RESONANCE RAMAN SCATTERING OF PHYCOBILISOMES AND PHYCOBILIPROTEINS FROM CYANOBACTERIUM *Westiellopsis prolifica**

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Phycobilisome is a kind of supramolecular light-harvesting pigment protein complex, which can facilitate the absorption and transfer of light energy to the photosynthetic reaction centers in cyanobacteria and red algae. The phycobilisomes (hereafter called PBS) of cyanobacterium *Westiellopsis prolifica* consist of three kinds of phycobiliproteins, i. e. phycoerythrocyanin (called PEC), C-phycocynin (called C-pc) and allophycocyanin (called APC) with absorption maximums at 570 nm, 615 nm and 650 nm, respectively[1]. Except that the PEC contains a biliviolinoid chromophore with undetermined structure (called PXB), all phycobiliproteins contain the same chromophore phcocyanobilin (called PCB)[1]. The structure of PCB is given in Fig. 1. The PCB and PXB are open-chain tetrapyrrol molecules which have linear extended conformation. They have extended π-electron system responsible for the absorption spectra as well.

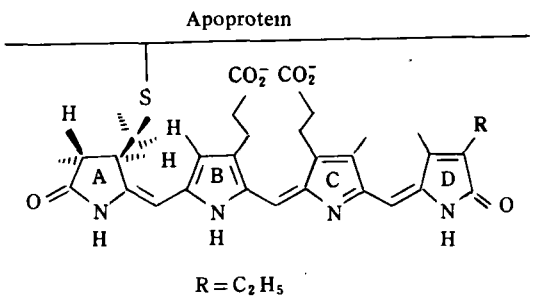

Fig. 1. Structural formulas of PCB. PXB has a similar structure[1].

Although there exist the difficulties caused by the light sensitivity and strong fluorescence of these compounds in studying the actual conformation of protein-controlled chromophores in PBS and phycobiliproteins by using resonance Raman spectroscopy, the resonance Raman spectroscopy has already been proved to be a powerful tool in studying the pigment structure in photosynthetic system[2].

This note reports the systematic study of the resonance Raman scattering of the chromophores PXB and PCB in PBS and phycobiliproteins, and attempt is made to analyse the spectral difference in phycobiliproteins by studying the PXB and PCB conformation,

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which may help further understand the energy transfer in PBS.

I. Materials and Methods

Cyanobacterial cultures for *Westiellopsis prolifica* were obtained from Indian Agricultural Research Institute, New Delhi, India, maintained in liquid media under the prescribed condition of light and temperature, and grown in culture medium BG-11[3] at 25±2°C, with constant illumination (2 W/m^2) for 21 days. The cultures were agitated using an air pump. For PBS isolation, the cells were washed twice in 1.0 mol/L K_2HPO_4/KH_2PO_4 (pH = 7.0) containing 0.001 mol/L NaN_3 and 0.1 mol/L NaCl (Hereafter all buffers contain the same concentrations of NaN_3 and NaCl), then incubated in 2% (*w/v*) Triton X-100 for 4 h under continuous stirring. The suspension was purified by centrifugation for 1 h at $40,000 \times 10^{-4}$ T (Backman 70 Ti rotor). The blue supernatant was placed in sucrose with continuous gradients 0.1 — 1.0 mol/L in 1.0 mol/L K_2HPO_4/KH_2PO_4(pH = 7.0), after ultracentrifuge for 2.5 h at $80,000 \times 10^{-4}$ T, the PBS bands were dialysed in 1 mol/L K_2HPO_4/KH_2PO_4 (pH = 7.0) buffer, concentrated by polyglycol 6000, and then centrifuge at $40,000 \times 10^{-4}$ T for 30 min again. The intact PBS was checked by absorption spectrum with $A_{622}/A_{280} = 3.5$. The dissociation PBS was got by dialysing intact PBS in 0.005 mol/L K_2HPO_4/KH_2PO_4 (pH = 7.0) buffer for 12 h, then centrifuged at $40,000 \times 10^{-4}$ T for 30 min. The dissociation PBS was checked by absorption spectrum with $A_{575}/A_{280} = 2.1$, $A_{618}/A_{280} = 3.4$ and $A_{650}/A_{280} = 3.1$. PEC, C-pc and APC were purified from dissociation PBS by column chromatography. Dissociation PBS was layered on DEAE-cellulose columns (Carl-Schloicher &. Schuell Co., U. S. A. φ1.5 cm, 1 15 cm) equilibrated with 5 mmol/L K_2PHO_4/KH_2PO_4 (pH = 7.0) buffer. Fractions PEC, C-pc and APC were eluted with 5 mmol/L, 100 mmol/L and 200 mmol/L K_2HPO_4/kH_2PO_4 (pH = 7.0) buffer, respectively. The proteins were concentrated by polyglyool 6000, centrifuged at $40,000 \times 10^{-4}$ T for 1 h, and fractionated again by the same column chromatography. Two repetitions were needed to obtain the purified PEC, C-pc and APC after the centrifugation at $40,000 \times 10^{-4}$ T for 30 min., and checked by absorption spectra with PEC : $A_{570}/A_{280} = 3.6$; C-PC : $A_{615}/A_{280} = 3.8$ and APC : $A_{650}/A_{280} = 3.7$, $A_{620}/A_{650} = 0.58$.

