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Effects of heat treatment on the protein secondary structure and pigment microenvironment in photosystem 1 complex

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Abstract

The protein secondary structure and pigments' microenvironment in photosystem 1 (PS1) complexes were studied in the temperature range of 25–80 °C using Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopy, respectively. Quantitative analysis of the component bands of the amide I band (1 700–1 600 cm^{-1}) showed no significant change below 50 °C. However, apparent conformational changes occurred at 60 °C and further continued at 70 and 80 °C accompanied with transitions of secondary structure mainly from α-helix to the β-sheet structures. CD analysis demonstrated that the regular arrangement, viz. protein microenvironment of pigments of PS1 complexes, was destroyed by heat treatment which might come from the changes of protein secondary structure of PS1. The CD signals at 645 nm contributed by chlorophyll (Chl) *b* of light-harvesting complex 1 (LHC1) were easily destroyed at the beginning of heat treatment (25–60 °C). When temperature reached 70 and 80 °C, the CD signals at 478 nm contributed mainly by Chl *b* of LHC1 and 498 nm contributed by carotenoids decreased most rapidly, indicating that LHC1 was more sensitive to high temperature than core complexes. In addition, the oxygen uptake rate decreased by 90.81 % at 70 °C and was lost completely at 80 °C showing that heat treatment damaged the regular function of PS1 complexes. This may be attributed to heat-induced changes of pigment microenvironment and protein secondary structure, especially transmembrane α-helix located in PsaA/B of PS1.

Additional key words: carotenoids; chlorophyll; circular dichroism; Fourier transform infrared spectra; oxygen uptake; photosystem 2; proteins; spinach; *Spinacia*.

Introduction

Photosystem 1 (PS1) is a large multi-subunit protein complex and functions as the light-driven plastocyanin-ferredoxin oxidoreductase in the thylakoid membranes of cyanobacteria and chloroplasts (Golbeck 1992, Chitnis *et al*. 1995, Chitnis 1996, 2001). In plants, PS1 is composed of a core complex and a light-harvesting complex (LHC1). The core complex consists of 12 core subunits and approximately 93 chlorophyll (Chl) *a* molecules and 22 β-carotene molecules (Scheller *et al*. 2001, Ben-Shem *et al.* 2003). It contains five different electron carriers, namely A_0 (primary electron acceptors of PS1), A_1 (secondary electron acceptors of PS1), and A_2 (iron-sulphur centre X) located in PsaA/B protein (core reaction centre), and A_3, A_4 (iron-sulfur centres A, B, respectively) located in peripheral protein-PsaC on the stromal side of the PS1 complexes (Nechushtai *et al.* 1996). LHC1 binds about 56 Chl *a*+*b* molecules and several carotenoid (Car) molecules, and additional 20 Chls are the "gap Chls" positioned between LHC1 and the core (Ben-Shem *et al.* 2003). Crystal structure from peas at 0.44 nm showed that the pigment density in LHC1 is high and the ratio of Chl/protein exceeds that of LHC2, which yields a more intricate set of close pigment-pigment interactions (Ben-Shem *et al.* 2003). When pigments in LHC1 absorb photons, their energy is transferred through antenna pigment molecules to the reaction centre (RC),

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Abbreviations: Car, carotenoid; CD, circular dichroism; Chl, chlorophyll; FTIR, Fourier transform infrared; LHC1, light-harvesting complex 1; P700, primary electron donor; PS1, photosystem 1; RC, reaction centre.
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P700, located in PsaA/B proteins of CC1. In RC, the excitation energy is trapped and used for energizing an electron, which is transferred to an acceptor Chl *a* molecule (A_0). Chl *a* molecule becomes oxidized while the primary electron acceptor becomes reduced, resulting in charge separation (Scheller *et al.* 2001). Both PsaA and PsaB subunits contain 11 trans-membrane α-helices (Klukas *et al.* 1999a,b, Fromme *et al.* 2001, Jordan *et al.* 2001), of which five are located in C-terminal and six in N-terminal. The C-terminal trans-membrane domain of PsaA and PsaB subunits surrounds the electron transfer chain and is called the RC domain (Fromme *et al.* 1994, Schubert *et al.* 1998). The C-terminal domains of PsaA and PsaB are also involved in the coordination of 25 Chls of the antenna system (12 in PsaA and 13 in PsaB). The 12 N-terminal α-helices of PsaA and PsaB coordinate 54 Chls (28 in PsaA and 26 in PsaB). In addition, most of the loop regions also contain secondary structure elements as α-helices and β-sheet. The subunits PsaC, PsaD, and PsaE do not contain transmembrane α-helices (Fromme *et al.* 2001).

