A quantitative secondary structure analysis of the 33 kDa extrinsic polypeptide of photosystem II by FTIR spectroscopy

A. Ahmed, H.A. Tajmir-Riahi, R. Carpentier*

Centre de recherche en photobiophysique, Université du Québec a Trois-Rivières, C.P. 500, Trois-Rivières, Québec, G9A 5H7, Canada

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Abstract In chloroplast photosystem II, the extrinsic polypeptide of 33 kDa is involved in the stabilization the Mn cluster in charge of water splitting and in the fulfillment of the Ca^{2+}-cofactor requirement for oxygen evolution. The conformational analysis of the purified 33 kDa extrinsic polypeptide was carried out using FTIR spectroscopy with its self-deconvolution and second derivative resolution enhancement as well as curve-fitting procedures. The FTIR spectroscopic results showed that the isolated polypeptide is characterized by a major proportion β-sheet conformation (36%) with 27% α-helix, 24% turn, and 13% β-antiparallel structures.

Key words: 33 kDa extrinsic; Protein; Conformation; PSII reaction center; FTIR spectroscopy

1. Introduction

Chloroplast photosystem II (PSII) constitutes a light-dependent water-plastoquinone oxidoreductase responsible for oxygen evolution [1]. The oxygen evolving process implicates a cluster of 4 Mn atoms and three extrinsic polypeptides of 16, 24, and 33 kDa, respectively, which are involved in a Cl^- and Ca^{2+} cofactor requirement [2]. Based on a correlation between the release of Mn^{2+} and the depletion of the 33 kDa extrinsic polypeptide from PSII particles, the latter protein has been considered as the main catalytic manganoprotein of the water oxidation system [3]. Further, a 33 kDa polypeptide containing two Mn atoms was also isolated [4]. However, more refined biochemical studies demonstrated that under certain conditions, the Mn cluster was retained with the membrane complexes even after complete removal of the 33 kDa polypeptide [5–7]. Subsequent spectroscopic measurements indicated that the structure of the Mn cluster was not significantly affected by the removal of the three extrinsic subunits [8,9]. The extrinsic 33 kDa polypeptide is more likely involved in the stabilization of two of the four Mn atoms in the cluster and in the modulation of Ca^{2+} cofactor requirement for oxygen evolution [10,11].

The exact conformational structure of this important polypeptide is unknown. An analysis of Chou and Fasman in 1978, based on the amino acid sequence of the polypeptide, indicated the protein to be composed of 28% α-helical, 25% β-sheet, and 12% turn structures, with other components representing 35% [12]. A recent structural analysis by Xu et al. [13] based on circular dichroism (CD) spectroscopy, exhibited a major β-sheet component of 38% with only 9% α-helical and 17% turn structures together with other conformers representing 35%. The above discrepancies between these two studies prompted us to analyze the secondary structure of the 33 kDa extrinsic polypeptide by FTIR spectroscopy. This technique can be advantageously used for the structural determination of isolated polypeptides due to the great sensitivity of the amide I band located between 1,700–1,600 cm^{-1} to protein conformation.

In this communication, the structural analysis of the 33 kDa protein is described, using FTIR spectroscopy with self-deconvolution and second derivative resolution enhancement method as well as curve-fitting procedures that has not been previously reported. It is shown that both α-helical and β-strand structures are present in a relatively large proportion (27% and 36%, respectively), which significantly differs from the previous conclusions of Chou and Fasman [12] and Xu et al. [13].

2. Materials and methods

2.1. Isolation and purification of the 33 kDa extrinsic polypeptide

PSII submembrane fractions freshly prepared using Triton X-100 according to the method of Berthold et al. [14] with some modifications [15], were washed three times in a buffer containing 10 mM NaCl, 300 mM sucrose, and 25 mM MES-NaOH (pH 6.5) by centrifugation at 36,000 × *g* at 4°C for 10 min. To remove the extrinsic 16 and 24 kDa polypeptides, PSII membranes were incubated in 1 M NaCl, 10 mM $MgCl_2$, 300 mM sucrose, and 50 mM MES-NaOH (pH 6.0) at a Chl concentration of 1 mg/ml for 1 h on ice and in the dark. The suspension was then centrifuged at 36,000 × *g* for 30 min. The pellet was resuspended in the same buffer and centrifuged at 36,000 × *g* for 20 min to remove any remaining loosely bound 16 and 24 kDa proteins.

