Heat-Induced Changes in the Photochemical Centres and the Protein Secondary Structures of Photosystem II Studied by Variable Fluorescence and Difference FT-IR Spectroscopy

M. Joshi[a] and M. Fragata[b]

[a] NHLBI/NIH, Building 3, Room B1–06, 9000 Rockville Pike, Bethesda, MD 20892, U. S. A.
[b] Département de chimie et biologie, Section de chimie et Groupe de recherche en énergie et information biomoléculaires (GREIB), Université du Québec à Trois-Rivières, Trois-Rivières, Que, G9A 5H7, Canada

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Variable fluorescence (F_v), i.e., $F_v = F_m - F_o$ where F_o is the minimal fluorescence and F_m the maximum fluorescence, and difference Fourier transform infrared (FT-IR) spectroscopy were used to study the effect of heat stress in the 25–55 °C range on photosystem II (PSII) structure and function. First, the F_v intensity reflects accurately the changes in the number of open photochemical centers in PSII. Secondly, the use of F_v in combination with FT-IR spectroscopy can disclose structure-function correlations in the heat inactivation of the PSII complex. Analysis of the midpoint temperatures of thermal denaturation, i.e., 50% inactivation, reported so far in investigations of the thylakoid membrane components has revealed that most of the thermal transitions attributed to PSII are in the 39–46 °C range. In this work, it is shown specifically that the midpoint temperature of PSII inactivation is at about 40 °C. Moreover, it was clearly demonstrated that the heat-induced changes above 40 °C are the result of a marked decrease in the number of open photochemical centers in PSII. It was also seen that above this same temperature the loss of photochemical centers has its structural counterpart in overall modifications of the secondary structures of the PSII proteins resulting from the decrease in the α-helix content concomitant with the increase in extended chain (β-strand) conformations. In brief, a novel finding reported here is that the number of open photochemical centers in PSII is dependent on a dynamic equilibrium between the contents of the PSII proteins in α-helix and extended chains (β-strands), but not in β-sheets and β-turn structures except for the antiparallel-β-sheet conformations. This therefore associates the thermal inactivation of the photochemical centers in photosystem II with distinct conformational changes in the proteins of the PSII supramolecular complex. In the particular context of the present study, these findings constitute a significant contribution to the investigation of structure-function correlations in the photosynthetic membrane. In a broader context, this information might be essential for the comprehension of the molecular arrangements or local structure order that are involved directly or indirectly in biological catalysis.

Introduction

Heat-induced changes in the thylakoid membrane of plant chloroplasts is one of the important factors that determine photosynthetic efficiency (see, e.g., Joshi *et al.*, 1995). A wide variety of heat-induced effects were observed. For example, the heat treatment causes the destacking of the thylakoid membranes (Gounaris *et al.*, 1983) and, as a result, alters the normal function of photosystem I and photosystem II (PSII). Heat stress is also responsible for the denaturation of the light-harvesting chlorophyll *a/b* proteins (Sundby and Andersson, 1985) and the oxygen-evolving complex (Katoh and San Pietro, 1967). It also causes the loss of Mn- and Ca-ions by the PSII complex (Cheniae and Martin, 1970; Nash *et al.*, 1985; Coleman *et al.*, 1988). Furthermore, Bukhov *et al.* (1990) and Cao and Govindjee (1990) found that the heat treatment hinders the rate of electron

Abbreviations: CF_1, chloroplast coupling factor subunit 1; Chl, chlorophyll; DCIP, 2,6-dichlorophenol indophenol; DSC, differential scanning calorimetry; F_m, maximum fluorescence; F_o, minimal fluorescence; F_v (= F_m – F_o), variable fluorescence; FT-IR, Fourier transform infrared; LHCII, light harvesting complex associated with PSII; PAM, pulse amplitude modulation; PSII, photosystem II.