The photosynthetic process is one of the most thermosensitive functions in the plant. The inhibitory effects of moderate to high temperatures in plants have been well documented (Berry and Björkman 1980, Morgan *et al.* 2002). PS1 is sensitive to temperature (Armond *et al.* 1980, Takeuchi and Thornber 1994, Ilík *et al.* 2000). But the detailed changes on the protein secondary structure and pigment microenvironment are unknown. Although the heat induced changes in protein secondary structure and thermal stability of photosystem 2 (PS2) complex are known (De Las Rivas and Barber 1997, Shi *et al.* 1998), very little is described about the effects of heat stress on the protein secondary structure of PS1. Therefore our paper focuses on the characterization of protein secondary structure and pigments' microenvironment of PS1 at heat treatment. The study of the process of protein heat denaturation can provide an insight into the structural organization and individual roles of proteins and cofactors.

Fourier transform infrared spectroscopy (FTIR) characterizes the secondary structure of soluble and membrane proteins, even of protein complexes having large molecular masses with high structural complexity (De Las Rivas and Barber 1997). The infrared amide I band in the 1 700–1 600 cm^{-1} region, arising mainly from the in-plane carbonyl (C=O) stretching vibration of the peptide bond (80 %) (Krimm and Bandekar 1986, He *et al.* 1991), is conformationally sensitive and is the region most commonly used in structural studies of proteins (Surewicz and Mantsch 1988, De Las Rivas and Barber 1997). Circular dichroism (CD) spectrum is the difference between levorotatory polarized radiation and dextrorotatory polarized radiation through samples. CD spectrum sensitively reflects the micro-environmental changes of the chromophore (Wei *et al.* 2001).

We studied the effects of heat treatment on the protein secondary structure and pigments' microenvironment in PS1 over the 25–80 °C temperature range using FTIR and CD spectra, respectively. The results revealed a clear conformational transition mainly from α-helix to the β-sheet structures, which may correspond to both the changes of pigments' microenvironment and functional inactivation of PS1 complexes.

Materials and methods

Isolation and purification of PS1 complexes: PS1 complexes were prepared from the fresh leaves of market spinach (*Spinacia oleracea* L.), using a modified procedure of Mullet *et al.* (1980) and Bassi and Simpson (1987), with only 6-h ultra-speed sucrose gradient centrifugation (100 000×*g*). The PS1 preparation was dialyzed for 3 h with 5 mM Tricine buffer (pH 7.8) and pelleted by centrifugation at 40 000×g for 30 min to remove excess *Triton X-100*. The pellet was re-suspended in de-ionized distilled water and stored at –80 °C until use. Chl concentrations were determined in 80 % (v/v) acetone solutions using the method of Arnon (1949).

Heat treatment: The PS1 complexes were re-suspended at about 200 g Chl m^{-3} in 50 mM Tricine-NaOH (pH 7.8) containing 0.1 M sorbitol, 10 mM NaCl, and 0.05 % (m/v) *Triton X-100*. Linear heating was performed by water bath from control (room temperature at 25 °C) to 80 °C using steps of 10 °C for 10 min.

FTIR spectroscopy was implemented according to Hu *et al.* (2004). The samples were washed twice in D_2O and re-suspended in D_2O at a concentration of 800 ng(Chl) m^{-3}. Then the samples were dropped on CaF_2 windows and dried in vacuum to form a layer of semi-hydrated film. Spectra were recorded with a *Nicolet* FTIR spectrometer (*Magna-550*, series *II*). All measurements were done at room temperature. 100 interograms were collected and the spectral resolution was 4 cm^{-1}. For data process, the extracted amide I band contour was subjected to second derivative calculation and Gaussian curve-fitting analysis as described in Ruan *et al.* (2000).

