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Protein Secondary Structure of the Isolated Photosystem II Reaction Center and Conformational Changes Studied by Fourier Transform Infrared Spectroscopy†

Wei-Zhong He,‡ William R. Newell,‡,§ Parvez I. Haris,‖ Dennis Chapman,‖ and James Barber*,‡

AFRC Photosynthesis Research Group, The Wolfson Laboratories, Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AY, U.K., and Department of Protein and Molecular Biology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, U.K.

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ABSTRACT: The secondary structure of the photosystem II (PSII) reaction center isolated from pea chloroplasts has been characterized by Fourier transform infrared (FTIR) spectroscopy. Spectra were recorded in aqueous buffers containing H_2O or D_2O; the detergent present for most measurements was dodecyl maltoside. The broad amide I and amide II bands were analyzed by using second-derivative and deconvolution procedures. Absorption bands were assigned to the presence of α-helices, β-sheets, turns, or random structure. Quantitative analysis revealed that this complex contained a high proportion of α-helices (67%) and some antiparallel β-sheets (9%) and turns (11%). An irreversible decrease in the intensity of the band associated with the α-helices occurs upon exposure of the isolated PSII reaction center to bright illumination. This loss of α-helical content gave rise to an increase in other secondary structures, particularly β-sheets. After similar pretreatment with light, sodium dodecyl sulfate polyacrylamide gel electrophoresis reveals lower mobility and solubility of constituent D1 and D2 polypeptides of the PSII reaction center. Some degradation of these polypeptides also occurs. In contrast, there is no change in the mobility of the two subunits of cytochrome b_{559}. In the absence of illumination, the PSII reaction center exchanged into dodecyl maltoside shows good thermal stability as compared with samples in Triton X-100. Only at a temperature of about 60 °C do spectral changes take place that are indicative of denaturation.

Crystallization and X-ray analyses of reaction centers from the purple photosynthetic bacteria *Rhodopseudomonas viridis* (Deisenhofer et al., 1984, 1985) and *Rhodobacter sphaeroides* (Allen et al., 1987a,b) have given detailed information about the organization of protein subunits and pigment molecules at atomic resolution. The core of the bacterial reaction center is made up of the L/M heterodimer. Both the L and M subunits possess a high level of α-helical secondary structure due to five transmembrane helices and some nontransmembrane helical regions. A third subunit, H, contains a single transmembrane helix. The remaining residues of the H subunit exist as predominantly parallel or antiparallel β-sheets.

Because of the sequence homologies between the bacterial reaction center L/M subunits and the higher plant D1/D2 polypeptides, it was proposed that the latter also form a heterodimer and constitute the reaction center of photosystem II (PSII)[1] (Trebst, 1986; Michel & Deisenhofer, 1986). This notion was supported by the isolation of a complex consisting of the D1 and D2 polypeptides but free of other chloro-

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* To whom correspondence should be addressed.
‡ AFRC Photosynthesis Research Group.
§ Present address: Department of Medicine, The Rayne Institute, University College London, University Street, London WC1E 6JJ, U.K.
‖ Department of Protein and Molecular Biology.

[1] Abbreviations: FTIR, Fourier transform infrared; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; PSII, photosystem II; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane.

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phyll-binding proteins (Nanba & Satoh, 1987; Barber et al., 1987), which is designated as the PSII reaction center hereafter. This PSII reaction center binds a small number of pigment molecules and is capable of carrying out the primary photochemical reactions as assayed by a variety of techniques [for a review, see Barber (1988)]. In addition to the D1/D2 polypeptides the isolated PSII reaction center is also composed of the α and β subunits of cytochrome b_{559} and the product of the *psbI* gene (Ikeuchi & Inoue, 1988; Webber et al., 1989). Hydropathy plots coupled with close homologies to the bacterial system have led to the suggestion that the D1 and D2 polypeptides each have five transmembrane helices (Michel & Deisenhofer, 1986; Barber, 1987), while the other three polypeptides each only have one (Tae et al., 1988; Ikeuchi & Inoue, 1988; Webber et al., 1989). These assignments of helical structure at present lack conclusive experimental support.

A striking difference between the reaction centers of purple bacteria and PSII is the apparent sensitivity of the latter to photodamage. In the intact organism this photodamage gives rise to the physiological stress condition known as photoinhibition. Accumulated evidence indicates tht vulnerability of the reaction center to high light stress is linked to the rapid turnover of the D1 polypeptide (Kyle et al., 1984). No such turnover is observed with the L subunit of the purple bacterial reaction center. The turnover of D1 identifies this protein as being involved in the photodamage, and a breakdown product is observed as a 23.5-kDa fragment (Greenberg et al., 1987). It seems that photoinhibition occurs when the rate of synthesis of D1 does not match its rate of photodegradation (Ohad et al., 1985). The precise molecular details that underlie the photodestruction of the PSII reaction center in vivo are not known. Interestingly, the isolated PSII reaction center is also highly vulnerable to photodamage (Chapman et al., 1989). Whether this damage is identical with that which occurs in vivo has yet to be established, but it involves the selective bleaching of chlorophylls that constitute the primary donor P680 (Telfer et al., 1990) and therefore a loss of photochemical activity (Chapman et al., 1989). The removal of oxygen from the suspension medium protects against photodamage (McTavish et al., 1989; Crystall et al., 1989), and studies of the P680 triplet lifetimes implicate the production of singlet oxygen as the underlying cause (Durrant et al., 1990).

In this paper we have used Fourier transform infrared (FTIR) spectroscopy to characterize the secondary structure of the isolated PSII reaction center complex and investigate conformational changes that relate to its vulnerability to photodamage.

