the dissociation medium.

With regard to the rates of phycoerythrin dissociation these anions can be ranked in the order $SCN^- > NO_3^- > Cl^- > HPO_4^{2-}$. Fig. 2 shows clearly that treatment with dimethyl suberimidate delays but does not prevent phycobilisome dissoci-

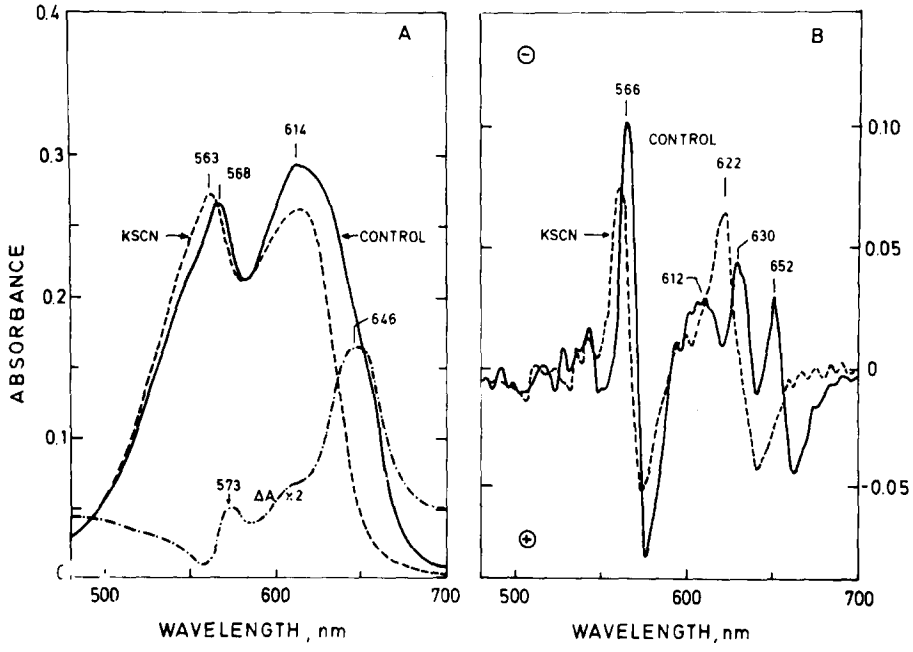


Fig. 4. Absorption (A) and inverted second-derivative absorption (B) spectra of intact phycobilisomes (———) and of phycobilisomes dissociated in the presence of 0.375 M KSCN (— — —). Difference absorption spectrum, intact-dissociated phycobilisomes (·–·–·).

ation. The moderately protective effect of this cross-linker is further supported by the results of Fig. 3. In this figure, the rate constant of the first dissociation process is plotted against the ionic strength of KNO_3 in the incubation medium. The structural stability of phycobilisomes is remarkably enhanced as a result of dimethyl suberimidate treatment. This is particularly evident at high ionic strengths of KNO_3.

These results definitely rule out the presence of interhexamer (i.e., interdisc) covalent cross-links in dimethyl suberimidate-treated phycobilisomes. Intrahexamer cross-links, however, cannot be ruled out on the basis of sensitized fluorescence experiments, such as those in Figs. 1 and 2. We examined the possibility of such cross-links with the help of absorption spectrophotometry and second-derivative absorption spectrophotometry, since they are capable of reporting on multimer-to-monomer transitions.

In the experiment presented in Fig. 4, intact phycobilisomes (80 μg phycobiliprotein/ml) were incubated for 1 h, at room temperature either in 0.75 M potassium phosphate buffer (pH 6.9), or in a solution of 0.375 M KSCN, 0.003 M potassium phosphate (pH 6.9). According to the criterion of phycoerythrin-sensitized allophycocyanin fluorescence, phycobilisomes suspended in the concentrated phosphate medium remained intact, while those suspended in the KSCN medium were dissociated. This dissociation is shown here to be accompanied by an absorption loss whose difference band implicates allophycocyanin (ΔA_{646}; Fig. 4A). No absorption changes that would signify denaturation of the α- and β-polypeptides of phycobiliproteins were detected in the near-ultraviolet region.