The resonance Raman spectra were obtained on a Jobin Yvon (JY-T800) spectrophotometer (Ramanor HG2S), using the 488 nm excitation wavelength from an argon ion laser (spectra physics 171). The slit width was always 360 μm with a resolution of 0.25 cm^{-1} (at 6328 Å). The detective system was RCA31034 photomultiplier and Model 1104 photon counter, while the time constant was always 1 s. The 488 nm excitation line lay at the high-energy-side of absorption band for PXB and PCB. In order to overcome the influence of the light sensitivity and strong fluorescence, the excitation power was about 13 mW for PEC, C-pc and APC, and 17 mW for PBS. The measurements were performed with a two-side-opened erect quartz curvette (φ1.5 mm, 1 6 cm) in which the sample flowed down slowly by the gravity of itself. The sample concentration was about 1.0×10^{-6} mol/L. The scan speed was always 50 cm^{-1}/min, and no accumulation had been done. The measurement accuracy of Raman shifts was about ±1.5 cm^{-1}. The discrimination of

Raman peaks under the 488 nm excitation was done by comparing them with the Raman peaks obtained under the 514.5 nm excitation and selecting the peaks with the same Raman shifts in both spectra. All spectra were recorded at room temperature.

II. Results

The biliverdin, a bile pigment, has been studied as a model compound for the biliverdin-type chromophores in phycobiliproteins and phytochrome which are found in cyanobacteria and higher plants[2]. The biliverdin dimethyl ester (called BVDE) (The molecular structure is shown in Fig. 2) is used as a model compound for PXB and PCB. The BVDE is of the cyclic open-chain conformation of tetrapyrrol ring in organic solvents and a free bile pigment, and the PXB and PCB are linear extending conformation linked with the apoprotein by thioether linkages in ring A[1], where the conformation of PXB and PCB is constrained by the surrounding protein matrix. The resonance Raman investigations of BVDE in detail have been reported in both calculations and experiments by L. Margulies et al.[4,5]

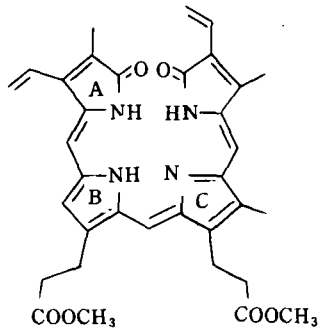

Fig. 2. Structural formula of BVDE.

Figs. 3 and 4 show the resonance Raman spectra of PBS and phycobiliproteins (PEC, C-pc and APC). The Raman peaks for the PBS and PEC arose from the PXB and PCB, and those for the C-pc and