FTIR spectroscopy has been shown to be a useful tool to elucidate the secondary structure of proteins (Susi & Byler, 1986; Surewicz & Mantsch, 1988; Haris & Chapman, 1988; Jackson et al., 1989) and is particularly good at monitoring small conformation changes that can occur with proteins in aqueous systems (Haris et al., 1986, 1990; Alvarez et al., 1987; Surewicz & Mantsch, 1988; Jackson et al., 1989). Information is obtained from the infrared active amide vibrations associated with the peptide groups; the amide I absorption band is principally due to an in-plane C=O stretching vibration weakly coupled with C–N stretching and in-plane N–H bending, whereas the amide II band is associated with in-plane N–H bending strongly coupled with C–N stretching. The transition frequencies of these two amide bands are dependent on secondary conformation (Susi & Byler, 1986; Surewicz & Mantsch, 1988; Jackson et al., 1989). A protein or protein complex containing several types of secondary structures gives several amide I and amide II maxima, which cause band overlap therefore giving rise to broad bands. Fourier self-deconvolution (Kauppinen et al., 1981; Yang et al., 1985) and second-derivative calculations (Susi & Byler, 1983; Lee et al., 1985) can provide accurate assessments of the number and positions of such underlying component peaks, so that information of a qualitative nature can be obtained.

Quantitative estimations of secondary structure by using infrared spectra were first attempted by least-squares optimization (Fraser & Suzuki 1966; Ruegg et al., 1975), but this approach was limited by the requirement for extensive input information. Quantification of infrared absorption data has also been made from consideration of peak intensities or band areas in either derivative spectra (Dong et al., 1989) or deconvoluted spectra (Byler & Susi, 1986). However, since these parameters are heavily influenced by differences in the original bandwidths of the overlapping components, these approaches have not proved to be reliable and reproducible (Surewicz & Mantsch, 1988; Lee et al., 1990). Very recently, alternative approaches have been developed with factor analysis based on reference protein spectra (Lee et al., 1990, Dousseau & Pezolet, 1990). It is this latter approach that is adopted in this communication.

Materials and Methods

Isolation of the PSII Reaction Center. The PSII reaction center complex was isolated from pea thylakoids by a procedure detailed in Chapman et al. (1988). Detergent exchange into dodecyl maltoside was carried out during the second column to improve the stability of the preparation.

Manipulation of the Samples for FTIR Spectroscopy. Tris was removed from the PSII reaction center by dialysis against 10 mM Hepes buffer, pH 7.2, for 24 h at 4 °C in the dark. The sample was then concentrated by placing the dialysis tubing in solid sucrose to give a final concentration of 20–50 mg protein/mL. Samples used in D_2O measurements were originally isolated in H_2O buffer containing 2 mM dodecyl maltoside. They were diluted with 10 mM Hepes buffer in D_2O, where the pD values was adjusted to 6.8 with NaOD to yield a deuteron concentration equivalent to the proton concentration in H_2O at pH 7.2 (Lee et al., 1985), and centrifuged at 290000*g* for 40 min with a Beckman TL-100 bench-top ultracentrifuge. The pellets were resuspended, spun a second time, and finally resuspended to give an approximate protein concentration of 20 mg/mL. For H–D exchange, the sample was left for 24 h at room temperature in darkness. This treatment had no effect on the position of the red absorption maximum, which was at 675 nm and is a measure of the intactness of the isolated PSII reaction center complex (Seibert et al., 1988; Chapman et al., 1988, 1989).

Illumination Pretreatment of the PSII Reaction Center. For high-intensity light treatment, PSII reaction centers were diluted to 100 μg/mL chlorophyll in 10 mM Hepes–NaOH, pH 7.2, containing 2 mM dodecyl maltoside. White light (150 W) was guided onto the sample, which was kept at 20 °C. The light intensity was 2000 μE m^{-2} s^{-1}. A Uniblitz shutter was used to control the exposure time. Following this treatment, samples were concentrated by using the Millipore Ultrafree-MC filter unit, with a 10000 NMWL low-binding PLGC membrane, centrifuged at 6500 rpm on an MSE microcentrifuge.

Visual Absorption Spectroscopy. After every exposure to light, 200-μL samples were transferred to a 0.1-cm path length cell for absorption measurements (samples were returned to the bulk solution afterward). These measurements were carried out at 4 °C on an SLM-Aminco DW-2000 spectro-

photometer equipped with an IBM PS/2 model 30 personal computer for data acquisition and analysis.

FTIR Spectroscopy. Infrared spectra were recorded on a Perkin-Elmer 1750 Fourier transform infrared spectrometer equipped with a TGS detector. A Perkin-Elmer Model 7300 data station was used for data acquisition, storage, and analysis. Samples were placed in a thermostated Beckman FH-01 CFT microcell fitted with CaF_2 windows and a 6-μm tin spacer for measurements in H_2O and a 50-μm teflon spacer for measurements in D_2O buffer. All spectra were recorded at 4 °C unless specified. Protein samples and buffer controls were measured with identical scanning parameters after equilibration for 15 min. Results are presented as absorption difference spectra (Haris et al., 1986). The sample compartment was continuously purged with nitrogen or dry air to eliminate absorption of water vapor in the spectral region of interest. Spectra were recorded in H_2O buffers (400 scans) or D_2O buffers (200 scans) and were signal averaged at a resolution of 4 cm^{-1}. Other details of methods used for spectral subtraction and for obtaining second-derivative and deconvoluted spectra are described in Haris et al. (1986). Quantitative analysis of the secondary structures were carried out by using the program CIRCOM supplied by Perkin-Elmer, as described in Lee et al. (1990).