Reprint requests to M. Fragata.
Fax: +1 819 376–5057.
E-mail: fragata@uqtr.uquebec.ca

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transfer from the primary electron acceptor Q_A to the secondary acceptor Q_B. These authors concluded that the molecular site of the heat effect might be at the acceptor side of PSII, or at least in its neighborhood. There is also evidence that the loss of PSII activity produced by heat stress is related to the transformation of the high potential cytochrome b_{559} into its low potential form (Nash *et al.*, 1985; Cramer *et al.*, 1981a,b). These observations suggest that the heat-induced perturbations of the PSII stability have their counterpart in structural changes in the PSII proteins. A recent infrared study of heat-treated PSII reaction centers (PSII-RC) (De Las Rivas and Barber, 1997) supports this assumption. In brief, De Las Rivas and Barber (1997) showed that the secondary structures in PSII-RC undergo a thermal transition at about 42 °C which is thought to be the reflection of changes occurring in the relative contents of the various conformations into which the PSII proteins are folded.

The identification of the molecular sites which are affected by high temperature has been attempted at first with a number of differential scanning calorimetry (DSC) studies designed to probe the heat-induced inactivation of specific photosynthetic processes. The DSC thermograms revealed about five to seven endothermic transitions in the region between 30 and 90 °C (Cramer *et al.*, 1981a,b; Thompson *et al.*, 1986; Smith *et al.*, 1989; Smith and Low, 1989; Shutilova *et al.*, 1995). These transitions indicate the presence in the thylakoid membrane of molecular components differing in their sensitivity to heat. The DSC transitions observed at about 42, 61, 65, 70 and 76 °C were identified with the perturbation, or denaturation, of the function of the oxygen-evolving complex (42 °C), the PSII reaction center (61 °C), the soluble subunit of the CF_1 complex (65 °C), the ribulose 1,5-biphosphate carboxylase (70 °C), and the LHCII complex (76 °C) (see discussions in Cramer *et al.*, 1981b; Thompson *et al.*, 1986; Smith *et al.*, 1989; Smith and Low, 1989; Shutilova *et al.*, 1995; De Las Rivas and Barber, 1997).

In this work, we used a combination of variable fluorescence (F_v) and difference Fourier transform infrared spectroscopy to study the effect of heat stress on the PSII structure and function in the temperature range from 25 to 55 °C. First, fluorescence measurements with the pulse amplitude modulation (PAM) technique (Schreiber *et al.*, 1986) have been shown to be a very sensitive method for the observation of small changes in the intensities of minimal fluorescence (F_o) and maximum fluorescence (F_m), and therefore for examining the levels of variable fluorescence F_v, where $F_v = F_m - F_o$. An important finding, in this connection, is the recognition that the F_v intensity reflects accurately the changes in the number of open photochemical centers in PSII (see discussions in Lavorel *et al.*, 1986). This, therefore, prompts the use of F_v in combination with techniques that are capable of detecting structural changes in the protein molecules, e.g., the Fourier transform infrared (FT-IR) spectroscopy.

FT-IR spectroscopy has been widely applied in structural studies of proteins (Arrondo *et al.*, 1993). A quite large number of FT-IR studies were dedicated to the examination of the structure-function relations in the photosynthetic membrane (see, e.g., He *et al.*, 1991; MacDonald and Barry, 1992; Berthomieu and Boussac, 1995; De Las Rivas and Barber, 1997). The spectral region which is most frequently used in infrared studies is the amide I from 1700 to 1600 cm^{-1}. This is because the amide I region is associated with an in-plane C=O stretching vibration (~80%) weakly coupled with some contributions from CN stretching and CCN deformation (Krimm and Bandekar, 1986). The amide I region is very sensitive to conformational changes in the proteins secondary structures (Surewicz and Mantsch, 1988), and, most interestingly for the present work, was shown recently to be particularly effective in the study of heat inactivation of proteins (Arrondo *et al.*, 1994).

From the above discussed considerations, one expects with reasonable confidence that the utilization of variable fluorescence in combination with FT-IR spectroscopy will provide new knowledge on the nature of the molecular alterations occurring in the PSII supramolecular complex during heat inactivation. In the work reported here, we applied concurrently the variable fluorescence and FT-IR spectroscopy methods. It is shown hereunder that this procedure has helped to shed new insight into the understanding of the thermal transition observed around 42 °C (see above). We shall see that the level of variable fluorescence in the PSII preparations used in the present study varies with the incubation temperature from 25 to 55 °C

according to a sigmoid curve with a midpoint temperature of thermal denaturation, i.e., 50% inactivation, at about 40 °C. What is more, this thermal transition coincides with the temperature that induces two significant infrared absorbance changes in the difference FT-IR spectra of heat-treated PSII samples, namely a maximum in the α-helix region and a minimum in the extended chain (β-strand) region.