More detailed information about the KSCN-induced dispersion of phycobilisome subunits is provided by the second-derivative absorption spectrum (Fig. 4B). Inverted second derivatives of Gaussian and Lorentzian curves have their major bands in the same position, and pointing to the same direction, as the original curves, and provide for superior band resolution in the case of overlaps [41]. Intact phycobilisomes are characterized by four major second-derivative absorption bands, centered at 652, 630, 612 and 566 nm. On the basis

of their spectral locations, the 652 and 566 nm bands can be assigned to allophycocyanin and phycoerythrin, respectively, while the 612 and 630 nm bands should be mixed absorptions, containing contributions from both phycocyanin and allophycocyanin chromophores [10,12,42]. After KSCN treatment, the second-derivative absorption bands at 612, 630 and 652 nm merge into a single band centered at 622 nm, while the phycoerythrin band at 566 nm shifts slightly to the blue. It has been shown that in the presence of powerful chaotropic agents, such as SCN^-, ClO_3^- and NO_3^-, phycocyanin and allophycocyanin multimers dissociate to monomers, whose phycocyanobilin chromophores have common absorption (616 nm) and fluorescence maxima (642 nm [10,11,40]). We may infer, accordingly, that the coalescence of the 612, 630 and 652 nm bands into a single band (622 nm) signifies the dissociation of these phycobiliproteins into monomers.

Using second derivative absorption spectrophotometry, we examined the effects of K_2HPO_4, KCl, KNO_3 and KSCN on phycobilisome structure. Control phycobilisomes (Fig. 5) and dimethyl suberimidate-treated phycobilisomes (Fig. 6) were incubated for 2 h, at room temperature, in isoionic solutions of these electrolytes ($I = 0.375$), buffered at pH 6.9 with 0.003 M potassium phosphate. It is quite clear from these figures that phycobilisome dissociation is a more complex event than the mere detachment of phycobiliprotein multimers. Even a mildy chaotropic medium, such as 0.125 M potassium phosphate buffer, causes appreciable fusion of phycocyanin and allophycocyanin ab-

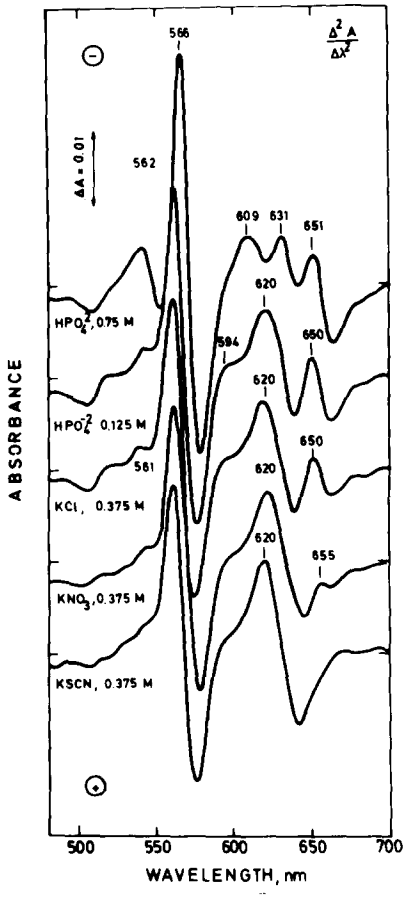


Fig. 5. Inverted second-derivative absorption spectra of phycobilisomes incubated for 2 h in the presence of the indicated electrolytes. $I = 0.375$.

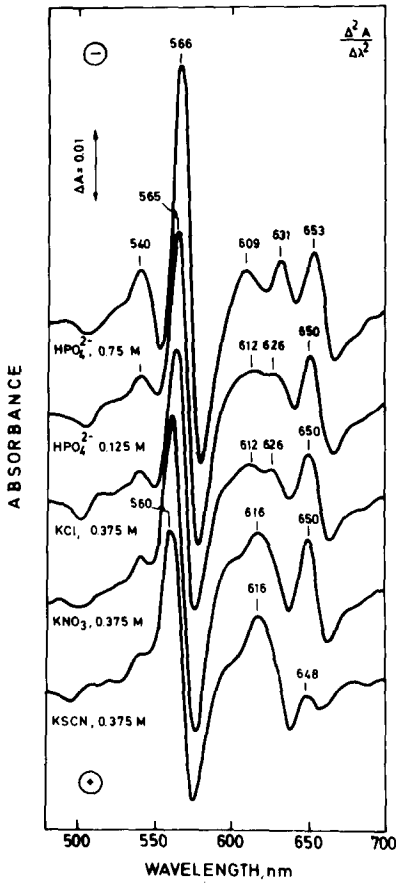
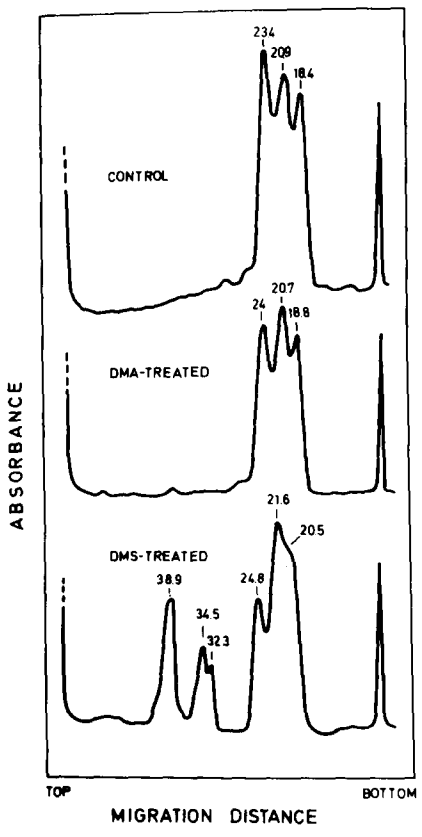