CD spectra were measured according to Wei *et al.* (2001) with a *Jasco J-500cs* spectro-polarimeter at a scanning speed of 3.33 nm s^{-1}, a bandwidth of 2 nm, a response time of 2 s, and an accumulation of four times. The samples were scanned at 30 ng(Chl) m^{-3}. The corresponding absorption spectra were obtained from the optical density conversion of the high tension voltage, which was recorded simultaneously with the CE data, using the Standard Analysis program provided by *Jasco*.

Oxygen uptake activity: PS1-mediated electron transfer

from reduced dichlorophenol indophenol ($DCIPH_2$) to methyl viologen (MV) was measured by O_2 consumption as described by Carpentier *et al.* (1984) using a Clark-type oxygen electrode at a photosynthetic photon flux density of 800 μmol m^{-2} s^{-1} and 25 °C. The reaction medium was composed of PS1 complexes treated with various temperatures and diluted at a Chl concentration of 10 ng m^{-3}, 20 mM Tricine-NaOH (pH 7.8), 0.5 mM MV, 1 mM NaN_3, and 20 μM $DCIPH_2$. Prior to measurements, samples were incubated for 2 min at 25 °C.

Results and discussion

Analysis of the protein secondary structure of PS1 complex: A series of spectral changes in the amide I band of PS1 complex after different temperature heat treatment is shown in Fig. 1. No significant change occurred below 50 °C in the peak position and bandwidth of the amide I band. For the amide I band at 60 °C, the peak position shifted to 1 649 cm^{-1} from 1 656 cm^{-1} at 25 °C, which indicated a change in the secondary structure of PS1 complexes. For higher temperature treatment, the bandwidth of the amide I band dramatically increased at 70 °C and the further increase was detected at 80 °C. This resulted in change of secondary structure of PS1 proteins initiated by the temperature rise at 60 °C and a serious damage occurred at around 70 °C. The above data are consistent with our prior results (Hu *et al.* 2004).

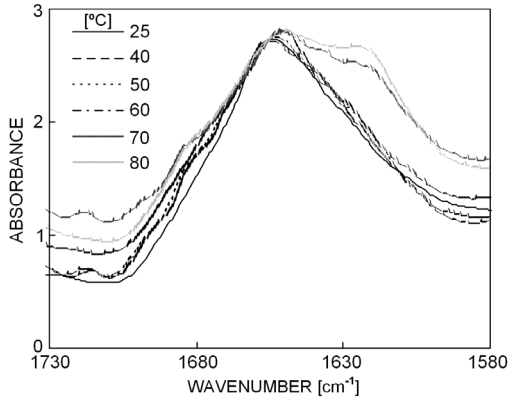

Fig. 1. Changes of the amide band I in the infrared spectra of the PS1 complex after heat treatments at different temperatures. The treatment temperatures were 25, 40, 50, 60, 70, and 80 °C, respectively, corresponding to curves arrayed from inside to outside.

The infrared absorption spectra (*thick line*) and band decomposition (*thin line*) of the PS1 complex amide band I at 25 °C between 1 700 and 1 600 cm^{-1} are shown in Fig. 2. In the infrared absorption spectra, the protein amide I bands of PS1 complex from approximately 1 700 to 1 600 cm^{-1} with the strongest absorption at about 1 658 cm^{-1} are attributed to the in-plane C=O stretching vibration (80 %) weakly coupled with C-N stretching and N-H bending (He *et al.* 1991). For the decomposition of the amide I band of PS1, second derivative and Gaussian curve-fitting spectral analysis methods were used to determine the actual secondary structure content. According to secondary derivative spectra of PS1 complex, nine sub-bands were found in the absorption spectra over the range of 1 700–1 600 cm^{-1}. The assignment of each sub-band between 1 700 and 1 600 cm^{-1}, which was used in the curve-fitting process, is summarized in Table 1. A minor sub-band at 1 608 cm^{-1} was ascribed to protein side chains as its frequency was too low to be assigned to any secondary structure. So its contribution is not included in the calculation of the secondary structures of PS1 complex (Zhang *et al*. 1999). Based on the above analysis methods, the secondary structure of PS1 complex at 25 °C contained 42 % of α-helix, 27 % of β-sheet, 19 % of turn, and 12 % of random structures. PS1 contained more α-helical structure than PS2 with about 35 % of α-helical structure. This different constitution of secondary structure showed more transmembrane helices located in the PS1 proteins.

