Photosynthetic Light-Harvesting Systems

Organization and Function

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COHERENT ANTI-STOKES RAMAN SPECTROSCOPY OF PHYCOBILISOMES, PHYCOCYANIN AND ALLOPHYCOCYANIN FROM MASTIGOCLADUS LAMINOSUS

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Introduction

In order to provide a large geometrical and spectral cross-section for the absorption of light, red and blue-green algae produce the so-called phycobilisomes (PBS), which contain the chromoproteins phycoerythrin (or phycoerythrocyanin), phycocyanin (PC) and allophycocyanin (APC). Although PC and APC contain the same chromophore, phycocyanobilin, the spectral properties of these biliproteins (tetrapyrrol chromophore bound to apoprotein) are quite different. Their excitation energies are modified according to their special function in the light harvesting system (see e.g. 1,2) by chromophore-protein interactions (14).
Resonance-enhanced Coherent Anti-Stokes Raman Spectroscopy (CARS) has proved to be a very suitable technique to produce vibrational spectra of highly fluorescing chromophores as e.g. light harvesting pigments (3,4,5). In this contribution CARS-spectra of room-temperature solutions of phycobilisomes and of phycocyanin and allophycocyanin trimers from Mastigocladus laminosus are presented and the implications to chromophore structure are discussed.

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Experimental

Resonance-enhanced CARS spectra are obtained by focussing two laser beams with different wavelengths λ_{pump} (fixed) and λ_{stokes} (variable) in the sample, where by a non-linear effect the CARS beam is generated, which holds the equality
$\tilde{\nu}_{CARS} = \tilde{\nu}_{pump} + (\tilde{\nu}_{pump} - \tilde{\nu}_{stokes})$
$\tilde{\nu}_{CARS} > \tilde{\nu}_{pump} > \tilde{\nu}_{stokes}$, with $\tilde{\nu} = 1/\lambda$ [cm^{-1}]
A plot of the CARS intensity as function of $\tilde{\nu}_{pump} - \tilde{\nu}_{stokes}$ reveals a vibrational spectrum of the chromophore. As the CARS frequency is higher than either pump and Stokes frequency, no flourescence problems as in spontaneous Raman spectroscopy can arise. (For more experimental details see ref. 3).

Preparation

PBS were isolated according to the method published by Nies and Wehrmeyer (6), except that the pH of the phosphate buffer was 6 and the purification on the sucrose gradient was done twice. APC was prepared very similar to a procedure described by Füglistaller et. al. (7).
The aggregation state of PC and APC was determined by sedimentation runs according to Martin and Ames (8). Both biliproteins were found to be trimeric. Linker peptides were not present as proved by absorption spectra, sedimentation coefficients and SDS-gel electrophoresis.

Results

As both the fingerprint (1100 - 1300 cm^{-1}) and the double bond stretching region (1500 - 1750 cm^{-1}) have proved to be most suitable for the investigation of chromophore geometry by CARS (3,4,5,9) or Resonance Raman Spectroscopy (10,11), both regions are considered in the following.

The phycobilisome spectra (PBS) are dominated by the contribution of the constituent phycocyanin trimers (PC) and are therefore rather similar to the spectra of the latter. Most of the vibrational frequencies are reproduced within experimental accuracy, though with different intensities (especially between 1200 and 1300 cm^{-1})(fig 1). At 1623 cm^{-1} an additional weak peak is found in PBS, which is due to allophycocyanin (APC). A second interesting feature is that for the pronounced bands a smaller width is found in PBS than in PC. It possibly indicates a lower structural inhomogeneity in PBS due to higher aggregation and the presence of linker peptides.