Fig. 6. Inverted second-derivative absorption spectra of dimethyl suberimidate-treated phycobilisomes, obtained after 2 h incubation in the presence of the indicated electrolytes. $I = 0.375$.



sorption, as well as a blue shift of the phycoerythrin absorptions in the control and diimido ester cross-linked preparations. This is quite remarkable, since reaction with dimethyl suberimidate, as performed here, results in the amidination of more than 90% of phycobiliprotein -NH_2 groups [15]. Qualitatively, with regard to the magnitude of the absorption changes they cause, the electrolytes studied are ranked in the order KSCN > KNO_3 > KCl > K_2HPO_4. Diimido ester-treated phycobilisomes appear, nevertheless, more resistant to chaotropic action. For example, the 652 nm second-derivative absorption band is virtually absent from the control preparation after KNO_3 treatment (Fig. 5), but still present in the diimido ester-treated preparation (Fig. 6). Incubation with KSCN, however, eliminates this band in both preparations.

Since intermonomer cross-links would have, most likely, prevented multimer-to-monomer transitions, we must conclude on the basis of Fig. 6 that such cross-links are absent from dimethyl suberimidate-treated phycobilisomes. The absence

Fig. 7. Electrophoresis of urea-SDS dissociated control, dimethyl suberimidate (DMS)-treated and dimethyl adipimidate (DMA)-treated phycobilisomes on polyacrylamide gels.

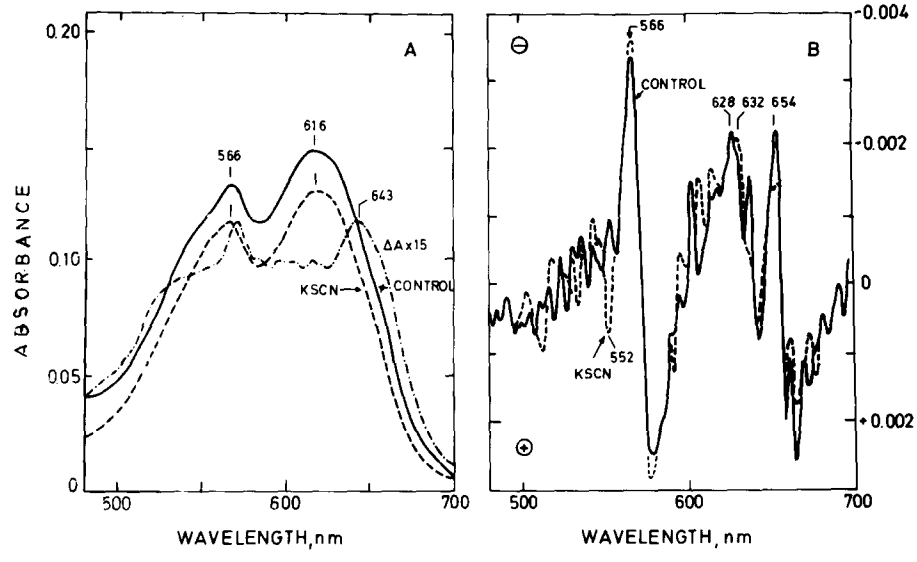


Fig. 8. Absorption (A) and inverted second-derivative absorption (B) spectra of glutaraldehyde-treated phycobilisomes after 2 h incubation in 0.75 M potassium phosphate (pH 6.9) (———), and in 0.375 M KSCN, 0.003 M potassium phosphate, pH 6.9 (— — —). Difference absorption spectrum (·–·–·–).