Table 1. Summary of the wavenumbers and proposed structural assignments of the bands for the decomposition between 1 700 and 1 600 cm^{-1} in the infrared spectra of PS1 complexes.

Frequency [cm^{-1}]	Assignment
1689	Turns
1676	Turns
1666	Turns
1658	α-helix
1650	Random coil
1645	Loops
1639	β-sheet
1628	β-sheet
1610	Side-chain (tyrosine)

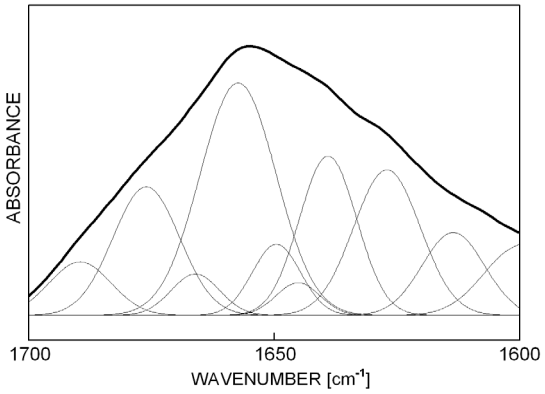

Fig. 2. Infrared absorption spectrum (*thick line*) in amide band I of PS1 complex between 1 700 and 1 600 cm^{-1} at 25 °C and its band decomposition (*thin line*) obtained by second derivative and Gaussian curve-fitting spectral analysis methods.

The changes of the secondary structure content of the PS1 complex after treatment with temperatures increased from 25 to 80 °C are displayed in Fig. 3. With the increase of temperature, all the four conformational components obviously changed. Most meaningful observation is that the α-helix content decreased significantly, which was accompanied with great increase of the β-sheet content. When comparing secondary structure at 80 °C to that at 25 °C, the content of α-helix decreased by 7.3 % accompanied with increase of β-sheet content by 5.7 % and turn structures decrease by 1.6 % while random structures increased by 3.2 %. So both the α-helical and turn structures decreased rapidly while the β-sheet and random structures increased. These changes are typical of protein conformational changes under heat stress, implying that a large portion of PS1 complexes was denatured. Especially the structure of trans-membrane domain and extra-membrane domain altered greatly under heat stress. Shi *et al.* (1998) already showed that great conformational changes in PS2 occur in the temperature range of 55–65 °C. However, the great changes of protein secondary structure in PS1 occurred at around 60–70 °C as shown in Figs. 1 and 3. This comparison demonstrates that PS1 is more stable to heat stress than PS2, and confirms that PS2 is the molecular target of the physiological process of photoinhibition (De Las Rivas and Barber 1997).

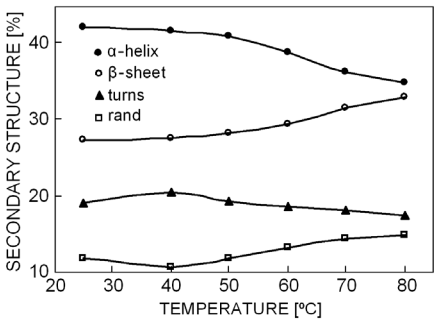

Fig. 3. Changes in the secondary structure content of the PS1 complex with different temperature treatments.