SDS-PAGE. After exposure to a preillumination, 20-μL samples were removed from the bulk solution and incubated in sodium dodecyl sulphate (SDS) solubilization buffer for 5 min at room temperature. After being resolved by electrophoresis on a 7–17% polyacrylamide gel (Laemmli, 1970), protein bands were visualized by staining with Coomassie blue.

Results and Discussion

Infrared Spectrum of PSII Reaction Centers in Buffer Made Up in H_2O. The infrared absorption spectrum of the isolated PSII reaction center obtained in H_2O buffer is shown in Figure 1A. The maxima of the amide I and amide II bands are at 1657 and 1548 cm^{-1}, respectively. The frequency of amide I band indicates that this complex is enriched in α-helical secondary structure (Byler & Susi 1986). Compared with the infrared spectrum of the reaction center complex isolated from the purple photosynthetic bacterium, *R. sphaeroides* (Nabedryk et al., 1982, 1988), the amide I band of the PSII reaction center appeared to be narrower in bandwidth and more symmetric. This is indicative of a higher content of α-helices in the PSII reaction center than in the bacterial counterpart. Calculation of the second-derivative spectrum and Fourier self deconvolution were used to reveal the overlapping components in the amide I region (Figure 1, B and C, respectively). Apart from the major α-helix component at 1657 cm^{-1}, several additional components are visualized in the amide I region. The bands at 1628 and 1638 cm^{-1} are consistent with the absorbance of β-sheet structures. The presence of 1693-cm^{-1} component indicates that some β-sheets are antiparallel. The band at 1678 cm^{-1} is tentatively assigned to turns. Bands at 1742 and 1725 cm^{-1} are too high in frequency to be assigned to any protein substructures. Presumably they are associated with C=O stretching bands from contiminating detergent and/or pigments bound in this complex (Nabedryk et al., 1982). It should be pointed out that any components that may be associated with unordered structures will overlap with the α-helical bands. In order to distinguish these two contributions, a spectrum of the PSII reaction centers exchanged into D_2O buffer was also recorded.

Infrared Spectrum of PSII Reaction Centers in Buffer Made Up in D_2O. The infrared spectrum of the PSII reaction centers exchanged into D_2O buffer is shown in Figure 2A. As

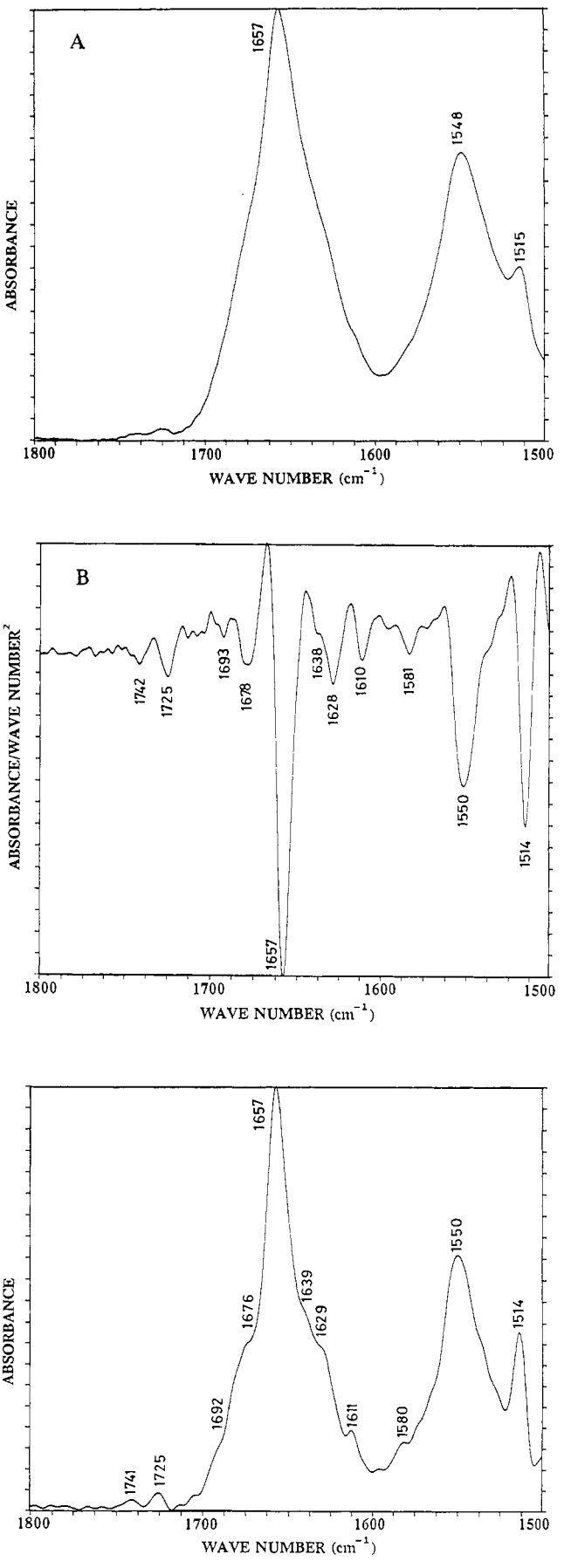

FIGURE 1: Infrared spectra in the amide I and amide II region of the isolated PSII reaction center exchanged into dodecyl maltoside (2 mM) in H_2O buffer containing 10 mM Hepes, pH 7.2. (A) Original spectrum, (B) second-derivative spectrum, and (C) deconvoluted spectrum.