The 33 kDa protein was isolated using a procedure similar to that reported previously [16] by incubating the NaCl-treated PSII preparations (1 mg Chl/ml) in 1.0 M $CaCl_2$, 15 mM NaCl, 10 mM $MgCl_2$, 300 mM sucrose, and 50 mM MES-NaOH (pH 6.0) for 30 min on ice and in the dark. The suspension was centrifuged at 36,000 × *g* for 30 min. The supernatant which contained the 33 kDa polypeptide was concentrated using an ultrafiltration device (Amicon, PM10 Diaflo membrane). The solution was then dialyzed against several changes of 5 mM MES-NaOH, pH 6.0, and a pale green precipitate was removed by centrifugation.

Protein concentration was determined using the Bio-Rad assay. Protein composition was analyzed by SDS-polyacrylamide gel electrophoresis using the buffer system of Laemmli [17] in miniature slab gels (Hoefer Scientific Instrument) containing 15% acrylamide. The gels were stained with Coomassie brilliant blue R-250 and scanned with an LKB Ultro Scan XL laser densitometer. The relative abundance of proteins was estimated from the peak area of the densitogram and the isolated extrinsic polypeptide of 33 kDa was pure at more than 97%. The resultant preparation is in fact characterized by the occurrence of a single band on SDS-polyacrilamide gels (Fig. 1).

2.2. Secondary structure determination

Infrared spectra were recorded on a Bomem DA3-0.02 Fourier Transform infrared spectrometer equipped with a nitrogen cooled HgCdTe detector and a KBr beam splitter. The spectra of thin films

*Corresponding author. Fax: (1) (819) 376 5057.

Abbreviations: PSII, photosystem II; SDS, sodium dodecyl sulfate; CD, circular dichroism; FTIR, Fourier transform infrared.

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(50 μl samples, 385 μg protein/ml) deposited on BaF_2 windows were recorded with a resolution of 2 to 1 cm^{-1} and 100 to 500 scans.

The protein secondary structure was analyzed from the shape of the amide I band [18]. Fourier self-deconvolution and second derivative resolution enhancement as well as curve-fitting procedures were applied, so as to increase the spectral resolution in the region of 1,700–1,600 cm^{-1}. The self-deconvolution was performed by using a Lorenzian line shape for the deconvolution and a Gaussian line shape for apodization [19]. In order to quantify the area of the different components of the amide I band revealed by the self-deconvolution, a least-square iterative curve-fitting was used to fit the Lorenzian line shapes to the spectrum between 1,700–1,600 cm^{-1}. Before curve-fitting was done, a straight baseline passing through the ordinates at 1,700 and 1,600 cm^{-1} was subtracted. The baseline was modified by the least-square curve-fitting, which allowed the horizontal baseline to be adjusted as an additional parameter to obtain the best fit. It is known that no meaningful curve-fitting can be performed by simple examination of the original infrared spectra, that is why the self-deconvolution procedure has to be carried out first. The resolution enhancement that results from the self-deconvolution is such that the number and the position of the bands to be fitted is determined [18–21]. A first curve-fitting was done on a spectra deconvoluted with $K = 1.5$–2.5. The initial input parameters of the curve-fitting were set as follows: (a) the frequency was adjusted manually by moving the cursor on the monitor screen of the computer; (b) the intensity were calculated to be two-thirds of the spectrum intensity at the frequency chosen and the full width at half-height was used to the extent of deconvolution applied. None of the input parameters were kept constant during the curve-fitting procedure.