Analysis of CD spectroscopy on pigment microenvironment in PS1 complex: A CD spectrum in the red region is sensitive to detect the location and tropism of pigments and is widely applied for analysis of thylakoid membrane pigment-protein complexes (Bassi *et al.* 1991, Somsen *et al.* 1996, Ruban *et al.* 1997). According to Haworth *et al.* (1983), Lam *et al.* (1984), and Bassi and Simpson (1987), the positive peak in Fig. 4*A* at 668 nm and negative peak at 684 nm, which was mainly caused by the excitonic interaction of Chl *a* in PS1, as well as positive peak at 448 nm were the characteristic peaks of Chl *a*. The negative peaks at 645 and 462 nm and the positive peak at 479 nm were probably contributed by Chl *b* of PS1. According to Bassi and Simpson (1987), when the LHC1 was removed from PS1 complexes, the negative peak at 645 nm disappeared, however, the peak at 479 nm still existed. This implies that the peak of 645 nm is the characteristic peak of LHC1 and the peak of 479 nm is partly contributed by other pigments besides main Chl *b*; the positive peak at 498 nm is induced by Cars. Fig. 4*A* shows that high temperature treatment significantly affected the PS1 CD spectrum. The CD signals of PS1 complexes decreased and peaks shifted during heat treatment, indicating that the protein microenvironment of pigments in PS1 complexes was destroyed, which may be a consequence of the changes of PS1 protein secondary structure.

The analysis of decreased percentage of CD signals (Fig. 4*B*) shows that the peak at 645 nm, which is contributed by Chl *b* of LHC1, was decreased more rapidly while other six peaks were little changed as the temperature increased from 25 to 60 °C. Hence the protein

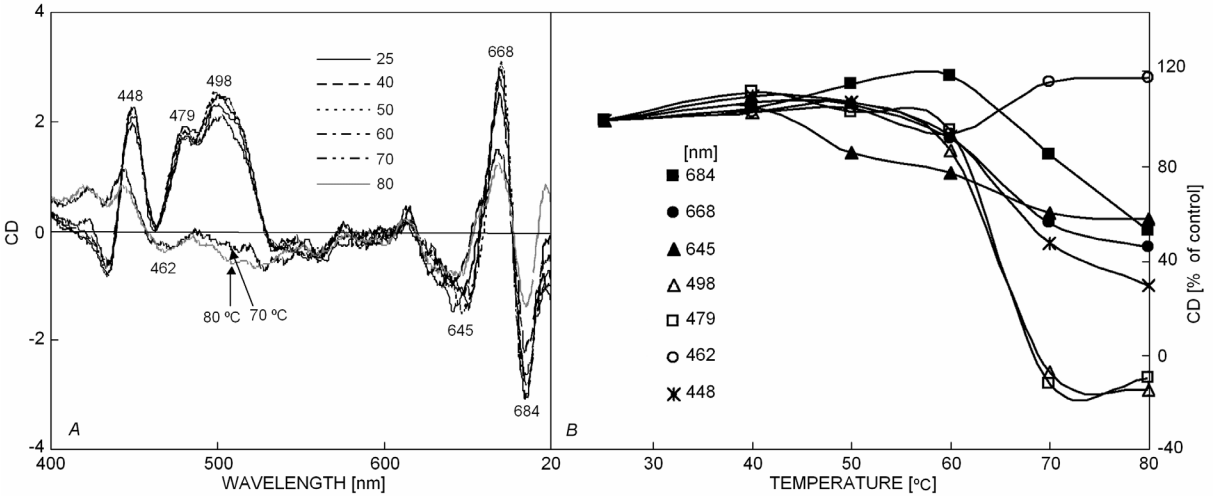

Fig. 4. *A*: The circular dichroism (CD) spectra of PS1 complexes after treatment with various temperatures. *B*: The decrease percent of CD signal intensities of PS1 complexes after treatment with various temperatures (PS1 complexes at 25 °C as control).