Table I: Structure Contents of Photosynthetic Reaction Center Complex

RC complexes	% α-helix	% β-sheet	% turns	fig/ref
PSII RC by FTIR	67.2 ± 4.8	9.3 ± 5.3	11.1 ● 4.7	1A
R. sphaeroides (L/M/H)	51	18	nd[a]	Allen et al. (1987b)
R. sphaeroides (L/M)	66	14	nd[a]	Allen et al. (1987b)
R. viridis (L/M/H)	50	9	nd[a]	Deisenhofer et al. (1985)
R. viridis (L/M)	64	4	nd[a]	Deisenhofer et al. (1985)

[a] Turns have not be reported in their papers.

can be seen, there is a dramatic reduction in the intensity of the amide II band at 1548 cm^{-1}, which shifts to a lower frequency. These changes are attributed to the isotopic substitution of the exchangeable N–H protons of the polypeptide by deuterons (Haris et al., 1986). There is also a relatively minor shift of the amide I band from 1657 to 1654 cm^{-1} due to H–D exchange. The second-derivative spectrum of the data is shown in Figure 2B. The presence of the band at 1657 cm^{-1} (due to α-helices) and the bands at 1628 and 1838 cm^{-1} (due to β-sheets) are consistent with previous assignments made from the data in Figure 1. The isotopic substitution of H to D exerts two opposite effects on the frequency of amide vibrational transitions that are attributed to the decoupling of the amide I and amide II modes in a single polypeptide group upon deuteration and to differences in coupling between groups linked across N—H--O=C and N—D--O=C bonds [see Cantor and Timasheff (1982)]. Consequently, the frequencies of the principle modes of the amide I band in the α-helices and antiparallel β-sheets are not affected by deuteration. In contrast, the high-frequency component of β-sheets, at 1693 cm^{-1} in H_2O, shifts toward a lower frequency and overlaps with the 1678-cm^{-1} component in D_2O to give a shoulder as seen in Figure 2B. Also, bands at 1742 and 1725 cm^{-1} in H_2O, associated with C=O stretching bands from contaminating detergent and/or pigments, shifted to 1739 and 1723 cm^{-1}, respectively, when exchanged into D_2O.

Quantitative Analysis and Comparison with Bacterial Reaction Centers. With the recently developed quantification method available (Lee et al., 1990), the above assignments can be verified, since this method is based on factor analyses but independent of band assignments. The results analyzed with CIRCUM using the amide I band (1700–1600 cm^{-1}) are listed in Table I. It seems that the results are consistent with the suggestion above that the isolated PSII reaction center contains a high proportion of α-helices relative to antiparallel β-sheets and turns. The structural data of reaction centers from purple photosynthetic bacteria determined by X-ray diffraction analyses (Deisenhofer et al., 1985; Allen et al., 1987b) are also listed. The α-helix content of the PSII reaction center is relatively higher than that of the bacterial reaction center. Such a difference can also be seen by comparing our IR spectrum with that of *R. sphaeroides* reported by Nabedryk et al. (1982). The amide I band of *R. sphaeroides* is rather asymmetric, and the interpretation of them having some β-sheets proved to be correct (Allen et al., 1987). Apparently, this is due to the presence of the H subunit, which has one large globular head protruding into the cytoplasm. Instead, the PSII reaction center contains three small-size polypeptides (α and β subunits of cyt b_{559} and *psbI* gene product). These three polypeptides each have one transmembrane α-helix, as predicted from their hydropathy plots. Therefore our conclusion that the PSII reaction center has a high percentage of α-helical structures is fully consistent with these predictions, bearing in mind that both D1 and D2 are likely to have five transmembrane α-helical domains (Michel & Deisenhofer, 1986; Barber, 1987).