Materials and Methods

Pea seedlings development

Pea seeds were first scarified in concentrated H_2SO_4 for 5 min and subsequently washed in running tap water for 15–20 h. Then, the seeds were germinated on sand in darkness inside a controlled environment cabinet kept at 293 K. Three to four day old seedlings were transferred to a liquid nutrition medium (Hoagland's solution) where they were grown under continuous white light (30 μE m^{-2} s^{-1}) for about 10 days.

Preparation of PSII particles

To obtain the PSII particles, type A chloroplasts were first extracted from pea seedlings leaves according to a modified method of Nakatani and Barber (1977). This was followed by the isolation of the thylakoid membranes and the PSII particles according to the following procedures. Fresh pea leaves were homogenized at 273 K in a medium containing 0.4 M sorbitol, 50 mM Tricine-NaOH (Tricine is N-Tris[hydroxymetyl]methylglycine) (pH 7.8) and 10 mM NaCl (buffer A). The homogenate was filtered through eight layers of cheese cloth and centrifuged at 300× *g* for 1 min. The pellet was discarded and the supernatant was centrifuged at 1000× *g* for 5 min at 277 K. The chloroplasts pellet was washed two times in 10 mM Tricine-NaOH (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer B). The pellet (thylakoid membranes) was suspended in 0.1 sorbitol, 20 mM MES-NaOH (MES is 2-[N-morpholino]ethanesulfonic acid) (pH 6.5), 10 $MgCl_2$ and 5 mM NaCl (buffer C). The chlorophyll (Chl) concentration was 2 mg ml^{-1} as determined by the method of Arnon (1949). Isolation of PSII particles was performed by a modified procedure of Berthold *et al.* (1981). The thylakoid suspension (2 mg Chl ml^{-1}) was mixed with 4% Triton X-100 in buffer C. The mixture was continuously stirred and was incubated in the dark for 20 min at 277 K. Thereupon, the mixture was diluted 10-fold and centrifuged at 750× *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 27000× *g* for 30 min at 277 K. The pellet (PSII particles) was suspended in buffer C and washed once in the same buffer. The PSII particles thus obtained were stored at 193 K in buffer C containing 30% glycerol.

Heat inactivation experiments

Heat inactivation studies were performed according to a procedure which is similar to methods described elsewhere (cf. Cramer *et al.*, 1981b; Thompson *et al.*, 1986). First, the PSII samples were washed in buffer C (see above) and resuspended in the same buffer at a concentration of 1 mg Chl ml^{-1}. Then, aliquots of a PSII sample were incubated in darkness for a period of 10 min at temperatures ranging from 25 to 55 °C. Finally, the PSII aliquots were kept in a ice bath in darkness till later use in infrared spectroscopy, or in variable fluorescence measurements.

Variable fluorescence measurements

Measurement of chlorophyll fluorescence in the PSII preparations was performed according to the procedure of Schreiber *et al.* (1986) using a PAM fluorometer (Walz GmbH, Effeltrich, Germany). A low frequency modulated beam (1.6 kHz) at very low light intensity was used to measure the minimal fluorescence (F_o). The saturating white light (10000 μE m^{-2} s^{-1}, 100 kHz) was used to determine the maximum fluorescence (F_m). The calculated variable fluorescence (F_v) is given by subtracting F_o from F_m, i.e., $F_v = F_m - F_o$.

Sample preparation for infrared studies and FT-IR measurements

The PSII samples (1.5 mg Chl ml^{-1}) were washed first in 1.2 ml of buffer C (see above), and then three times in D_2O and finally resuspended in D_2O. This preparation was precipitated and the pellet was layered on 25 mm diameter ZnSe plates. The infrared absorbance measurements were made in a Nicolet Fourier transform infrared (FT-

IR) spectrophotometer, model 420. In general, 100 interferograms were collected and co-added using the Omnic software facility of the Nicolet FT-IR instrument. The infrared spectra were obtained upon subtraction of the spectrum of the ZnSe plates used for deposition of the samples. The spectral resolution was between 1 and 2 cm^{-1}. Prior to data processing the FT-IR spectra were corrected for their content in D_2O by a standard subtraction procedure (Gabashvili *et al.*, 1998). The spectra were processed using the GRAMS/386 Spectra-Calc Program, version 1.06A, of Galactic Industries Corporation (Salem, NH).