environment of Chl *b* in LHC1 was easily perturbed at the beginning of heat treatment. When the treatment temperatures reached 70 and 80 °C, the positive peaks of 498 nm (contributed by Cars) and 479 nm (contributed mainly by Chl *b* of LHC1) were decreased most rapidly. Thus the Cars in PS1 may be sensitive to high temperature, which may reflect a role of Cars in protecting PS1. At the same time, the decrease of peak at 479 nm indicated that the regular interaction between pigments and proteins in LHC1 was seriously disturbed at 70 and 80 °C. The decrease of positive peaks of Cars at 498 nm and Chl *b* at 479 nm demonstrated the destruction of the function of energy-absorbing and energy-transferring in PS1 complexes. Fig. 4*B* also shows that three peaks contributed by Chl *a* (namely the positive peak at 668 nm, the negative peak at 684 nm, and the positive peak at 446 nm) were decreased more slowly, indicating that the regular arrangement of Chl *a* microenvironment together with the integrity of proteins in PS1 was gradually destroyed. The protein microenvironment of Chl *a* in PS1 complexes was relatively more stable than that of Chl *b* and Cars. Hence the functional LHC1 is more sensitive than PS1 RC upon high temperature and protects PS1 RC, which might contribute to the thermostability of PS1. So it is probable that the thermostability of protein secondary structure in PS1 and the protection of PS1 RC by LHC1 might contribute to physiological resistance to stress environment. For example, a cyanobacterium *Nostoc flagelliforme* Born. *et* Flah contains much more PS1 than higher plants and is highly adapted to drought, cold, and irradiance stresses, and is suitable for growing in the unfavourable areas (Wang *et al*. 2000, Zhong *et al*. 2000).

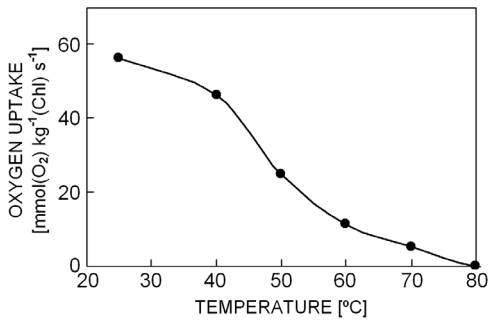

Fig. 5. Oxygen uptake rates of PS1 complex during heat treatment process (for measuring conditions see Materials and methods).

Effects of heat treatment on the oxygen consumption rates in PS1 complexes: Fig. 5 shows the changes of the oxygen uptake rates of PS1 after various temperature treatments. The higher the temperature applied, the larger was the oxygen uptake inhibition. The capability of oxygen uptake was minimal and decreased by 90.8 % at 70 °C. When the temperature arrived at 80 °C, the oxygen uptake was completely inhibited. Thus heat treatment might induce changes of pigment microenvironment and PS1 protein secondary structure (especially, trans-membrane α-helix located in PsaA/B of PS1 core complexes). The oxygen consumption rates decreased gradually and this trend may be related to the integrity of PS1 function (Barber *et al*. 2000). However, the oxygen-evolution activity of PS2 decreased severely and quickly, and was completely lost at about 60 °C under heat stress. Barber *et al*. (2000) found that the D1 and D2 subunits in PS2 contained five trans-membrane portions, structurally homologue with the five C-terminal helices of PsaA and PsaB in PS1, and the CP43 and CP47 subunits in PS2 contained six trans-membrane portions, similar to the six N-terminal helices of PsaA and PsaB in PS1. CP43 and CP47 are bound to PS2 RC as core antenna proteins and play roles of maintaining the stabilization of PS2 RC and keeping the activity of oxygen evolution of PS2. D1 and D2 are core proteins and bind most cofactors related to electron transfer in PS2. So we deduce that the conformational changes of trans-membrane α-helix located in PsaA/B protein have pronounced effects on electron transfer activity in the PS1 complexes.

In this paper we elucidate that during heat treatment great changes of PS1 polypeptide secondary structure and the pigment microenvironment occurred and the oxygen uptake activity of PS1 complex was significantly affected. However, the oxygen uptake activity came from integrity of PS1 complexes. Membrane lipids and lipid bilayer are functionally important constituents and involve in functions of proteins and pigments in PS1. As reviewed by Fromme *et al.* (2001), in the 0.25 nm structure of PS1 four lipid molecules are identified. They are bound by PsaA/B and located at the stromal side of the membrane. The two fatty acid chains of each lipid are anchored between trans-membrane α-helices of PsaA/B and extend to the middle of the membrane. Thus more detailed studies on the interaction between membrane lipids, proteins, and pigments should be carried out in order to better elucidate the mechanism responsible of PS1 complexes under heat stress.

References

Armond, P.A., Björkman, O., Staehelin, L.A.: Dissociation of supramolecular complexes in chloroplast membranes. A manifestation of heat damage to the photosynthetic apparatus. – Biochim. biophys. Acta **601**: 433-442, 1980.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Barber, J., Morris, E., Buchel, C.: Revealing the structure of the photosystem II chlorophyll binding proteins, CP43 and CP47. – Biochim. biophys. Acta **1459**: 239-247, 2000.