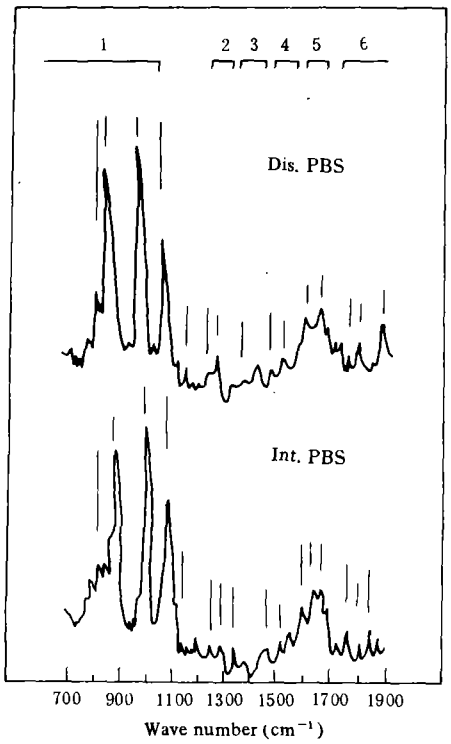

Fig. 3. Resonance Raman spectra of phycobilisomes.

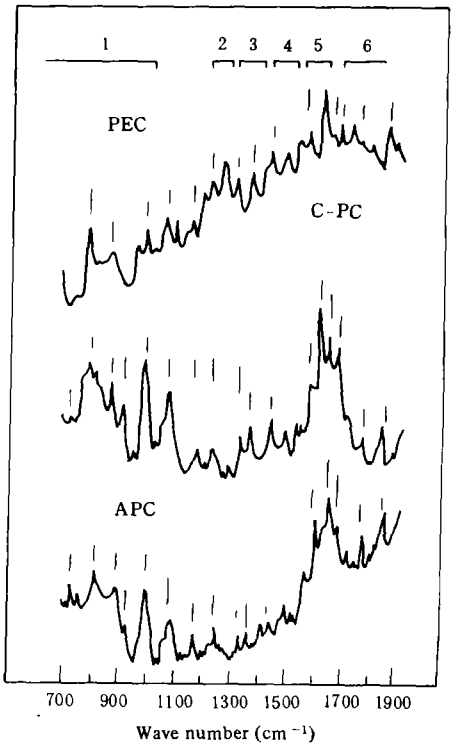

Fig. 4. Resonance Raman spectra of phycobiliproteins.

APC were from the PCB. Comparing both the measured and calculated resonance Raman spectra of BVDE, we find that besides the enhancement in the 1590—1650 cm^{-1} region for BVDE, PXB and PCB, there is an enhancement region in 700—1100 cm^{-1} for PXB and PCB, but not for BVDE. Table 1 lists the frequencies and assignments of modes by comparing them with the normal modes analysis.

Table 1

Raman Frequencies (cm^{-1})*

Int. PBS	Dis. PBS	PEC	C-pc	APC	Assignment
1865 w	1870 m	1860 m			
1775 w	1780 w		1770 w	1770s	
1675 m			1675 s	1675 ssh	lactam C=O
1645 m	1650 m	1650 wsh	1640 s	1650 es	C=C
1615 msh	1615 msh	1620 vs			
1590 m	1595 m	1595 m	1610 es	1600 s	C=O
			1585 msh	1585 m	pyr. C=C
		1530 m	1530 w	1530 w	pyr. C=C
		1495 m	1495 mw	1490 w	pyr. C—C
1460 vw	1460 vw				
		1445m	1445 m	1440 vw	
		1370 w	1370 m	1360 vw	ν_4[6]
		1240 m	1240 w	1245 w	C—H rocking
1175 w		1175 m	1175 m	1175 m	skeleton (995cm^{-1}
1080 es	1080 es	1080 s	1080 m	1075 m	probably due to
995 es	995 es	995 m	995 s	995 m	N—H stretching in
880 es	880 es	875 w	875 m	880 m	ring A or D)[7]

* 1. Based upon [5], except the noted one; 2. abbreviations: s, strong; sh, shoulder; m, medium; w, weak; v, very; e, extremely; Int., intact; Dis., dissociation.

