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RESONANCE RAMAN SPECTRA OF PHYCOCYANIN, ALLOPHYCOCYANIN AND PHYCOBILISOMES FROM BLUE - GREEN ALGA *ANACYSTIS NIDULANS*

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Abstract: Resonance Raman spectra of native C-phycocyanin, allophycocyanin and whole, intact phycobilisomes from the blue-green alga *Anacystis nidulans* (Synechococcus 6301) are reported. A tentative assignment for the more prominent resonance Raman bands is suggested. The possibly sensitive regions for inter-chromophore interactions in the case of phycobilisomes are also discussed.

Introduction: Phycobilisomes are supramolecular pigment aggregates which gather and funnel the light to reaction centers in blue-green (cyanobacteria) and red algae (1). Phycobilisomes of *Anacystis nidulans* (Synechococcus 6301) contain only phycocyanin and allophycocyanin (2). Both proteins form aggregates within the phycobilisome. The aggregates are organized into rods (phycocyanin) or make up the core (allophycocyanin) of the phycobilisomes. The absorption and the emission spectra of the two pigments are modulated by linker proteins to achieve a very effective light harvesting system. Both phycocyanin and allophycocyanin contain the same chromophore, phycocyanobilin (2). The spectroscopic differences between the two pigments are accounted for alterations in the apoprotein-chromophore interaction.

Phycocyanobilin is an open tetrapyrrol molecule which has a linear conformation in native phycobiliproteins. It has an extended π-electron system responsible for the observed ab-

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complex. The inactivation may proceed through a multi-step mechanism, however, the minimum scheme presented in equation 1 was sufficient to describe the observed behavior of the kinetic data.

The two to three-fold difference between the K_I determined from the inactivation experiment and the K_{is} from the kinetic experiments most likely reflects the slightly different experimental conditions, e.g., glutamate and NH_3 are absent in the inactivation experiments.

Analysis of the composition of CrATP isomers by HPLC leads to the conclusion that glutamine synthetase is inactivated by the Λ isomer(s) of CrATP. In considering the enironment of the n_2 metal-nucleotide binding site of glutamine synthetase one must remember that the metal ion and nucleotide can bind independently.[12,13,14] It is not surprising therefore that when levels of Mg(II) or Mn(II) are added to ensure saturation of the n_2 site(see Table I), an alteration occurs in the inactivation rate produced by CrATP. Magnetic resonance data are consistent with the simultaneous binding of CrATP and Mn(II) at the n_2 site[14] and our current experiments suggest that the Cr(III) moiety of the Λ isomer of enzyme-bound CrATP is positioned near an enzyme residue with which it can interact. From previous work in our laboratory,[15] the B isomer of Ado-5'-(2-thioPPP) was established as a substrate for glutamine synthetase in the presence of Mg(II). Thus, the stereochemistry of the chelate ring of the Λ isomer of β,γ-$Cr(H_2O)_4$ATP and the B isomer of Mg(II)-Ado-5'-(2-thioPPP) are identical. This correlation may mean that an amino residue which is a ligand for the Mg(II) could displace a water molecule of CrATP leading to inactivation by the formation of a new stable Cr(III) complex. Identification of the peptide and amino acid residue(s) that may be altered by reaction with CrATP is currently underway in our laboratory.

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Raman spectroscopy: It was carried out on a Coderg PHO spectrometer equipped with an argon ion (Spectra Physics) laser. The excitation power was about 15 mW. The measurements were taken with the use of a home made flow cell. The total volume of the samples were 5 ml, which circulated around about 5 times during the recording of a spectrum. Samples were checked with absorption and fluorescence spectroscopy before and after the Raman measurments. No deterioration was found in any case except for a very slight change in the fluorescence spectrum of phycobilisomes. This may indicate a decreased efficiency of energy transfer in phycobilisomes caused by the laser irradiation. Nevertheless the spectra were reproducible. Instrumental setting was always 8 cm^{-1} resolution. Data were collected by an intelligent data aquisition system (6) in 1 cm^{-1} steps and stored in a computer (TPA 1140, KFKI, Hungary). The only data processing was a five point smoothing and for the phycocyanin spectrum a linear baseline substruction because of the strong fluorescence background. Band positions are accurate within ±5 cm^{-1}. All the spectra were recorded at room temperature.

