RAMAN SPECTROSCOPIC EVIDENCE FOR PHYCOCYANIN–CAROTENOID INTERACTION IN *ANACYSTIS NIDULANS*

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1. Introduction

In all algae and higher plants carotenoids accompany the chlorophylls as pigments of the photosynthetic apparatus. Their function is probably multifold. One is energy transfer (for example in brown algae and diatoms [1]). A generally accepted second function is to provide photoprotection of chlorophylls against oxidation. In many cases, however, their function is not clear because on the one hand they do not take part in the excitation energy transfer [2] but on the other hand they were found necessary to the structure of the photosynthetic apparatus [3,4].

The migration of the energy within the photosynthetic unit is usually monitored by measuring chlorophyll fluorescence or absorption changes of certain components of the system. The fluorescence yield of carotenoids is extremely low and the absorption changes measured around 515 nm are attributed to electrochromic shifts and thus reflect changes in the membrane potential rather than carotenoid absorption. Therefore it is difficult to identify any particular function of carotenoids in intact systems unless it can be measured via other components as in the case of energy transfer and photoprotection.

Carotenoids are known to exhibit a very strong resonance enhanced Raman spectrum [5] which was found to be sensitive to the conformation [6] and the environment [7,8] of the molecule. It was determined by resonance Raman spectroscopy that in *Anacystis nidulans* the photosensitivity of carotenoids was related to the decomposition of phycocyanin fluorescence during nitrate starvation [9].

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Phycocyanin is an auxiliary pigment of the blue–green algae which is believed to be organised in phycobilisomes attached to the surface of the thylakoid membrane. (Carotenoids are located in the thylakoid membrane.) It transfers the absorbed energy via allophycocyanin to the photosystem II reaction center [10]. It was indicated earlier that in vivo direct excitation energy transfer between phycocyanin and chlorophyll may exist [11]. This should involve a close contact between phycocyanin and the thylakoid membrane.

Here we present a more accurate and direct information about phycocyanin–carotenoid interaction than the parallel measurement of the decomposition of Raman and fluorescence spectra in [9]. Measuring the effect of the additional 625 nm light absorbed by phycocyanin on the resonance Raman spectrum of carotenoids we found evidence of a specific phycocyanin–carotenoid interaction in *Anacystis nidulans*. This interaction probably involves the π-electron system both of the phycocyanin and the carotenoid.

2. Materials and methods

Anacystis nidulans was cultured in medium C [12] in glass vessels at 27°C in a New Brunswick incubator shaker. Vigorously growing log-phase cultures were used for Raman experiments without any further treatment. The sample was put into a 1 cm glass cuvette. The unfocused laser beam irradiated the sample with the following intensities: 530.8 nm $\approx$ 5 mW, 625 nm $\approx$ 30 mW and 670 nm $\approx$ 40 mW. Both the 530.8 nm and the 568.1 nm lines of a Coherent Radiation CR-3000 K krypton ion laser were used for excitation. The set up used in the experiments can be seen in fig.1. At the output of the ion laser a beam-

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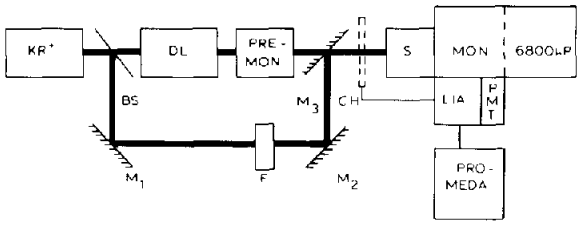

Fig.1. The experimental set-up; Kr^+, ion laser; DL, dye laser; PREMON, premonochromator; F, filter; BS, beam splitter; M_1, M_2, mirrors; M_3, mirror with a central hole; S, sample compartment; MON, monochromator; 6800 μP, microprocessor; CH, chopper blade; LIA, lock-in amplifier; PMT, photomultiplier; PROMEDA, multichannel analyzer; for details see the text.

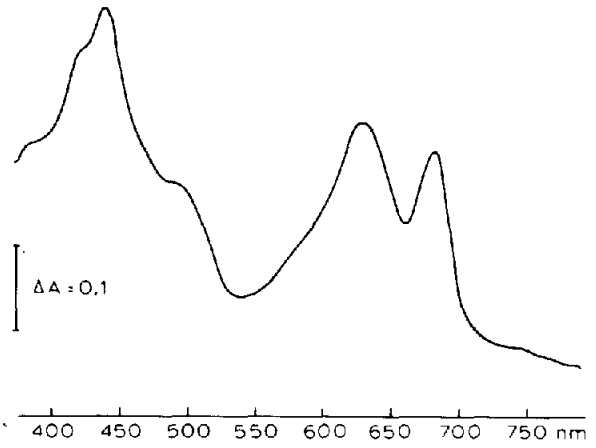

Fig.2. Absorption spectrum of *Anacystis nidulans*.

splitter deflected a small fraction of the light, the rest of it excited the Rhodamine 101 dye in a Coherent Radiation 599 jet-stream dye laser. The dye laser could be tuned in the 615–685 nm region with a 3 element birefringent filter. The side bands of the birefringent filter were eliminated with an Anaspec 300 S premonochromator. The plasma lines and the 568.1 nm line were eliminated from the deflected light with an interference filter centered at 528 nm. The output of the dye laser and the deflected light were incident on the same spot of the sample. Raman spectra were detected on a microprocessor-controlled Jobin Yvon Ramanor HG2S double monochromator equipped with an RCA 31034 A02 cooled photomultiplier. Every spectrum was the average of 9 scans. The spectra were digitized and stored in an Elscint Promeda multichannel analyzer. The only computer processing of the spectra was a linear base line subtraction in the case of the (530.8 + 625) nm illumination.