In the second stage, the same set of initial input parameters, but with widened full width at half-height were used to undeconvoluted spectrum for a new curve-fitting, in order to obtain results free of any possible artifact introduced by the deconvolution procedure, in the integrated intensities. This is very important for dichroic ratio measurements. The resulting curve-fitted was analyzed as follows. Each Lorenzian band was assigned to a secondary structure according to the frequency of its maximum: α-helix (1,647–1,660 cm^{-1}); β-sheet (1,615–1,640 cm^{-1}); turn (1,660–1,680 cm^{-1}); random (1,646–1,641 cm^{-1}) and β-antiparallel (1,681–1,692 cm^{-1}). The area of all the component bands assigned to a given conformation were then summed and divided by the total area. The number obtained was taken as the proportion of the polypeptide chain in that conformation. These assignments are according to the previous values determined theoretically [22] and experimentally [18]. The accuracy of this method was texted on several proteins of the known secondary structures [23], such as cytochrome *c* (α-helix 49%) and bacteriorhodopsin (α-helix 63%), which resulted in error of ±3–5%.

The spectral manipulations were performed with SpectraCalc program (Galactic, Industries Co., Salem, NH, USA).

3. Results and discussion

The vibrational spectra of the 33 kDa extrinsic polypeptide consists of several characteristic bands in the mid-infrared region. The primary spectrum of the protein exhibits absorbance maxima for amide I and amide II near 1,652 and 1,545 cm^{-1}, respectively. The amide A (N–H stretching) occurs as a strong and broad band at 3,000 cm^{-1}, while the presence of several sharp bands with medium intensities in the region of 2,950–2,850 cm^{-1} is assigned to the C–H stretching modes (spectrum not shown). The vibrational mode most useful for the analysis of protein secondary structure is the amide I band between approximately 1,600 and 1,700 cm^{-1}. The amide I region of the infrared spectrum of 33 kDa polypeptide shows a maximum at about 1,652 cm^{-1} and a very intense shoulder between 1,620 and 1,635 cm^{-1} (Fig. 2). While these two spectral features already indicate the presence of both α-helical and β-type conformations [24,25], further details are likely to be hidden by the partial overlapping of bands that are characteristic of various components of protein secondary structure [26–31]. Indeed, the Fourier self-deconvoluted spectrum and the second derivative resolution enhancement shown in Fig. 2A reveals the presence of at least five component bands in the amide I region of the 33 kDa protein. The frequencies of the component bands identified in the resolution enhancement spectrum can be used subsequently as input parameters for curve-fitting of the original broad amide I band contour. The results of such curve-fitting analysis of the amide I spectral region of 33 kDa protein is shown in Fig. 2B. The frequencies of the best fitted component bands correspond closely to those identified in the resolution enhanced spectrum (Fig. 2A).

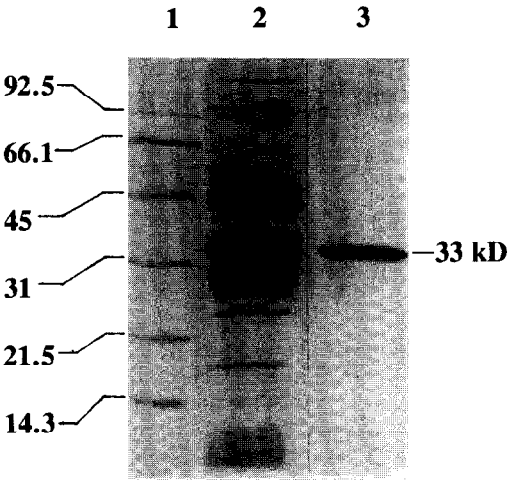

Fig. 1. Polyacrylamide gel electrophoresis of the pure 33 kDa extrinsic polypeptide showing that the preparation exibit a single protein band. Lane 1, molecular weight standards for phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybeal tripsin inhibitor, and lysozyme; lane 2, polypeptide profile of the PSII submembrane fraction; lane 3, isolated 33 kDa extrinsic polypeptide (15 μg). Conditions are described in section 2.