Effect of Triton X-100. The PSII reaction center is isolated

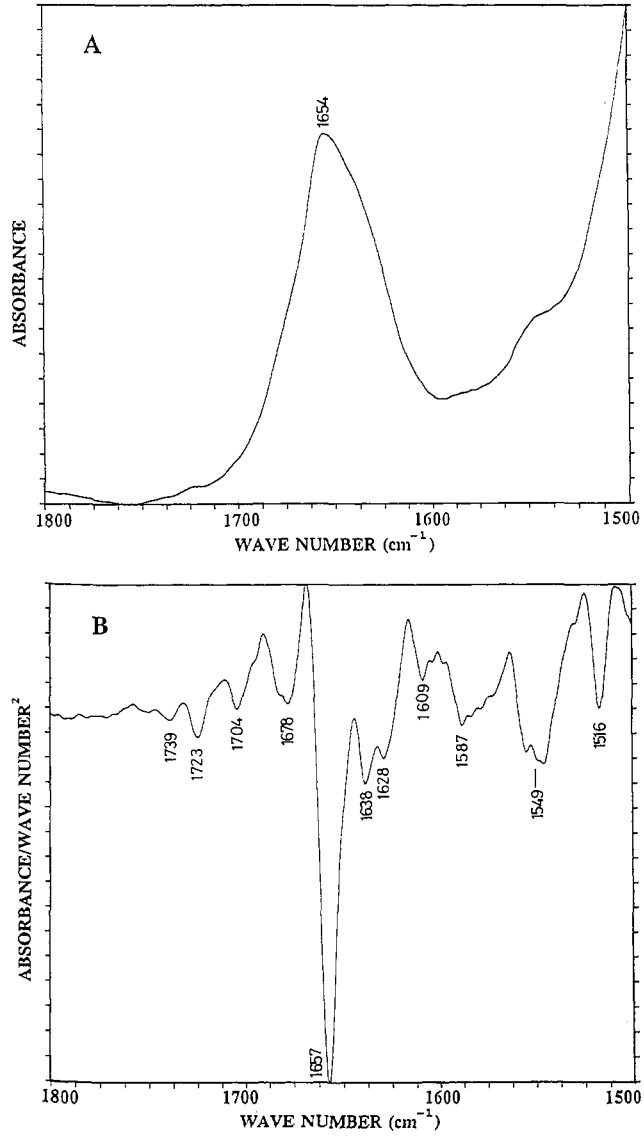

FIGURE 2: Infrared spectra in the amide I and amide II region of the isolated PSII reaction center in D_2O buffer containing 2 mM dodecyl maltoside and 10 mM Hepes, pD 6.8 (see Materials and Methods). (A) Original absorption spectrum and (B) second-derivative spectrum.

by an initial solubilization with Triton X-100. If the reaction center is left in this detergent, even under dark conditions, it readily degrades at room temperature as observed by a blue shift in the red absorption maximum and a loss of photochemical activity (Chapman et al., 1988; Seibert et al., 1988; Braun et al., 1990). Interestingly, we observed the infrared spectral changes, as shown in Figure 3A, occurring with a PSII reaction center preparation in the presence of Triton X-100. The changes that occurred over a 30-min period were mainly in the amide I region. The magnitude of the shoulder at 1627 cm^{-1} increased relative to the peak at 1656 cm^{-1}. One interpretation of this is that the PSII reaction center undergoes a conformational transition involving a conversion of some of its α-helices into other secondary structures, which is also

Table II: Structural Contents of the PSII Reaction Centers

PS II RC samples		% α-helix	% β-sheet	% turns	% random	fig
samples in Triton X-100	control	62.5 ± 4.8	15.7 ± 5.3	17.0 ± 4.3	9.5 ± 2.9	3A (a)
	15 min	31.0 ± 4.9	38.4 ± 5.3	24.2 ± 6.5	12.5 ± 3.0	3A (b)
	30 min	20.6 ± 5.1	44.7 ± 5.3	22.2 ± 7.1	14.4 ± 3.1	3A (c)
samples in dodecyl maltoside	control	67.2 ± 4.8	9.3 ± 5.3	11.1 ± 4.7	11.5 ± 2.8	1A and 4A (a)
	1 min light	64.4 ± 4.8	11.2 ± 5.3	18.7 ± 4.8	11.8 ± 2.8	not shown
	2 min light	61.6 ± 4.8	12.9 ± 5.3	18.5 ± 4.8	12.3 ± 2.8	not shown
	30 min light	23.8 ± 4.8	41.4 ± 5.1	24.4 ± 6.0	14.7 ± 3.0	4A (b)

suggested by the results of the quantitative analysis tabulated in Table II. The second-derivative and deconvoluted spectra of the 30-min sample (corresponding to spectrum c in Figure 3A) are shown in Figure 3, B and C, respectively. By comparing these two spectra with those in Figure 1, B and C, respectively, bands at 1628 and 1638 cm^{-1} are replaced by a single strong band at 1626 cm^{-1} after a 30-min incubation. Such protein conformational changes may well, in part, account for the spectral changes of pigments observed in the UV–Vis region and for photochemical inactivation (Seibert et al., 1988; Chapman et al., 1989).

Photomodification of the PSII Reaction Center. When the PSII reaction center is exchanged into dodecyl maltoside as described under Materials and Methods, it is significantly stabilized such that the treatment given above does not bring about any change in its visible and infrared absorption spectra, and the preparation maintains full photochemical activity. However, if subjected to strong illumination (2000 $\mu E\ m^{-2}\ s^{-1}$) at room temperature and in the presence of atmospheric oxygen, there is also a blue shift in the red absorption maximum due to a selective irreversible bleaching of a long-wavelength chlorophyll absorption (Telfer et al., 1990). As a result of this, there is a loss of photochemical activity (Chapman et al., 1989; Crystall et al., 1989; McTavish et al., 1989). Figure 4A shows the infrared absorption spectrum before and after exposure to bright white light (2000 $\mu E\ m^{-2}\ s1^{-1}$) at 20 °C for 30 min. The spectral changes are remarkably similar to those observed with the effect of Triton X-100. This similarity is emphasized by comparison of the second-derivative spectra (Figure 4B with 3B) and deconvoluted spectra (Figure 4C with 3C). Therefore it seems that both treatments give rise to a similar conformational change in which the amount of α-helices relative to β-sheets decreases. This relative change could be a conversion of α-helices into other secondary structures or simply a preferential loss of α-helices by a degradation process. To investigate these possibilities further, samples preilluminated with 2000 $\mu E\ m^{-2}\ s^{-1}$ at 20 °C were subjected to SDS–PAGE.

SDS–PAGE Analysis. As seen in Figure 5, the effect of the preillumination treatment was to slightly decrease the electrophoretic mobility of both the D1 and D2 polypeptides without affecting the mobility of the α subunit of cytochrome b_{559}. Also of note is the apparent decrease in the intensities of the D1 and D2 bands and a compensation of this loss by an increase in the intensity of the high molecular weight diffuse band (60–70 kDa) together with the appearance of a new band at about 40 kDa. Like the higher molecular weight diffuse band, the new band was immunologically reactive to an antibody raised to the D1 polypeptide. It was, however, immunologically silent to an antibody raised to the D2 polypeptide (Shipton & Barber, unpublished). We therefore concluded that the pretreatment with white light brings about both a conformational change and a partial degradation. Apparently, according to the FTIR data, these changes involve a shift in favor of other secondary structures (particularly β-sheet) relative to α-helices.