Results and Discussion: Fig. 1. shows the resonance Raman spectra of the phycobiliproteins and the phycobilisome prepared from *Anacystis nidulans*. The resonance Raman spectrum originates in each case from the chromophore phycocyanobilin.(For detailed structure see ref. 2) The characteristic Raman frequencies are listed in Table I.

Detailed calculations and experiments were carried out on biliverdin-dimethyl-esther (7,8), which compound can be regarded as a model for phycocyanobilin in certain aspects. The most important difference is that the biliverdin-dimethyl-esther has a helical arrangement of the four tetrapyrrol rings in contrast to the linear structure of the phycocyanobilin in the native protein.

Based on the similarities found in either the measured or calculated resonance Raman spectra of biliverdin-dimethyl-esther and phycocyanobilins we tentatively assign the dominating band of the spectrum around 1635-1645 cm^{-1} to the ν(C=C) vibration of the methin bridge, the strong band at 1585 cm^{-1} to ν(C=C) in the pyrrol rings, the medium band around 1535 cm^{-1} to a pyrrol ring vibration involving the two C=C bonds in the ring. The strong band always present in the spectra around 1365-1380 cm^{-1} could be the ν_4 pyrrol ring mode (9).

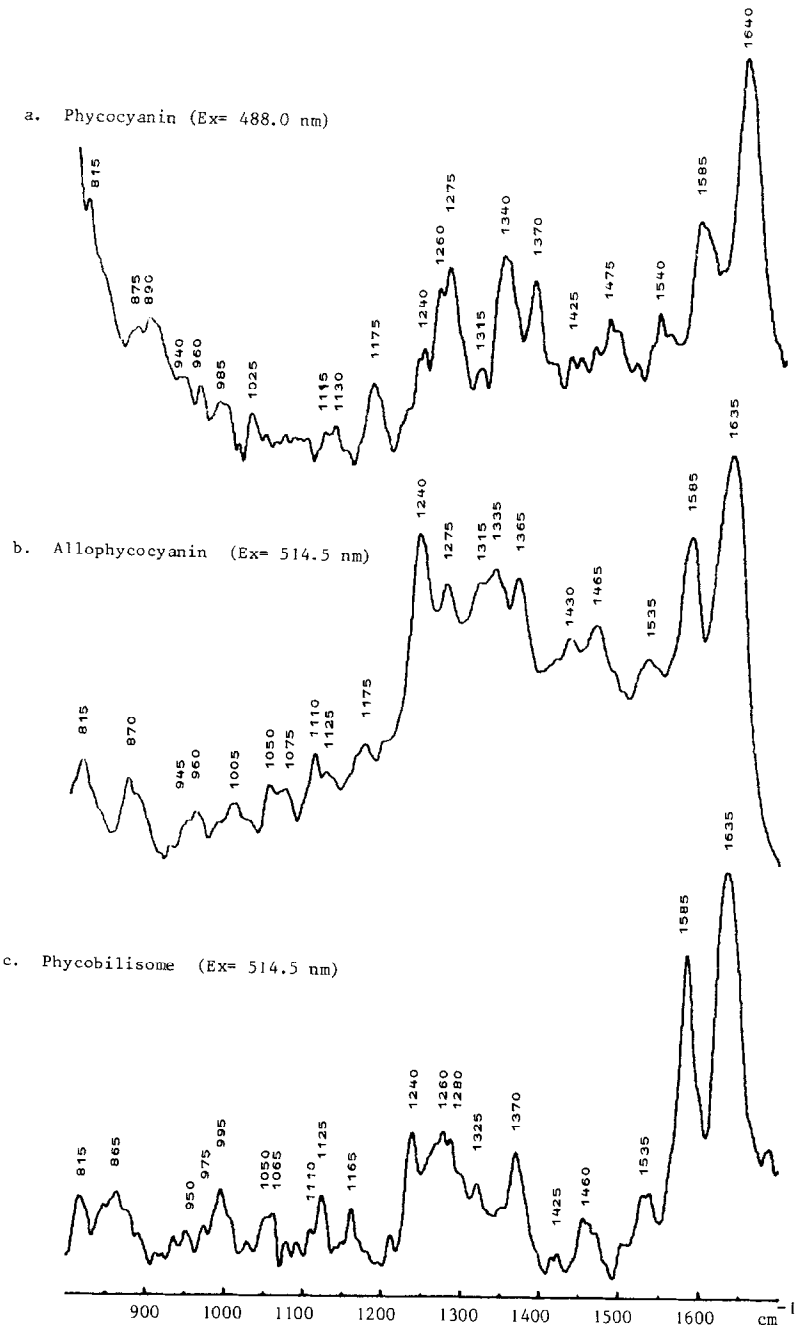

Fig. 1. Resonance Raman spectra of phycobiliproteins and phycobilisomes from the blue-green alga *Anacystis nidulans*.

There are features in the spectra which we can not assign at this moment but they might be important in studying intra- or

Table 1

Characteristic resonance Raman frequencies of phycobiliproteins and phycobilisomes from *Anacystis nidulans* (Synechococcus 6301)

Phycocyanin		Allophycocyanin		Phycobilisome		Assignment
815	w	815	m	815	m	
		870	m	865	m	
880	wsh					
