ABSTRACT

In higher plants, carotenoid molecules play particularly important roles in harvesting light, stabilizing protein structures, regulating energy flow, and dissipating excess energy not required by the organism for photosynthesis. Low-temperature resonance Raman spectroscopy was used to study the spectral properties, binding sites and composition of major carotenoids in spinach Photosystem I particles. Photosystem I is a supercomplex of a reaction centre, core and light-harvesting complexes. Resonance Raman spectra of carotenoid molecules have four main groups of intense bands, which provide information on the conformation and configuration of these molecules. The most sensitive to their molecular configurations relaxed or distorted is \( \nu_4 \) band exhibiting an increase in both intensity and structure indicating out-of-plane distortion of the molecule. During prolonged exposure to high-light intensities various pigments in Photosystem I exhibited different susceptibility to photodestruction. Resonance Raman technique allowed us to recognize the type and conformation of photobleached carotenoid molecules and to reveal the involvement of these pigments in the photoprotection. Raman data revealed a nearly full photobleaching of the long-wavelength lutein molecules. The observed similar bleaching rate of the lutein molecules and the most red-shifted long-wavelength chlorophyll \( a \), located in the antenna membrane protein \( Lcha4 \), suggested that these molecules are located closely. Our results showed that the photobleached antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls are involved in photoprotection of PSI core complex. We investigated the effect of histidine on the photobleaching of pigments in isolated particles of photosystem I. Our preliminary results showed that histidine reduces the photobleaching of antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls located in PSI antenna complex.

Keyword: Carotenoids, Photosystem I, Raman spectroscopy

Introduction

The plant photosystem I (PSI) supercomplex is the most efficient nano-photochemical machine in nature. It is remarkable that PSI exhibits a quantum yield of nearly 1, and almost every captured photon is eventually trapped and results in electron translocation (4). The PSI supramolecular complex consists of a chlorophyll (Chl) \( a \) binding core complex (PSI core) and a Chl \( a/b \) binding peripheral light-harvesting complex (LHCI), located on one side of the core (4-6). The LHCI consists of four different membrane proteins (Lhca1-4), which bind about 110 Chl \( a \) and all Chl \( b \) molecules and approximately 20 xanthophyll molecules (4,6). Data about the content, type and conformational state of carotenoids in the LHCI are scarce and controversial to some extent (9,11,17). In higher plants, carotenoid molecules play particularly important roles in harvesting light, stabilizing protein structures, regulating energy flow, and dissipating excess energy not required by the organism for photosynthesis. The knowledge of carotenoid composition and organization of light-harvesting and core complexes is important for understanding in detail the mechanisms of their main function in the photosynthetic apparatus: the protection against high-light stress and reactive oxygen species, realized via the quenching of electronic excited states of chlorophyll \( a \) molecules, quenching of singlet oxygen and scavenging of free radicals.

Resonance Raman spectroscopy (RRS) has been proven to be a non-destructive method providing precise information on the type and conformation of carotenoid molecules in...
Materials and methods

The PSI particles were isolated from spinach chloroplasts by mild digitonin treatment (12). The submembrane particles obtained by this method originated mainly from stroma exposed regions of thylakoids and consist of PSI core complex and LHCl, so called PSI-200 complexes. The final pellet was resuspended in a medium containing 20 mM Tricine-KOH (pH 7.8), 10 mM NaCl, 10 mM NaCl, and 5 mM MgCl₂. Pigment concentration was determined by using the method of Lichtenthaler (10).

Pigment extraction of spinach PSI particles was performed as described by Ikegami et al. (7) with diethyl ether. Prior to ether extraction PSI particles were repeatedly washed with distilled water in order to remove salts and detergent. The ether treated material was dried under N₂ flux and then dissolved to appropriate concentration in pyridine. The latter was chosen as its polarizability was close to that of lipid and membrane protein environments (14).

Resonance Raman spectra measurements

For the RR spectra measurements the Chl concentration was 500 μg/ml. RR spectra at 77K were obtained in a translucent Dewar using 0.85 m double spectrophotometer Spex (model 1403; Spex Industries, Inc., Edison, NJ, USA), equipped with a cooled photomultiplier tube (model R943, Hamamatsu Photonics, K. K., Shizuoka, Japan). The excitation was provided by an argon ion laser (Innova 307, Coherent) at 514.5, 502, 496.5, 488, 476.5 and 457.9 nm. Five to ten successive Raman spectra were averaged for each experiment. To compare absolute RR amplitudes, exactly the same amount of Chl in each sample was used and signal arising from the buffer at the 814 cm⁻¹ was used for normalization of the measured spectra as described in (3).

High-light treatment

The illumination of isolated PSI particles was carried out in a temperature-controlled vessel under continuous stirring at room temperature (22°C). The Chl concentration during illumination was 500 μg/ml. Samples were placed in a flat vessel to ensure equal illumination of all sample layers. High intensity illumination was provided by a 1000 W halogen projector lamp giving intensity of 1800 μE m⁻² s⁻¹ on the vessel surface. The light was passed through a heat filter. Samples for analysis were taken at different time intervals (0, 60, 90 and 120 min) during illumination and where it is indicated 25 mM histidine was added.