Difference FT-IR spectra

It is important to note that before the calculation of the difference FT-IR spectra, the baselines of the original spectra were corrected at about 2000 cm^{-1} using the GRAMS/386 Spectra-Calc Program. In this respect, it is emphasized that in the great majority of the proteins spectra the region around 2000 cm^{-1} presents no significant infrared absorbance. To obtain the difference spectra, i.e., the [PSII heated above 25 °C]-minus-[PSII at 25 °C] spectra, we applied the methods developed by Heimburg and Marsh (1993) and Lee *et al.* (1990). In short, the area under the amide I band, i.e., the 1700–1600 cm^{-1} region, is first normalized according to the expression $A_n = kA_o$, where A_o is the integral of the observed surface under the band envelope calculated with the Spectra-Calc Program, k is a scale factor that normalizes A_o to 100, and A_n is the normalized area, i.e., 100. Then, the [PSII heated above 25 °C] – [PSII at 25 °C] subtraction of the spectra was performed (see also discussions in Gabashvili *et al.*, 1998).

Results and Discussion

Temperature effect on the variable fluorescence

Fig. 1A displays the effect of temperature (t) on the minimal (F_o) and maximum fluorescence (F_m) of PSII particles incubated at temperatures between 25 and 55 °C. The figure shows that initially F_o increases slowly from 25 to 35 °C, then undergoes a rapid increase with a thermal transition at about 40 °C, and attains finally a maximum at 55 °C. Fig. 1A indicates also that in the same temperature range, the F_m intensity undergoes a weaker but steady decrease with a minimum which coincides with the maximum observed in the F_o *vs.* t curve. The study of the variable fluorescence, F_v, is done in Fig. 1B. The figure represents the ratio $F_v(t)/F_v(25)$ as a function of t, where $F_v(t) = F_m(t) - F_o(t)$ is the variable fluorescence at the temperature t in °C, and $F_v(25) = F_m(25) - F_o(25)$ the variable fluorescence at 25 °C which is used as the control temperature. One sees that $F_v(t)/F_v(25)$ decreases with t according to a sigmoid curve which exhibits an inflexion point at about 40 °C as was observed also in the F_o *vs.* t curve displayed in Fig. 1A.

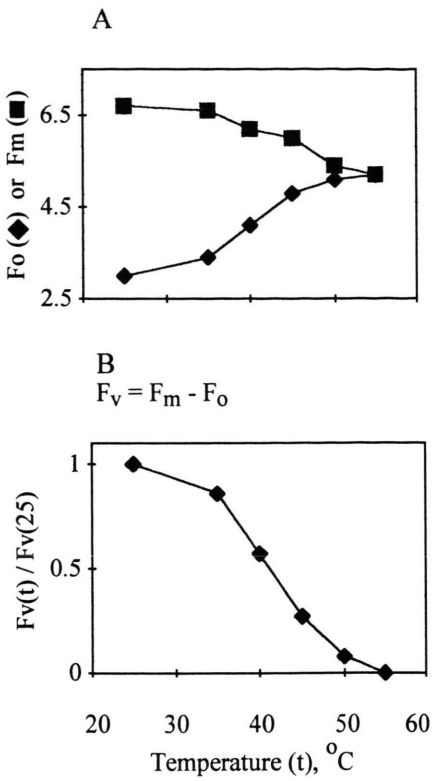

Fig. 1. Effect of temperature on (A) the minimal fluorescence, F_o, and the maximum fluorescence, F_m, and (B) the variable fluorescence, F_v (= F_m – F_o), in photosystem II particles incubated at temperatures (t) between 25 and 55 °C.

The $F_v(t)/F_v(25)$ ratio at the temperature of the inflexion point is 0.5, indicating that the number of open photochemical centers in response to heat stress are reduced by about 50%. Temperatures above the inflexion point temperature enhance the

thermal inactivation of the photochemical centers, or their complete denaturation at 50–55 °C. As a consequence, the photochemistry is brought to its lowest value and the trapping of excitation energy is progressively raised to his highest value which is visible in the maximum fluorescence yield observed at about 50–55 °C.