900	w					
940	w	945	wsh	950	w	
960	w	960	w	975	w	
		1005	w	995	w	
		1050	m	1050	m	
		1070	m	1065	m	
		1110	m	1110	w	
1120	wsh	1125	w	1125	m	
1130	w					
1175	m	1175	w	1165	m	
1240	m	1240	vs	1240	s	δ(C-H)pyrrol
1260	s			1260	msh	
1275	s	1275	s	1280	s	
1315	w	1315	msh	1310	msh	
1340	s	1335	s			
1380	s	1365	s	1370	s	ν_4(pyrrol)
1430	ew	1430	w	1425	w	
1480	m	1465	m	1460	m	ν(C-C)pyrrol
1540	m	1535	m	1535	m	ν(C=C)pyrrol
1590	s	1585	vs	1585	vs	ν(C=C)pyrrol
1645	vs	1635	vs	1635	vs	methin C=C

abbreviations: e-extremely; m-medium; s-strong; sh-shoulder; v-very; w-weak

intermolecular interactions:

(i) The band at 1240 cm^{-1} is relatively weak in phycocyanin (see also ref. 3) and rather strong in both allophycocyanin and phycobilisome. It coincides with a pyrrol δ(C-H) vibration but its intensity is higher than it could be expected considering pyrrol alone.

(ii) Both phycocyanin and allophycocyanin spectra contain a relatively strong band around 1335 cm^{-1} which is totally missing from the phycobilisome spectrum.

(iii) The resonance Raman spectrum of phycobilisomes has the highest I_{1585}/I_{1635} ratio in the three spectra.

Presuming that phycobilisome resonance Raman spectrum should be the summation of the phycocyanin and allophycocyanin resonance Raman spectra weighted with the stochiometry of the two compounds in the phycobilisome plus alterations caused by molecular interactions in the supramolecular organisation of the phycobilisome. Therefore the regions mentioned above can be interesting in this respect.

These results may facilitate the finer analysis of the phycobilisome organisation to achieve a better unerstanding its way of action. Further studies in this direction are under way in our laboratory.

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