Bassi, R., Silvestri, M., Dainese, P., Moya, I., Giacometti, G.M.: Effects of a non-ionic detergent on the spectral properties and aggregation state of the light-harvesting chloro-

phyll *a/b* protein complex (LHCII). – J. Photochem. Photobiol. B **9**: 335-354, 1991.
Bassi, R., Simpson, D.: Chlorophyll-protein complexes of barley photosystem I. – Eur. J. Biochem. **163**: 221-230, 1987.
Ben-Shem, A., Frolow, F., Nelson, N.: Crystal structure of plant photosystem I. – Nature **426**: 630-635, 2003.
Berry, J., Björkman, O.: Photosynthetic response and adaption to temperature in higher plants. – Annu. Rev. Plant Physiol. **31**: 491-543, 1980.
Carpentier, R., Larue, B., Leblanc, R.M.: Photoacoustic spectroscopy of *Anacystis nidulans*. III. Detection of photosynthetic activities. – Arch. Biochem. Biophys. **338**: 534-543, 1984.
Chitinis, P.R.: Photosystem I. – Plant Physiol. **111**: 661-669, 1996.
Chitinis, P.R.: Photosystem I: function and physiology. – Annu. Rev. Plant Physiol. Plant mol. Biol. **52**: 593-626, 2001.
Chitnis, P.R., Xu, Q., Chitnis, V.P., Nechushtai, R.: Function and organization of Photosystem I polypeptides. – Photosynth. Res. **44**: 23-40, 1995.
De Las Rivas, J., Barber, J.: Structure and thermal stability of photosystem II reaction centres studied by infrared spectroscopy. – Biochemistry **36**: 8897-8903, 1997.
Fromme, P., Jordan, P., Krauß, N.: Structure of photosystem I. – Biochim. biophys. Acta **1507**: 5-31, 2001.
Fromme, P., Schubert, W.D., Krauß, N.: Structure of Photosystem I: Suggestions on the docking sites for plastocyanin, ferredoxin and the coordination of P700. – Biochim. biophys. Acta **1187**: 99-105, 1994.
Golbeck, J.H.: Structure and function of photosystem I. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 293-324, 1992.
Haworth, P., Watson, J.L., Arntzen, C.J.: The detection, isolation and characterization of a light-harvesting complex which is specifically associated with photosystem I. – Biochim. biophys. Acta **724**: 151, 1983.
He, W.-Z., Newell, W.R., Haris, P.I., Chapman, D., Barber, J.: Protein secondary structure of the isolated photosystem II reaction centre and conformational changes studied by Fourier transformation infrared spectroscopy. – Biochemistry **30**: 4552-4559, 1991.
Hu, Z.H., Xu, Y.N., Jiang, G.Z, Kuang, T.Y.: Degradation and inactivation of photosystem I complexes during linear heating. – Plant Sci. **166**: 1177-1183, 2004.
Ilík, P., Kouřil, R., Fiala, J., Nauš, J., Vácha, F.: Spectral characterization of chlorophyll fluorescence in barley leaves during linear heating analysis of high-temperature fluorescence rise around 60°C. – J. Photochem. Photobiol. B **59**: 103-114, 2000.
Jordan, P., Fromme, P., Klukas, O., Witt, H.T., Saenger, W., Krauß, N.: Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. – Nature **411**: 909-917, 2001.
Klukas, O., Schubert, W.D., Jordan, P., Krauß, N., Fromme, P., Witt, H.T., Saenger, W.: Localization of two phylloquinones, QK and QK', in an improved electron density map of photosystem I at 4-Å resolution. – J. biol. Chem. **274**: 7361-7367, 1999a.
Klukas, O., Schubert, W.D., Jordan, P., Krauß, N., Fromme, P., Witt, H.T., Saenger, W.: Photosystem I, an improved model of the stromal subunits PsaC, PsaD, and PsaE. – J. biol. Chem. **274**: 7351-7360, 1999b.