Temperature effect on the infrared absorbance

In spite of the fact that the infrared spectra of most proteins display usually an apparent lack of fine structure in the amide I region from 1700 to1600 cm^{-1} (cf. Fig. 2, curve A), recent developments in the FT-IR methodology made possible the decomposition of the infrared spectra into several regions which were successfully correlated with the various molecular conformations into which the proteins are folded (see, e.g., Krimm and Bandekar, 1986; Arrondo *et al.*, 1993). These studies have revealed five major spectral regions within the amide I envelope at:

(i) 1696–1665 cm^{-1}: turns (e.g., β-turns), antiparallel β-sheet (~1693 cm^{-1});
(ii) 1658–1654 cm^{-1}: α-helix;
(iii) 1648–1641 cm^{-1}: random structures, loops;
(iv) 1640–1620 cm^{-1}: β-sheet (~1636 cm^{-1}), β-strands (extended chains: ~1626 cm^{-1});
(v) 1620–1600 cm-1: aromatic side chains (e.g., tyrosine), chlorophyll molecules.

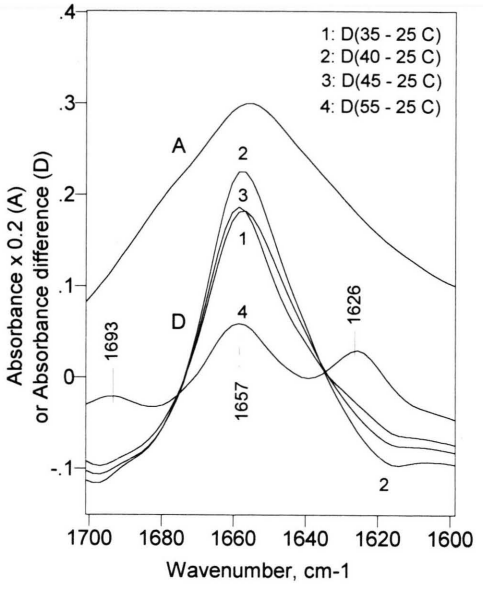

Fig. 2. Absorbance FT-IR spectrum (A) of PSII particles incubated at 25 °C, and difference FT-IR spectra (D) of PSII particles incubated at temperatures from 25 to 55 °C. The difference FT-IR spectra were obtained by subtracting the spectrum of the PSII particles incubated at 25 °C from the PSII spectra obtained at temperatures from 35 to 55 °C upon baseline correction (see Materials and Methods). The isosbestic points in the absorbance difference curves are at approximately 1675 and 1635 cm^{-1}. *Abbreviations:* FT-IR, Fourier transform infrared; PSII, photosystem II.

Fig. 2 displays the absorbance difference FT-IR spectra (D) of the PSII particles incubated at temperatures from 25 to 55 °C. We note the presence of three major regions exhibiting significant absorbance changes caused by heat treatment of the PSII particles. That is to say, from 1700 to 1675 cm^{-1}, from 1675 to 1635 cm^{-1} and from 1635 to 1600 cm^{-1}. These spectral regions are delimited by two isosbestic points observed at approximately 1675 and 1635 cm^{-1} which are situated at the boundary of, respectively, the β-turn to α-helix area and the β-sheet to β-strand area. From the above considerations (Krimm and Bandekar, 1986; Arrondo *et al.*, 1993) and the spectral analyses that we performed using second derivative and Fourier self-deconvolution methods (cf. De Las Rivas and Barber, 1997; Gabashvili *et al.*, 1998), we were able to identify the major PSII structures which are affected by temperature: (a) turns (e.g., β-turns) and the antiparallel β-sheet (~1693 cm^{-1}) from 1696 and 1665 cm^{-1}, (b) α-helix conformations around 1657 cm^{-1}, and (c) two different bands in the 1640–1620 cm^{-1} region which are assigned to β-structures.