For PCB and PXB, the band 1675 cm^{-1} corresponds to C=O stretching in the lactam groups of rings A and D; C=C stretching in methin bridge A—B appears at 1640 cm^{-1} (C-pc), 1650 cm^{-1} (APC), 1650 cm^{-1} (PEC), 1645 cm^{-1} (intact PBS) and 1650 cm^{-1} (dissociation PBS), and in bridge C—D at 1610 cm^{-1} (C-pc), 1600 cm^{-1} (APC), 1595 cm^{-1} (PEC), 1590 cm^{-1} (intact PBS) and 1595 cm^{-1} (dissociation PBS), respectively. Band 1585 cm^{-1} is from C=C stretching in rings D and C which contain one C=C bond, and band 1530 cm^{-1} from C=C stretching in ring B which contains two C=C bonds; bands 1490 cm^{-1} (APC), and, 1495 cm^{-1} (C-pc, PEC) correspond to C—C stretching in ring B; Bands 1370 cm^{-1} (C-pc) and 1360 cm^{-1} (APC) are pyrrol mode ν_4[6], which are mainly due to the stretching of the C—N and C—C bonds of ring C in the ring plane[7]. However, Margulies and Toporowicz[5] insisted by calculation that this mode should occur at 1408 cm^{-1} in BVDE. This mode is very sensitive to the geometry of pyrrol ring. In our experiment, we have found that it is polarization. The weak bands 1240 cm^{-1} (C-pc) and 1245 cm^{-1} (APC) are attributed to the C—H rocking in methin bridges B—C and C—D. They are sensitive to the environment such as solutions[2].

III. Discussion

The conformations of PCB and PXB in phycobiliproteins depend on the conformations of apoproteins. As a result of apoprotein-chromophores interaction, in the region of 1590—1650 cm^{-1} corresponding to the C=C stretching in methin bridges A—B and C—D of PXB and PCB, the variation of the relative intensity between the C=C stretching in bridge A—B and that in bridge C—D may reflect the linear extending degree of the tetrapyrrol chromophore in various phycobiliproteins (i.e. cyclic, semiextended or linear conformation). The conformation is more extended, the π-electron system of conjugation tetrapyrrol ring is more delocalized. Therefore, the overlap of π-electron system between PCBs or PXBs is larger and the interaction between them is stronger. The fact that the value of I_{1610}/I_{1640} in C-pc is larger than that of I_{1600}/I_{1650} in APC indicates that the linear extending degree of the tetrapyrrol chromophore PCB conformation in APC is larger than that in C-pc. That is to say, a more "extended" conformation for the tetrapyrrol ring in APC than that in C-pc results in the stronger delocalization of π-electron system of PCB in APC. Therefore, the interaction among the PCB chromophores in APC is much stronger than that in C-pc. This is the reason why there is a characteristic absorption peak at 650 nm and a red shift of the fluorescence for APC, which arises from chromophore-chromophore interaction. With the comparison of the C-pc and APC, the value of I_{1595}/I_{1650} for PEC indicates that the linear extending degree of the conformations of PCB and PXB in PEC is less than that in C-pc and APC, which causes a blue shift of absorption.

In the range of 700—1100 cm^{-1}, three main resonance Raman peaks at about 880, 995 and 1080 cm^{-1} are probably due to the "breath" vibrations of the conjugation tetrapyrrol macrocyclic ring (skeleton) with periodic stretching and contracting, which are caused by strong interaction of conjugation π-electron system of PCB and PXB chromophores in PBS and phycobiliproteins (PEC, C-pc and APC)[8].

References

[1] Glazer, A. N., *Biochim. Biophys. Acta*, **768**(1984), 29.

[2] David L. Farrens, Randall E. Holt, Bernard N. Rospendowski, Pill-soon Song & Therese M. Cotton, *J. Am. Chem. Soc.*, **111**(1989), 9162.

[3] Stanier, R. Y., Kunisawa R., Mandel, M. & Cohen-Baire, G., *Bacteriol. Rev.*, **35**(1971), 171.

[4] Margulies, L. & Stockburger, M., *J. Am. Chem. Soc.*, **101**(1979), 743.

[5] Margulies, L. & Toporwicz, N., *J. Am. Chem. Soc.*, **106**(1984), 7331.

[6] Szalontai, B., Gombos, Z. & Csizmadia, V., *Biochim. Biophys. Res. Comm.*, **130** (1985), 358.

[7] Hsieh, Y. -Z. & Morris, M. D., *J. Am. Chem. Soc.*, **110**(1988), 62.

[8] Xia Andong, Zhang Xinyi, Hong Qiang, Meng Jiwu, Hou Shanggong & Sudha Mahajan, *Proc. Fourth Asia Pacific Phys. Conf.*, Vol. 2 (eds. Ahn, S. H. et al.), Seoul, Korea, World Scientific, Singapore, 1990, pp. 1433—1436.