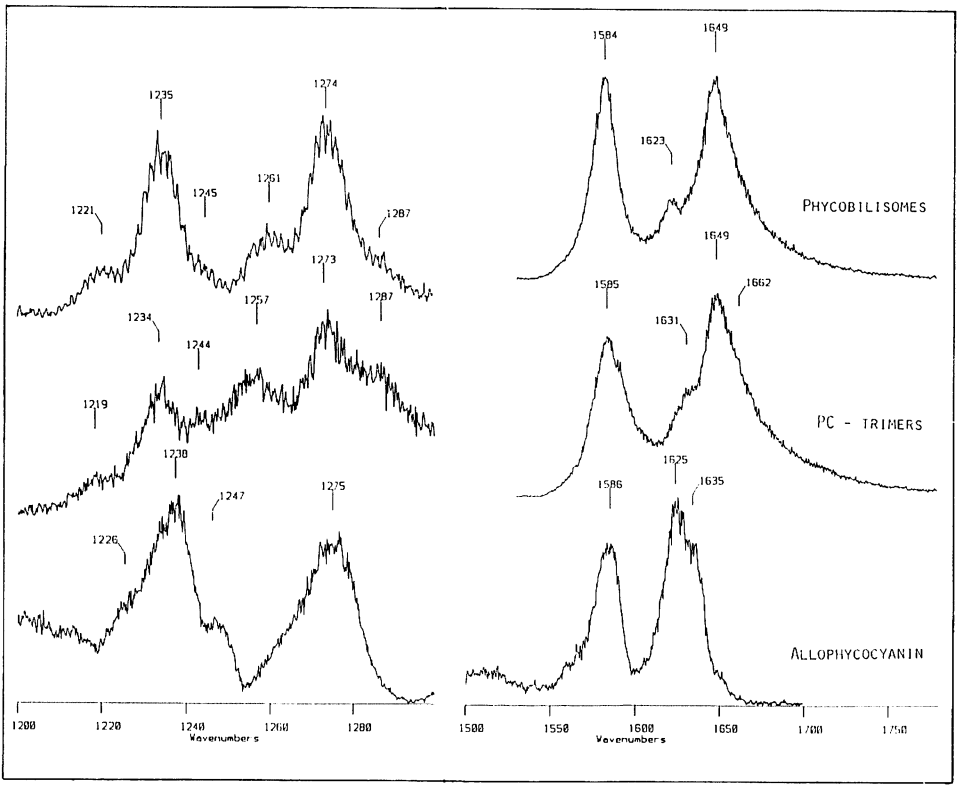

Fig. 1: Resonance-enhanced CARS spectra of phycobilisomes, phycocyanin trimers and allophycocyanin trimers from Mastigocladus laminosus. Pump wavelength 640 nm.

APC spectra are very different from PC spectra (see also ref. 10). The 1257 cm^{-1} and 1287 cm^{-1} bands observed in PC are absent in APC. In the double bond stretching region the dominant peak in PC is found at 1649 cm^{-1} with shoulders at ≈1631 and ≈1662 cm^{-1}. In APC spectra, however, the dominant peak is found at 1625 cm^{-1} with a strong shoulder at ≈1635 cm^{-1} and a weak one at ≈1650 cm^{-1}.

Discussion

According to preliminary normal mode calculations using the coordinates provided by X-ray analysis (12,13), the bands found between 1200 and 1300 cm^{-1} represent extensively mixed C-C stretch / C-H in plane bending modes and can presently not be interpreted in a straight-forward manner. The band around 1585 cm^{-1} is related to C=C stretching vibrations in the ring B/C fragment. That this band exhibits nearly no change in frequency, most likely indicates a conservation of the geometry of this part of the chromophore.
The C=C double bonds of the methine bridges between ring A-B and ring C-D, on the other hand, should give rise to localized modes with frequencies between 1650 and 1700 cm^{-1}. The C=O stretch mixes with the C=C stretch in ring D to produce vibrational frequencies around 1630 cm^{-1}. The actual value of the calculated frequencies is found to be sensitive to structural changes.
From these theoretical results and the comparison of CARS spectra of model compounds (9) with the presented CARS spectra a significant difference in chromophore geometry and/or Π-electron distribution between PC and APC must be postulated. Interpretation of e.g. fluorescence decay data of APC should therefore not be based on structural information gained from PC.

Additional measurements on antenna pigments and a more elaborate normal mode analysis are in preparation.

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