of such intermonomer cross-links is further supported by the urea-polyacrylamide gel electropherograms shown in Fig. 7. Control and dimethyl adipimidate-treated phycobilisomes contain only 18–24 kDa polypeptides, which should be the α- and β-polypeptides of *Nostoc* phycobiliproteins. In contrast, the electropherogram of the dimethyl suberimidate-treated preparation displays also molecular masses approximately twice as large as that of polypeptides α and β. These could originate, of course, from both intramonomer and intermonomer cross-links, but in view of the complete dispersion of dimethyl suberimidate-treated phycobilisomes into monomers upon treatment with KSCN, the second possibility should be ruled out. Larger molecular masses, which would evidence the presence of both intra- and intermonomer cross-links, were not detected.

It should be noted, here, that amidines, the products of the reaction of imido ester groups with $-NH_2$, carry a positive electric charge which makes possible the correlation of the molecular size of unmodified and modified proteins to their electrophoretic mobility. This is not the case with glutaraldehyde-reacted proteins, where electrophoretic mobility is influenced both by molecular mass and by the disappearance of bound electric charge [33]. Therefore, a comparison of the molecular masses of control and glutaraldehyde-treated phycobiliproteins by means of polyacrylamide gel electrophoresis is impossible.

Absorption and inverted second-derivative absorption spectra indicate that glutaraldehyde-treated phycobilisomes are, more or less, insensitive to KSCN (Fig. 8). Minor differences appearing after incubation with this chaotropic agent must be attributed to a small fraction of incompletely fixed phycobilisomes.

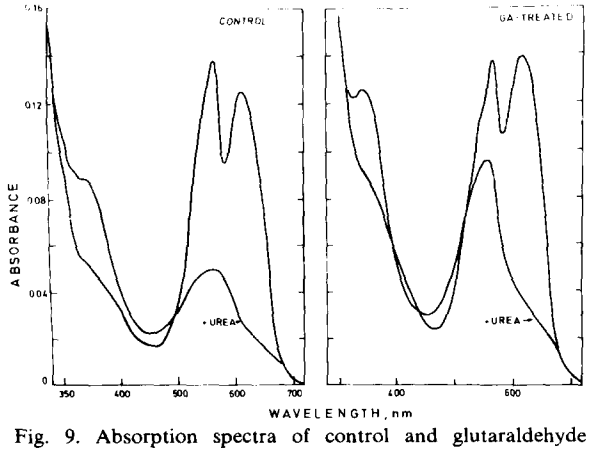


Fig. 9. Absorption spectra of control and glutaraldehyde (GA)-greated phycobilisomes, before and after 2 h incubation in the presence of 8 M urea.

Cross-linking with glutaraldehyde, however, does not prevent the unfolding of phycobiliproteins in concentrated urea solutions. As shown in Fig. 9, incubation of glutaraldehyde-treated phycobilisomes in 8 M urea results in the suppression of the visible absorption bands and the enhancement of the near-ultraviolet absorption. Such changes are ascribed to the unfolding of phycobiliprotein polypeptides [9] and the attendant transformation of prosthetic tetrapyrroles from the extended to the cyclic configuration [22].

Discussion

Although phycoerythrin-sensitized allophycocyanin fluorescence is a convenient criterion for detecting phycobilisome dissociation [5], multimer-to-monomer transitions and denaturation of phycobiliproteins are more aptly reported by their absorption properties. This reflects the fact that the electronic energy levels of the tetrapyrroles are influenced both by the local protein environment and by the exciton coupling between chromophores belonging to adjacent monomers [9–11,16, 43]. In the present work we have employed absorption and fluorescence spectrophotometry in order to identify such transitions, and through them to characterize the types of covalent cross-links which glutaraldehyde and dimethyl suberimidate introduce in the phycobilisome structure.