First, the band around 1636 cm^{-1} in the difference FT-IR spectra (Fig. 2) arises from intramolecular carbonyl vibrations of β-sheets. Secondly, the spectral band at 1626 cm^{-1} is attributed to β-strands, i.e., extended chains, according to Arrondo *et al.* (1994) and De Las Rivas and Barber (1997). The assignment of the 1626 cm^{-1} band to protein chains in extended chain (β-strand) configurations is particularly interesting in the study of structure-function in macromolecules since these structures are directly related to a hydrogen-bonding network formed of hydrogen-bonding donors and acceptors in amino acid residues which do not participate in intramolecular β-sheets. These molecular patterns are for the most part involved in

hydrogen-bonding interactions with other molecular structures. Moreover, it is plausible to assume that the heat-induced effects observed in this work may as well result from thermal perturbations affecting the three-dimensional structures of the PSII proteins either globally or, most likely, in well-delimited regions in the supramolecular complex.

We remark finally that the absorbance difference FT-IR spectra in Fig. 2 show clearly that the temperature-induced absorbance changes recorded in the region around 1657 cm^{-1} are first an increase up to a temperature of the order of 40 °C, then is followed by an absorbance decrease at higher temperatures. In contrast with this observation, the spectral regions with maxima at 1693 cm^{-1} and 1626 cm^{-1} follow opposite trends. That is, an infrared absorbance decrease is observed up to 40 °C followed by an increase at higher temperatures. This is best seen in Fig. 3 which presents the variation with temperature of the infrared absorbance at 1657 cm^{-1}, i.e., in the α-helix region (Fig. 3A), and at 1626 cm^{-1}, i.e., in the β-strand region (Fig. 3B). Interesting enough, the curves in Figs. 3A and 3B display respectively a maximum and a minimum at about 40 °C which is exactly the same midpoint temperature of thermal inactivation in the minimal, F_o, and variable fluorescence, F_v, curves represented in Figs. 1A and 1B, i.e., F_o vs. t and $F_v(t)/F_v(25)$ vs. *t*.

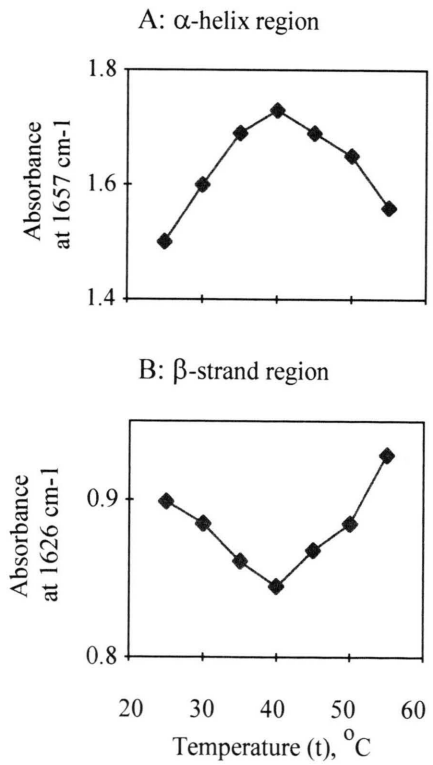

Fig. 3. Infrared absorbance in the α-helix region at 1657 cm^{-1} (A) and the extended chain (β-strand) region at 1626 cm^{-1} as a function of temperature in photosystem II particles incubated between 25 and 55 °C.

Temperature-dependent structure-function correlations in PSII

A major finding in this work is the similitude of the transition temperature at the inflexion points in Figs. 1A and 1B, and the maximum and the minimum observed in Figs. 3A and 3B. That is, the fluorescence transitions in the curves of Fig. 1 detected at approximately 40 °C, i.e., at F_o = 4.1 (Fig. 1A) and at $F_v(t)/F_v(25)$ = 0.5 (Fig. 1B), are seen at exactly the same transition temperature in the infrared absorbance *vs. t* curves of Fig. 3. This is a straightforward indication that the midpoint temperatures around 40 °C correspond to a main thermal transition which is most likely related to the inactivation, or denaturation, of the PSII units. This interpretation is clearly sustained by the $F_v(t)/F_v(25)$ *vs. t* curve which describes the inactivation of open photochemical centers with increasing temperature. Moreover, comparison of these results with data available in the literature (Table I) shows that the thermal transition around 40 °C is of general occurrence. Table I shows that most thermal transitions attributed to PSII are in the 39–46 °C range and are in general assigned to the denaturation of the oxygen-evolving complex and the electron transport chain. Our results reported in this paper support these conclusions.