In anaerobic conditions the reaction center is far more stable to prolonged illumination with bright light (McTavish et al., 1989; Telfer et al., 1990). Flash absorption and emission studies (Crystall et al., 1989; Durrant et al., 1990) indicate that when O_2 is present, singlet oxygen is formed via photosensitization by the triplet state of P680. It seems that this very reactive free radical can rapidly attack the chromophore and protein components of the reaction center. Indeed, recent observations indicate that damage to the D1 and D2 polypeptides due to strong illumination is avoided if the treatment is carried out under anaerobic conditions (C. A. Shipton and J. Barber, unpublished). The preferential photodamage of the D1 and D2 polypeptides compared with cytochrome b_{559} is consistent with these two polypeptides binding the reaction center pigments.

Effect of Heat on the PSII Reaction Center. Exchange from Triton X-100 to dodecyl maltoside significantly increases the thermal stability of the isolated reaction center as long as it is kept in the dark (Seibert et al., 1988; Chapman et al., 1988). Making use of this property, we investigated the occurrence of conformational changes that may occur when increasing the temperature above ambient. FTIR spectra were recorded for PSII reaction center preparations in D_2O buffer measured at 20, 40, and 60 °C (see Figure 6). No major changes were detected at 20 and 40 °C compared with spectra recorded at 4 °C (see Figure 2). However, at 60 °C there were significant changes. As can be seen by comparing Figure 6 with Figure 2B, there is an appearance of peaks in the second-derivative spectrum at 1624 and 1686 cm^{-1} that are indicative of the formation of intermolecular β-sheets typically found when proteins undergo thermal denaturation (Jackson et al., unpublished). Therefore both photo- and thermoinduced damage of the isolated PSII reaction center seems to bring about an overall increase in β-sheet conformation.

Conclusions

FTIR spectroscopy has revealed that the isolated PSII reaction center contains a high proportion of α-helices (67%), some β-sheets (9%), and some turns (11%). Compared with the secondary structures of bacterial reaction centers determined by X-ray diffraction analyses (Deisenhofer et al., 1985; Allen et al., 1987b), the α-helix content is higher while the β-sheet content is lower in the PSII reaction center than that of purple bacteria. This difference may well be attributed to the fact that the H subunit possesses a large globular portion intruding into the cytoplasm. In fact, when only L/M heterodimer subunits are considered, the α-helix content is 66% in *R. sphaeroides* and 64% in *R. viridis*, which is comparable to the estimations for the isolated PSII reaction center.

Effects of Triton X-100, light, and heat on the conformation of the PSII reaction center were investigated. Incubation with 0.2% Triton X-100 at room temperature caused a considerable change in the conformation of the PSII reaction center resulting in a decrease of its α-helical content. Such a conformational change probably partly accounts for the spectral

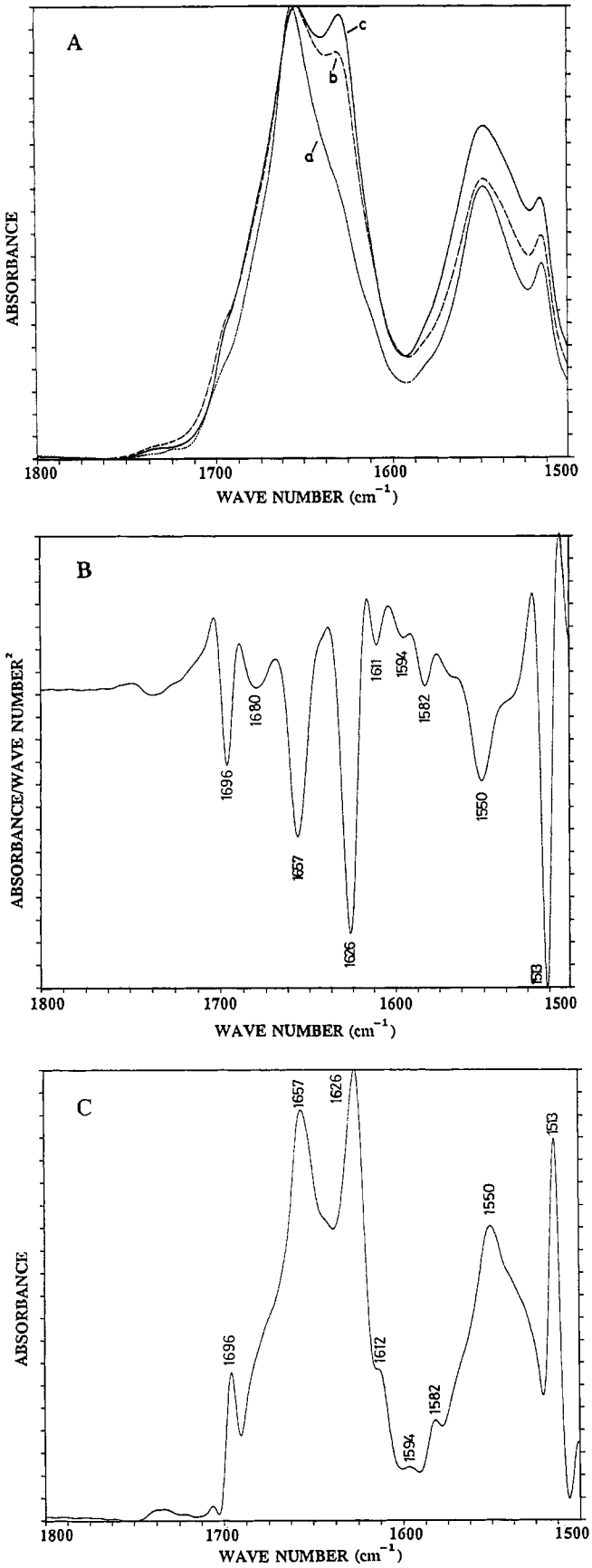

FIGURE 3: (A) Infrared spectra of PSII reaction centers, isolated in Triton X-100 (in H_2O buffer), (a) before and (b) after 15 min and (c) 30 min incubation at room temperature in the dark; (B) second-derivative of spectrum c; and (C) deconvoluted spectrum of c.