Krimm, S., Bandekar, J.: Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. – Adv. Protein Chem. **38**: 181-364, 1986.
Lam, E., Ortiz, W., Malkin, R.: Chlorophyll *a/b* proteins of photosystem I. – FEBS Lett. **168**: 10-14, 1984.
Morgan, R., Ivanov, A.G., Williams, J., Khan, M., Huner, N.P.A.: Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga. – Biochim. biophys. Acta **1561**: 251-265, 2002.
Mullet, J.E., Burke, J.J., Arntzen, C.J.: Chlorophyll proteins of photosystem I. – Plant Physiol. **65**: 814-822, 1980.
Nechushtai, R., Eden, A., Cohen, Y., Klein, J.: Introduction to photosystem I: reaction centre function, composition and structure. – In: Ort, D.R., Yocum, C.F. (ed.): Oxygenic Photosynthesis: The Light Reactions. Pp. 289-311. Kluwer Academic Publ., Dordrecht – Boston – London 1996.
Ruan, X., Wei, J., Xu, Q., Wang, J.S., Gong, Y.D., Zhang, X.F., Kuang, T.Y., Zhao, N.M.: Comparison of the effects of Triton X-100 treatment on the protein secondary structure of photosystem I and photosystem II studied by FT-IR spectroscopy. – J. mol. Struct. **525**: 97-106, 20000.
Ruban, A.V., Calkoen, F., Kwa, S.L.S., Grondelle, R.V., Horton, P., Dekker, J.P.: Characterization of LHCII in the aggregated state by linear and circular dichroism spectroscopy. – Biochim. biophys. Acta **1321**: 61-70, 1997.
Scheller, H.V., Jensen, P.E., Haldrup, A., Lunde, C., Knoetzel, J.: Role of subunits in eukaryotic photosystem I. – Biochim. biophys. Acta **1507**: 41-60, 2001.
Schubert, W.D., Klukas, O., Saenger, W., Witt, H.T., Fromme, P., Krauß, N.: A common ancestor for oxygenic and anoxygenic photosynthetic systems: a comparison based on the structural model of photosystem I. – J. mol. Biol. **280**: 297-314, 1988.
Shi, H., Xiong, L., Yang, K.Y., Tang, C.Q., Kuang, T.Y., Zhao, N.M.: Protein secondary structure and conformational changes of photosystem II during heat denaturation studied by Fourier transform infrared spectroscopy. – J. mol. Struct. **446**: 137-147, 1998.
Somsen, O.J., Grondelle, R.V., Amerongen, H.V.: Spectral broadening of interacting pigments: polarized absorption by photosynthetic proteins. – Biophys. J. **71**: 1934-1951, 1996.
Surewicz, W.K., Mantsch, H.H.: New insight into protein secondary structure from resolution-enhanced infrared spectra. – Biochim. biophys. Acta **952**: 115-130, 1988.
Takeuchi, T.S., Thornber, J.P.: Heat-induced alterations in thylakoid membrane protein composition in barley. – Aust. J. Plant Physiol. **21**: 759-770, 1994.
Wang, M., Zhong, Z.P., Wang, K.B., Li, L.B., Kuang, T.Y.: Isolation of chlorophyll protein complexes from the thylakoid membrane in *Nostoc flagelliforme* and their spectroscopic characterization. – Acta bot. sin. **42**: 684-688, 2000.
Wei, J., Yu, H., Li, L.B., Kuang, T.Y., Wang, J.S., Gong, Y.D., Zhao, N.M.: The photodamage process of pigments and proteins of PS I complexes from *Spinacia oleracea* L. – Chin. Sci. Bull. **46**: 1812-1816, 2001.
Zhang, H., Yamamoto, Y., Ishikawa, Y., Carpentier, R.: Characterization of the secondary structure and thermostability of the extrinsic 16 kilodalton protein of spinach photosystem II by Fourier transform infrared spectroscopy. – J. mol. Struct. **513**: 127-132, 1999.
Zhong, Z.P., Wu, Y.H., Xu, J., Feng, L.J., Shi, D.J.: Differentiation of hormogonia and photosynthetic characterization of *Nostoc flagelliforme*. – Acta bot. sin. **42**: 570-575, 2000.