At approximately neutral pH, and in the presence of strongly chaotropic anions, phycocyanin and allophycocyanin exist as monomers [11,40], while in dilute phosphate they are predominantly trimers and hexamers [6,44]. The absorption, fluorescence and fluorescence excitation spectra of the monomeric forms of these two phycobiliproteins are closely similar, in contrast to the spectra of their multimeric forms, which differ with regard to the band shape and the position of the band maximum. Dissociation of phycoerythrin from a higher to a lower state of aggregation (most likely

to a monomer) is accompanied by a blue shift and suppression of the 573 nm absorption [12], as well as a decrease of the 575 nm and increase of the 555 nm circular dichroism bands [16]. We have shown here that the second-derivative absorptions of phycocyanin and allophycocyanin at 612, 630 and 652 nm merge into a single band at 622 nm, while the second-derivative absorption band of phycoerythrin at 566 nm is blue shifted when intact isolated phycobilisomes are incubated in NO_3^-- or SCN^--containing media (cf. Fig. 5). The absorption changes in the visible range were not attended by increases in the near-ultraviolet absorption of phycobiliproteins that would signify protein denaturation. We may, therefore, infer that the observed second-derivative absorption changes reflect the dispersion of phycobilisomes into monomeric phycobiliprotein subunits under the influence of the chaotropic electrolytes of the medium.

On the basis of these spectrophotometric criteria, it appears that glutaraldehyde-treated phycobilisomes resist dissociation even after prolonged incubation in KSCN-containing media. The increased phycobilisome stability after glutaraldehyde treatment is, in all likelihood, due to a network of inter- and intrahexamer covalent cross-links, which prevents the detachment of hexameric discs, as well as their dissociation into smaller subunits. Glutaraldehyde, however, affords no protection against secondary structure randomizers, such as concentrated urea solutions, which modify the absorption spectrum of glutaraldehyde-treated phycobilisomes in a manner signifying denaturation of phycobiliproteins (cf. Fig. 9).

In contrast to glutaraldehyde, the 11.5 nm long imidate dimethyl suberimidate appears to be able to introduce only intramonomer cross-links in the phycobilisome structure. Such phycobilisomes dissociate into monomeric subunits in the presence of KSCN, and on the basis of urea-polyacrylamide gel electrophoresis they appear to contain polypeptides with molecular masses corresponding to the sum of a- and β-polypeptides. Dimethyl suberimidate, nevertheless, confers moderate stability to isolated phycobilisomes, which dissociate more slowly in the presence of chaotropic electrolytes than untreated phycobilisomes.

A possible cause for this moderate stability is the increased hydrophobicity of phycobiliprotein subunits due to monofunctional alkylation of protein $-NH_2$ groups by half-hydrolysed diimido ester molecules. Such hydrolysis is quite possible in view of the fact that the cross-linking reaction was performed in medium which was not sufficiently alkaline. Moderate phycobilisome stabilization has been observed also after treatment with the monofunctional methyl acetimidate (Sofrova, D. and Papageorgiou, G.C., unpublished observations), and this can also be accounted for by the increased surface hydrophobicity of the alkylated phycobiliprotein subunits. It should be noted that increased hydrophobicity contributes also to the stabilization of glutaraldehyde-treated phycobilisomes, but its importance is overshadowed by covalent cross-linking, whose extensiveness should be attributed to the ability of glutaraldehyde and its polymers to modify several polypeptide side-chain groups [33].

The biphasic first-order rise of phycoerythrin fluorescence during phycobilisome dissociation (cf. Fig. 2) expresses probably the fact that monomers have a higher fluorescence yield compared to multimers. The biphasic kinetics are particularly evident in dimethyl suberimidate-treated phycobilisomes, where increased monomer hydrophobicity slows down the dissociation of phycoerythrin multimers. In contrast, as observed also by Zilinskas and Glick [32] earlier, untreated phycobilisomes dissociate in a single first-order process, when incubated in the presence of strongly chaotropic electrolytes, such as KSCN.

References

1 Gantt, E. (1980) Int. Rev. Cytol. 66, 45–80
2 Gantt, E. (1981) Annu. Rev. Plant Physiol. 32, 327–347
3 Glazer, A.N. (1982) Annu. Rev. Microbiol. 36, 173–198
4 Cohen-Bazire, G. and Bryant, D.A. (1982) in The Biology of Cyanobacteria (Cair, N.G. and Whitton, B.A., eds.), pp. 143–190, Blackwell, Oxford
5 Gantt, E. and Lipschultz, C.A. (1973) Biochim. Biophys. Acta 292, 858–861
6 Hattori, A., Crespi, H.L. and Katz, J.J. (1965) Biochemistry 4, 1225–1237
7 Neufeld, G.J., and Riggs, A.F. (1969) Biochim. Biophys. Acta 181, 234–243
8 Glazer, A.N., Fang, S. and Brown, D.M. (1973) J. Biol. Chem. 248, 5679–5685