changes occurring in the UV–Vis region as previously reported. This effect of Triton X-100 is overcome by exchange into dodecyl maltoside. However, even then the action of strong

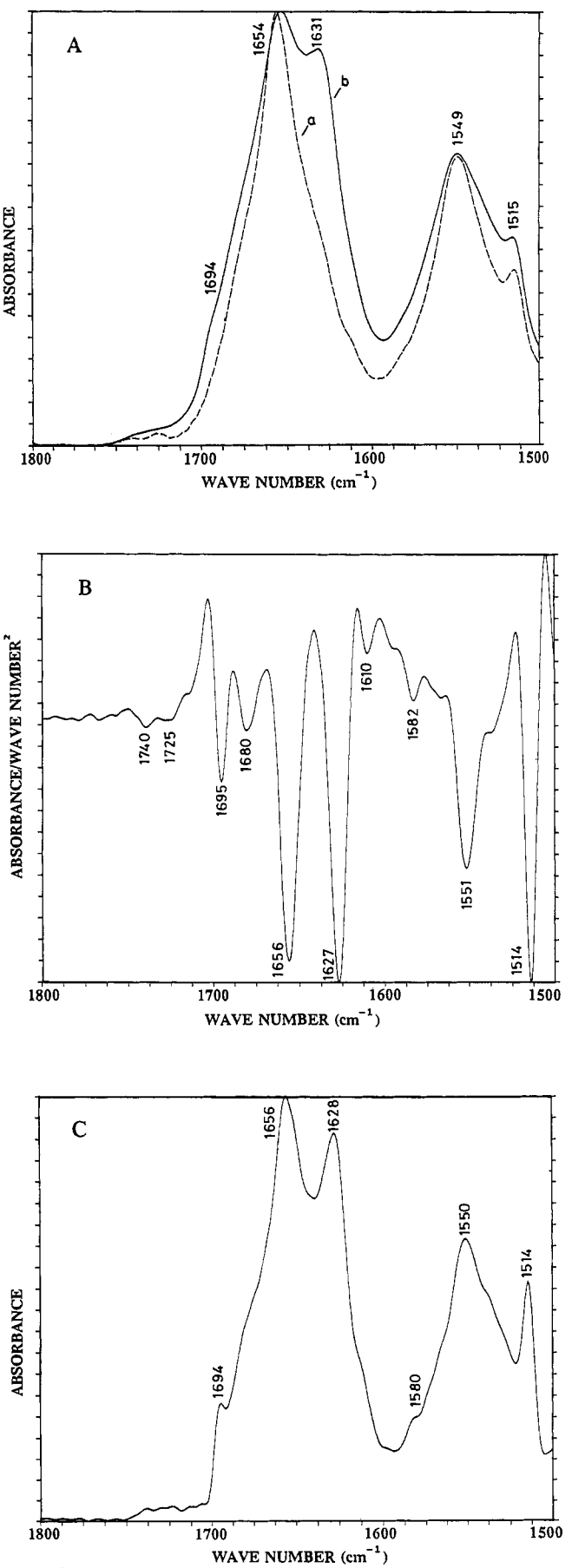

FIGURE 4: (A) Infrared spectra of PSII reaction centers exchanged into 2 mM dodecyl maltoside (in H_2O buffer) (a) before and (b) after 30 min exposure to white light (2000 μE m^{-2} s^{-1}) at 20 °C, (B) second-derivative of spectrum b, and (C) deconvoluted spectrum of b.

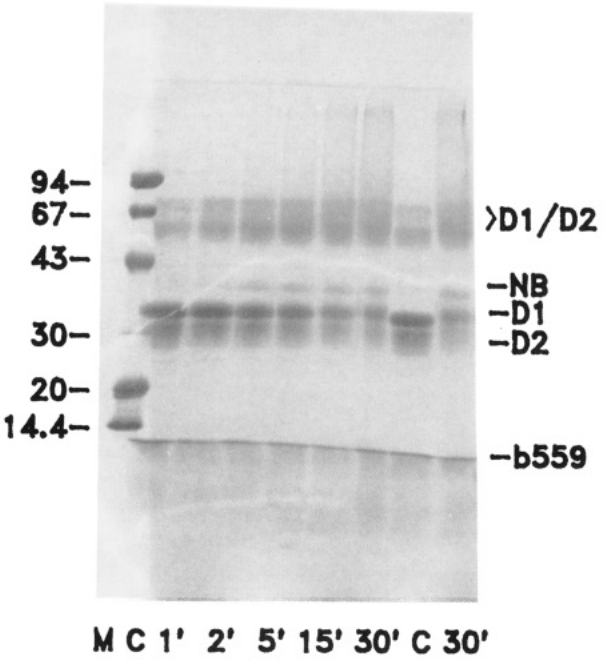

FIGURE 5: SDS–PAGE and PSII reaction center complexes exchanged into 2 mM dodecyl maltoside exposed to various periods of preillumination with white light (2000 μE m^{-2} s^{-1}) at 20 °C. Lane M is the molecular weight markers as indicated on the left in kDa. Lane C is the control. NB stands for a new band that appeared after photodamage.

