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EFFECT OF AGGREGATION ON CHROMOPHORE STRUCTURE IN ALLOPHYCOCYANIN STUDIED BY RESONANCE CARS-SPECTROSCOPY

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Photosynthetic organisms absorb light and convert the photonic energy into chemical energy by the process of photosynthesis. Usually, the photosystems are composed of an intra-membrane reaction center and an associated antenna. In cyanobacteria and red algae, the antenna proteins contain an open-chain tetrapyrrole bilinchromophore covalently bound to the apoprotein. By chromophore - protein interaction, the spectral properties of the biliproteins are tuned for their biological function. Recently the structure of the biliproteins phycocyanin (PC) and allophycocyanin (APC) and especially the geometry of the chromophores has been determined by high-resolution X-ray spectroscopy. The knowledge of the chromophore geometry and the arrangement of the chromophores within the natural building blocks, the so-called trimers, proved to be essential for the understanding of the energy transfer processes within the antenna complexes (phycobilisomes). Until today no information is, however, available for chromophore geometry and arrangement in APC.

In contrast to the above mentioned biliproteins, the UV-VIS absorption spectrum of APC changes dramatically upon aggregation from the monomeric to the trimeric aggregation state. Whereas one deals with well isolated chromophores in the monomeric unit one could have excitonic coupling between pairs of chromophores in the trimer, a fact which could explain the completely different spectroscopic behaviour of the trimeric unit compared to the monomeric unit. Alternatively, the geometry of the chromophore(s) could change upon aggregation. In an attempt to clarify this important open question, we have recorded resonance-enhanced CARS-spectra of monomers and trimers of APC from the cyanobacterium *Mastigocladus laminosus*. In Fig. 1 the CARS-spectra of monomers and trimers of APC recorded with a pump-wavelength $\lambda = 640$ nm are shown for direct comparison. Each solid line represents the best fit, applying the usual expression for $|\chi^3|^2$ [1]. Although it is difficult to compare resonance-CARS-spectra just by eye inspection due to the fact that line shapes can be rather complex, it is obvious that the prominent bands, which show up in the spectra of APC-trimer and APC-monomer respectively, are very different. Such a statement is not true, if one compares the CARS-spectra of PC monomer and PC trimer [2].

If the UV/VIS spectra change due to excitonic coupling, one would not expect the vibrational frequencies to change since they describe the electronic distribution in the ground state. A change of the electronically excited state, to which the

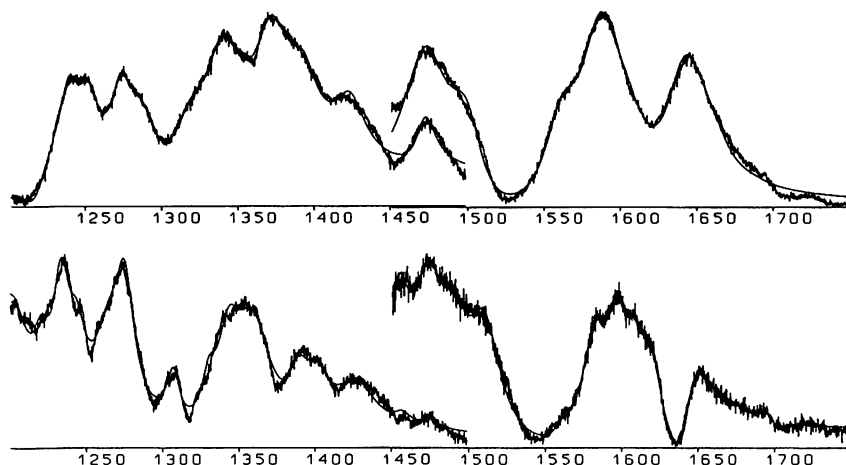


Fig. 1 CARS-spectra ($\Delta\nu$ in cm^{-1}) of APC monomer (upper) and trimer (lower) recorded at a pump wavelength of $\lambda = 640$ nm with different phasematch angles (right and left).

pump radiation is in resonance, would (in accordance with the experiments of resonance-enhanced spontaneous Raman spectroscopy) change the intensities of the various normal modes (since the Franck-Condon factors could be different). Since the analysis proves that the differences between the monomer and trimer spectra are not produced by a variation of the relative intensities of bands with constant frequencies [2], it must be concluded that the chromophore, which gives rise to the spectrum, does change its geometry upon aggregation. One should mention at this point that the appearance of CARS-spectra of APC-trimers (from *Mastigocladus laminosus*) covering the double bond region [3] proved to be very sensitive with respect to the chosen pump wavelength. An analysis shows, however, that upon changing the pump wavelength only the relative intensities of the bands change, but not the frequencies. This can be understood, if the two different type of chromophores, which are present, experience a different resonance-enhancement. It can furthermore be stated that PC and APC originating from different organisms exhibit similar CARS-spectra, although the exact frequency values differ slightly. Since the chemical structure of the chromophores is the same, the frequency differences image the variation in protein - chromophore interaction [2].

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