Results and Discussion

The RR spectra of carotenoid molecules in multipigment PS I particles, excited selectively at 514.5, 502, 496.5, 488, 476.5 and 457.9 nm are compared in Fig. 1 (2). They contained four known groups of bands (called from ν₁ to ν₄) characteristic for carotenoids (8,13,14). The bands have been assigned (8,13) as follows: ν₁ – to C=C bonds in phase stretching vibrations; ν₂ – to C₁₄=C₁₅ stretches coupled to C₁₅=H in plane bending; ν₃ – to methyl CH₃ in plane rocking vibrations; ν₄ – to C-H out of plane bending modes coupled with C₇=C₈ torsion.

The most intensive band in the carotenoid resonance Raman spectrum (called ν₁) is located at ~ 1530 cm⁻¹. Its precise position is strongly sensitive to both the number of conjugated double bonds (the length of polyene chain) and the carotenoid configuration (8,13,14). Each additional double bond produces ~ 3 cm⁻¹ down shift of this parameter (8,14) and therefore can be used to identify the specific carotenoid absorption transitions in complexes containing carotenoids with distinguished number of conjugated double bonds. As Ruban et al. (14) have found, ν₁ position depends strongly on the excitation lines being quite different for each carotenoid. Thus, by ν₁ position it is possible to recognize which carotenoid dominates the RR spectrum obtained at a given wavelength matching the absorption of this carotenoid molecule.
In PS I particles excitation lines at 514.5, 502, 496.5, 488, 476.5 and 457.9 nm yielded the following ν₁ positions: 1526.5, 1531, 1528.5, 1533, 1527 and 1533 cm⁻¹, respectively (Fig. 2a). They were very similar to those of lutein at 514.5, 496.5 and 476.5 nm, violaxanthin – at 502 nm, and neoxanthin – at 488 nm and 457.9 nm. In addition, the ν₁ bandwidths in PS I particles at all excitation lines except at 457.9 nm were between 12.5 cm⁻¹ and 14.6 cm⁻¹, equal or slightly smaller than those of corresponding carotenoids in pyridine (14) confirming that at these wavelengths only one type of carotenoid contributes to the band. The lack of expected intensive ν₁ band at 1522-1524 cm⁻¹, a position characteristic for all-trans β-carotene (8,14,15) was surprising taking into account its abundance in core complex of PSI (4-6). However, the discernible shoulder at smaller wavenumbers and wider bandwidth (15.4 cm⁻¹) at 457.9 nm excitation is indicative for the presence of some all-trans β-carotene.

Its very small contribution could be explained by the assumption that at these wavelengths in PS I particles the excitations did not reach the core pigments and only the major xanthophylls belonging to LHCl were excited (1). Most probably, the formed LHCl belt (4) surrounding the PSI core could shield the core β-carotene molecules thus hindering their direct excitation. To test this possibility we compared the spectra of PSI particles with the spectra of pigment extracts from the same PSI particles, dissolved in pyridine (Fig. 2b). In latter the contribution of all-trans β-carotene is more clearly pronounced at 457.9 excitation (curves 1 and 2 in Fig. 2b), compared to 488 nm (curves 3 and 4 in Fig. 2b) but still not so great as we expected. Results obtained confirmed that LHCl belt shielded some all-trans β-carotene molecules. However, this could be not the only reason for ν₁ band small intensity at 1522-1524 cm⁻¹. There are several reasons that could explain these experimental results considered in detail in (2).
The absorption transitions for lutein, violaxanthin and 9-cis neoxanthin in spinach photosystem I particles are characterized and their binding are discussed in (2). Resonance Raman data (Fig. 2) suggest that β-carotene molecules are also present in all-trans and probably in 9-cis configurations.

We used RR scattering in an attempt to recognize the type and conformation of photobleached carotenoid molecules during prolonged exposure to high-light intensities (3). Resonance Raman spectra were measured with excitation at 488 and 514.5 nm lines, which coincide with the absorption, maxima of 9-cis neoxanthin and long-wavelength lutein respectively (1,14-17). The absolute RR band amplitudes are normalized as explained in Materials and Methods. RR spectra in ν₁ region as a function of the time of illumination are compared in Fig. 3a and 3b for lutein and 9-cis neoxanthin molecules, respectively. Their dependencies on the time of illumination are compared in Fig. 3c. Analysis of RR data revealed nearly a full photobleaching of the long-wavelength lutein molecules, whereas the bleaching of the neoxanthin molecules was negligible (Fig. 3c).

The observed in (3) similar bleaching rate of the lutein molecules and the most red-shifted long-wavelength Chl a, located in the antenna membrane protein Lcha4, suggested that these molecules are located closely. Our results showed that the photobleached antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls, are involved in photoprotection of PSI avoiding formation of triplet chlorophyll states. They confirm that the photoprotection is an intrinsic property of light-harvesting systems.

We employed the RR spectroscopy in order to obtain direct information about the effect of histidine on the photobleaching of the lutein molecules (18). The RR spectra in the ν₁ region as a function of the time of illumination are compared in Fig. 4a and 4b for lutein molecules in control and histidine-containing PSI particles. The most intensive band ν₁ in the lutein RR spectrum is located at 1526.5±1 cm⁻¹. The discernible shoulder at smaller wavenumbers and its weakly increasing intensity suggest an origin from zeaxanthin, which is known (15) to exhibit a band at 1524 cm⁻¹ and accumulates under high-light illumination. The ν₁ intensity dependencies for lutein molecules in control and histidine-containing PSI particles on the time of illumination are compared in Fig. 4c. The results revealed nearly full photobleaching of lutein in control samples, while in histidine-containing samples the bleaching is markedly retarded. Histidine expressed protective effect against photodestruction carotenoids thus indicating that photobleaching process in photosystem I particles was mediated by singlet oxygen.
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