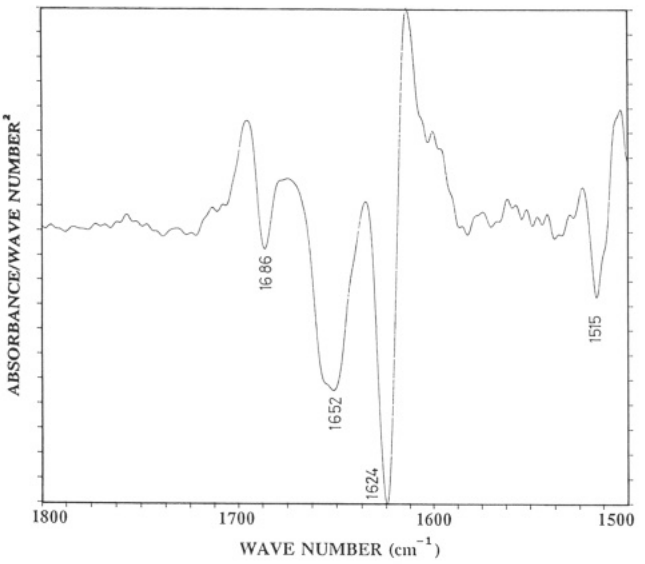

FIGURE 6: Second-derivative spectrum of PSII reaction centers in D_2O buffer recorded at 60 °C.

light and heating is to cause conformational changes that are similar to those induced by Triton X-100 in that there is a reduction in the content of α-helices.

The question arises whether the sensitivity of the structure of the isolated complex to these various treatments has any relevance to the phenomenon of photoinhibition and the related instability of the PSII reaction center in the intact organism. Using thermoluminescence as a technique to monitor the damage of PSII reaction centers during photoinhibition, Ohad et al. (1990) have recently suggested that a protein conformational change is involved and that it occurs in two steps: a reversible conformational change followed by an irreversible modification of the D1 protein. Clearly a more detailed examination of the light-induced FTIR spectral changes induced in the isolated PSII reaction center could be useful in relation to their hypothesis.

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Laser Flash Photolysis Study of Intermolecular and Intramolecular Electron Transfer in Trimethylamine Dehydrogenase†

James T. Hazzard,‡ William S. McIntire,§ and Gordon Tollin*,‡

Department of Biochemistry, University of Arizona, Tucson, Arizona 85721, Molecular Biology Division, Veterans Administration Medical Center, San Francisco, California 94121, and Department of Biochemistry and Biophysics and Department of Anesthesia, University of California, San Francisco, California 94143

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ABSTRACT: Laser flash photolysis has been used to investigate the kinetics of reduction of trimethylamine dehydrogenase by substoichiometric amounts of 5-deazariboflavin semiquinone, and the subsequent intramolecular electron transfer from the FMN cofactor to the Fe_4S_4 center. The initial reduction event followed second-order kinetics ($k = 1.0 \times 10^8$ M^{-1} s^{-1} at pH 7.0 and 6.4×10^7 M^{-1} s^{-1} at pH 8.5) and resulted in the formation of the neutral FMN semiquinone and the reduced iron–sulfur cluster (in a ratio of approximately 1:3). Following this, a slower, protein concentration independent (and thus intramolecular) electron transfer was observed corresponding to FMN semiquinone oxidation and iron–sulfur cluster reduction ($k = 62$ s^{-1} at pH 7.0 and 30 s^{-1} at pH 8.5). The addition of the inhibitor tetramethylammonium chloride to the reaction mixture had no effect on these kinetic properties, suggesting that this compound exerts its effect on the reduced form of the enzyme. Treatment of the enzyme with phenylhydrazine, which introduces a phenyl group at the 4a-position of the FMN cofactor, decreased both the rate constant for reduction of the protein and the extent of FMN semiquinone production, while increasing the amount of iron–sulfur center reduction, consistent with the results obtained with the native enzyme. Experiments in which the kinetics of reduction of the enzyme were determined during various stages of partial reduction were also consistent with these results, and further indicated that the FMN semiquinone form of the enzyme is more reactive toward the deazariboflavin reductant than is the oxidized FMN. The results of these experiments have been evaluated in terms of the X-ray structure of the enzyme and have been compared with previous results obtained with other multi-redox-center enzymes.

Trimethylamine dehydrogenase (TMAD)[1] is an iron–sulfur flavoprotein from a methylotrophic bacterium (W3A1) which catalyzes the conversion of trimethylamine to dimethylamine and formaldehyde, using as an acceptor an FAD-containing electron-transfer protein (Steenkamp & Mallinson, 1976; Steenkamp & Gallup, 1978). It is a tightly associated dimer

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* To whom correspondence should be addressed.
‡ University of Arizona.
§ Veterans Administration Medical Center and University of California, San Francisco.

[1] Abbreviations: dRf and dRfH•, oxidized and neutral semiquinone species of 5-deazariboflavin, respectively; FADH•, neutral semiquinone species of flavin adenine dinucleotide; FMN and FMNH•, oxidized and neutral semiquinone forms of flavin mononucleotide, respectively; Fe_4S_4, 4Fe–4S cluster; TMAC, tetramethylammonium chloride; TMAD, trimethylamine dehydrogenase.

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