3. Results and discussion

In [9] the carotenoid photosensitivity correlated with the change of the fluorescence of phycocyanin. This indicates that the electronic excitation/relaxation of the phycocyanin may play a role in the interaction with the carotenoids. Therefore we recorded the resonance Raman spectrum of carotenoids in intact *Anacystis nidulans* cells with and without an additional illumination at 625 nm (the maximum of phycocyanin absorption (fig.2)). As the carotenoid absorption is not measurable at 625 nm, this light can not have direct effect on the carotenoid resonance Raman spectrum. Any change observed in the presence of the red light can only be the result of molecular interactions.

The Raman spectrum of *Anacystis nidulans* cells excited in the green is dominated by the resonance-enhanced peaks of carotenoids [9]. One of them, ν_1 (C=C stretching) around 1525 cm^{-1}, is sensitive in its position to π-electron delocalisation [5].

We recorded the 1500–1550 cm^{-1} region of the Raman spectrum excited at 530.8 nm (fig.3c). Upon

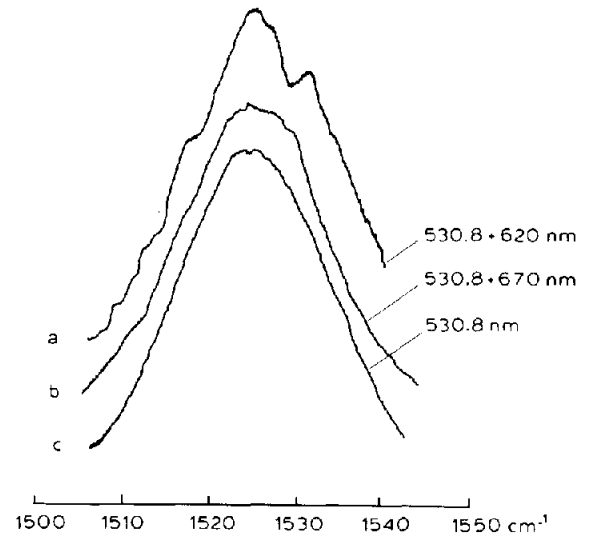

Fig.3. Resonance Raman band of carotenoid ν_1 (C=C stretching) vibration in intact *Anacystis nidulans*. For details see the text.

the addition of 625 nm light the Raman band was split and a new band (sometimes only a strong shoulder) appeared at higher wave-number shifts (fig.3a). Since the excitation energy is transferred from the phycocyanin to the chlorophyll this shift in the Raman band could be the result of a chlorophyll–carotenoid interaction as well. This latter possibility can be ruled out by measuring the effect of a 670 nm additional illumination. At this wavelength chlorophyll absorbs predominantly (fig.1). No effect of this light was found (fig.3b).

To avoid the photodegradation of carotenoids the intensity of the Raman probe light was kept very low (≈5 mW). The additional 625 and 670 nm illumination was not absorbed by the carotenoids and therefore the considerably higher intensities (30 mW and 40 mW, respectively) could not cause damage to the carotenoids. They may have damaged however the phycocyanin and the chlorophyll, respectively. Even if this were the case it would not effect our conclusion. In order to keep the local overheating of the sample at a minimum we used a 1 cm^2 cuvette to be able to generate convective currents within the sample.

On the basis of the above results, in our opinion there is a specific interaction between phycocyanin and certain carotenoids in *Anacystis nidulans*.

The particular carotenoid which takes part in this interaction is not known. β-Carotene can be excluded since it was found accompanied only to photosystem I in *Anacystis nidulans* [2] and phycocyanin is connected to photosystem II. The remaining major carotenoid components are all xanthophylls: zeaxanthin (39%), caloxanthin (25%) and nostoxanthin (10%) [13]. Unfortunately Raman spectroscopy is unable to differentiate among them [7].

A shift in the ν_1 towards higher wave-number shifts is always caused by a decrease in the π-electron delocalisation. Such a decrease can be the result of conformational changes. For example *trans–cis* isomerisation of the β-carotene was found to cause an upward shift of 10 cm^{-1} in cyclohexane solution [6].

Here we prefer a different explanation: a decrease in the π-electron delocalisation caused by the disturbance of the neighbouring molecules. The chromophore of the phycocyanin is the most suitable partner for this role since it has a π-electron system itself as well.

The fact that in [9] the carotenoid photosensitivity changed together with the phycocyanin fluorescence which was effected earlier and stronger by the nitrate starvation than the phycocyanin absorption we can ascribe to the importance of a well-defined structure. From the results of this paper that carotenoids sense the excited state of the chromophore we can conclude that a very close packing occurs.

These results however, raise the possibility of a hitherto unobserved function of carotenoids in blue–green algae: They may influence the coupling between phycobiliproteins and the chlorophyll without transfering their own absorbed energy to the reaction center.

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