9 Glazer, A.N. (1976) Photochem. Photobiol. Rev. 1, 71–115
10 MacColl, R., Csatorday, K., Berns, D.S. and Traeger, E. (1980) Biochemistry 19, 2817–2820
11 MacColl, R., Csatorday, K., Berns, D.S. and Traeger, E. (1981) Arch. Biochem. Biophys. 208, 42–48
12 Bennett, A. and Bogorad, L. (1971) Biochemistry 10, 3625–3634
13 Gantt, E., Lipschultz, C.A. and Zilinskas, B. (1976) Biochim. Biophys. Acta 430, 375–388
14 Erokhina, L.G., Shubin, L.M. and Krasnovsky, A.A. (1980) Fisiol. Rast. 27, 483–490
15 Papageorgiou, G.C. and Sofrova, D. (1983) in Photosynthetic Prokaryotes: Cell Differentiation and Function (Papageorgiou, G.C. and Packer, L., eds.), pp. 43–57, Elsevier, New York
16 Rigbi, M., Rosinski, J., Siegelman, H.W. and Sutherland, J.C. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1961–1965
17 Canaani, O.D. and Gantt, E. (1980) Biochemistry 19, 2950–2956
18 Teale, F.W.J. and Dale, R.E. (1970) Biochem. J. 116, 161–167
19 Dale, R.E. and Teale, F.W.J. (1970) Photochem. Photobiol. 12, 99–117
20 Kobayashi, T., Degenkolb, E.O., Bersohn, R., Rentzepis, P.M., MacColl, R. and Berns, Q.S. (1979) Biochemistry 5073–5078
21 Wong, D., Pellegrino, F., Alfano, R.R. and Zilinskas, B. (1981) Photochem. Photobiol. 33, 651–662
22 Scheer, H. and Kufer, W. (1977) Z. Naturforsch. 32C, 513–519
23 Gantt, E. and Conti, S.F. (1966) Brookhaven Symp. Biol. 19, 393–405
24 Cohen-Bazire, G. and Lefort-Tran, M. (1970) Arch. Microbiol. 71, 245–247
25 Papageorgiou, G.C. and Tzani, H. (1980) J. Appl. Biochem. 2, 230–240
26 Rosinski, J., Hainfeld, J.F., Rigbi, M. and Siegelman, H.W. (1981) Ann. Bot. 47, 1–12
27 Koller, K.P., Wehrmeyer, W. and Mörschel, E. (1978) Eur. J. Biochem. 91, 57–63
28 Papageorgiou, G.C. (1977) Biochim. Biophys. Acta 461, 379–391
29 Sofrova, D. and Papageorgiou, G.C. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), Vol. 3, pp. 167–174, Balaban, Philadelphia
30 Papageorgiou, G.C. and Isaakidou, J. (1981) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), pp. 727–736, Balaban, Philadelphia
31 Gantt, E., Lipschultz, C.A., Grabowski, J. and Zimmerman, B.K. (1979) Plant Physiol. 63, 615–620
32 Zilinskas, B.A. and Glick, R.E. (1981) Plant Physiol. 68, 447–452
33 Papageorgiou, G.C. (1979) in Photosynthesis in Relation to Model Systems (Barber, J., ed.), pp. 211–241, Elsevier, Amsterdam
34 Hesse, G. (1973) Acta Histochem. 46, 253–266
35 Browne, D.T. and Kent, S.B.H. (1975) Biochem. Biophys. Res. Commun. 67, 126–132
36 Chua, N.H. (1980) Methods Enzymol. 69, 434–446
37 Bennett, A. and Bogorad, L. (1973) J. Cell Biol. 58, 419–435
38 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.I. (1951) J. Biol. Chem. 193, 265–275
39 Böhlen, P., Stein, S., Dairman, W. and Udenfriend, S. (1973) Arch. Biochem. Biophys. 155, 213–220
40 MacColl, R., Berns, D.S. and Koven, N.L. (1971) Arch. Biochem. Biophys. 155, 213–220
41 Butler, W.L. (1979) Methods Enzymol. 56, 501–515
42 Gantt, E. and Lipschultz, C.A. (1974) Biochemistry 13, 2960
43 Canaani, O.D. and Gantt, E. (1980) Biochemistry 19, 2950–2956
44 Cohen-Bazire, G., Beguin, S., Rimon, S., Glazer, A.N. and Brown, D.M. (1977) Arch. Microbiol. 111, 225