Secondly, we showed that the variation in the α-helix content in the PSII complex is accompanied particularly by an opposite variation in the extended chains (β-strands) content but not in β-sheet and β-turn structures, except for the antiparallel-β-sheet conformations seen at 1693 cm^{-1} (cf. Fig. 2). This is clearly seen in the isosbestic points observed around respectively 1635 and 1675 cm^{-1} in the difference FT-IR spectra shown in Fig. 2. The presence of these two isosbestic points is a good indication that the PSII contents in β-turns and β-sheet conformations are not altered signifi-

Table I. Comparison of midpoint temperatures of thermal denaturation of function and structure observed between 39 and 46 °C in photosystem II.

Plant material	Parameter measured	Termal denaturation, °C		Refs.[e]
		TD_f[b]	TD_s	
PSII reaction center[a]	Infrared bandwidth (amide I; 1700–1600 cm^{-1})		42	1
PSII particles	Variable fluorescence	40[c]		*This work*
PSII particles	Infrared intensity (α-helix region; 1658–1654 cm^{-1})		40[d]	*This work*
PSII particles	Infrared intensity (β-strand region; ~ 1626 cm^{-1})		40[d]	*This work*
PSII particles	Oxygen evolution	39–42		2
PSII particles	DCIP reduction	40–46		2
Thylakoid membranes	Oxygen evolution	43		3, 4
Thylakoid membranes	Cyt b_{559} content	45		3, 4
Thylakoid membranes	Mn-ions release	45		3, 4

[a] *Abbreviations:* cyt b_{559}, cytochrome b_{559}; DCIP, 2,6-dichlorophenol indophenol; PSII, photosystem II; TD_f and TD_s, thermal denaturation of function and structure, respectively.
[b] Analysis of the midpoint temperatures of thermal denaturation of function (Td_f) disclosed that most of the thermal transitions attributed to PSII are in the 39–46 °C range. Moreover, the Td_f's were assigned to the denaturation, i.e., 50% inactivation, of the oxygen-evolving complex and the electron transport chain, and to the loss of Mn-ions. The transition involving cyt *b*559 corresponds to the tansformation of the high potential cyt b_{559} into its low potential form.
[c] See Fig. 1.
[d] See Fig. 3.
[e] *References:* 1, De Las Rivas and Barber (1997); 2, Thompson *et al.* (1986); 3, Cramer *et al.* (1981a); 4, Cramer *et al.* (1981b).

cantly with the increase of the incubation temperature. But, the isosbestic points in Fig. 2 indicate that one is most likely dealing with a phase transition phenomenon which, in the present case, materializes as a α-helix to extended chain (β-strand) transition. Most importantly, it is plausible that these changes in the secondary structures of the proteins constitute an efficient means of control of the PSII activity in the thylakoid membrane.

A corollary from the above discussed considerations is that at temperatures below the thermal transition at about 40 °C, the PSII proteins conformations capable of assuring the maximum number of open photochemical centers is intimately dependent on a dynamic equilibrium between the α-helix and the extended chain (β-strand). Above the thermal transition which induces an extreme loss of open photochemical centers, one observes the decrease in the α-helix content accompanied by the increase in the β-strands content. One may therefore expect spatio-temporal fluctuations in the PSII supramolecular complex caused by local, or more generalized, temperature changes. This may give rise to simple background noise effects in the PSII function, or to more important functional deviations.

As a final conclusion it is interesting to remark that the structural changes occurring in the course of protein folding and unfolding provide useful information about the intra- and intermolecular interactions between specific amino acid residues and/or protein domains that are essential to maintain the physiologically active macromolecular conformations (see, e.g., Mombelli *et al.*, 1997). Such information is fundamental for the comprehension of the molecular arrangements or local structure order that are involved directly or indirectly in biological catalysis. This has been the scope of a wide range of investigations on the function of the thylakoid membrane proteins. The present study is a novel step in this direction as it demonstrates that the stability of the open photochemical centers in photosystem II is dependent on a overall dynamic equilibrium between the PSII proteins contents in α-helix and extended chain (β-strand) conformations. The study of these structure-function relations is now pursued in specific protein components of the PSII complex with

the intent of identifying the thylakoid membrane structures (e.g., thylakoid lipids and/or proteins) that could intervene in the onset of the heat effect described above by opposing or facilitating it.

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