The most prominent feature in the amide I region of the infrared spectrum of the 33 kDa protein is the band at 1,629 cm^{-1} (Fig. 2B). This component, which accounts for 36% of the total area of the amide I band, can be assigned unambiguously to protein segments in the β-sheet conformation [24–29]. Fourier resolution enhancement reveals that the other bands observed in the original spectrum of Fig. 2A consists of five bands with maxima at 1,652 and 1,658 cm^{-1} and three smaller at 1,666, 1,675, and 1,685 cm^{-1}. The frequencies of 1,652 and 1,658 cm^{-1} bands are highly characteristic of α-structures [18–23]. The assignment of the three minor components at 1,666, 1,673, and 1,685 cm^{-1} is less certain as the two bands due to turn structure (1,666 and 1,675 cm^{-1}) are coupled with high frequency vibration of β-segments (1,685 cm^{-1}), which might contribute to the spectral region between 1,666 and 1,690 cm^{-1} [18–23]. The frequencies of bands due to these different structures may in certain cases be very close or even coincide. In previous analyses of infrared spectra obtained from water soluble proteins, the turn structures have been assigned to bands around 1,666 cm^{-1} as well as to frequencies above 1,680 cm^{-1} [18–28]. A usually weak band occurring at around 1,675 cm^{-1}, on the other hand, has been attributed to in-phase vibrations of β-segment in antiparallel conformation [18].

Table 1 shows the secondary structural analysis, which were

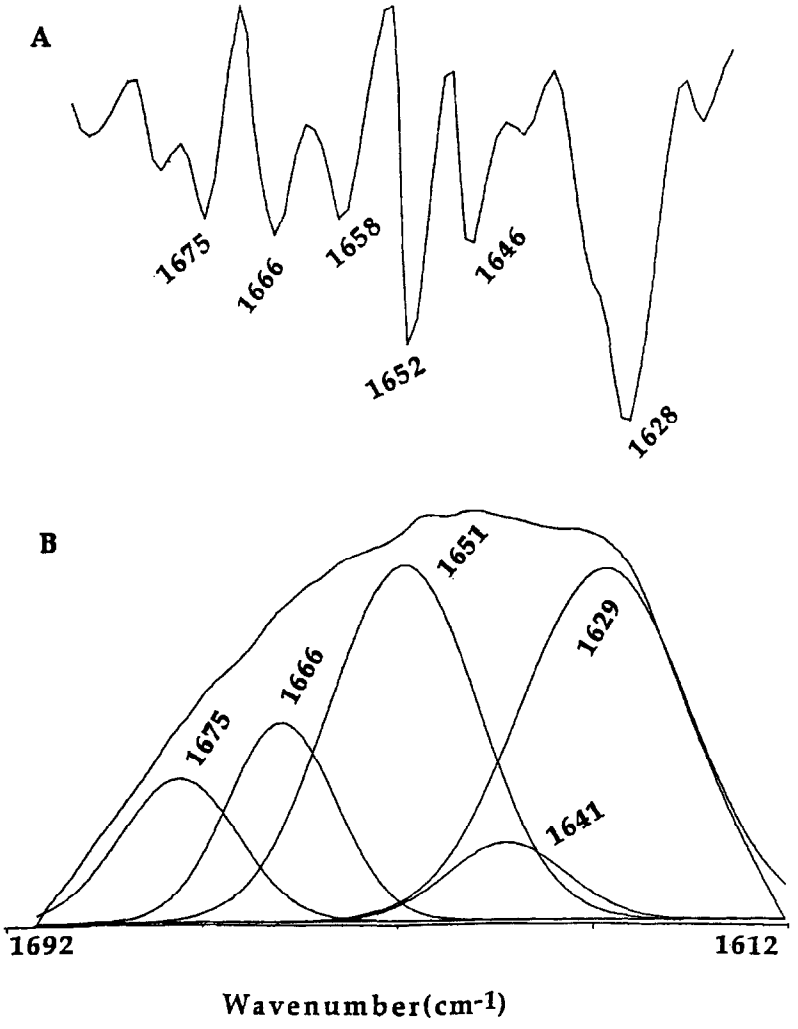

Fig. 2. Second derivative resolution enhancement spectrum (A) of the amide I region (1,692–1,612cm^{-1}) with curve-fitted spectrum (B) used for secondary structure determination of the 33 kDa extrinsic polypeptide as described in section 2.

determined from FTIR spectroscopy using resolution enhancement and curve-fitting procedures. It is obvious from the data presented in Table 1 that the 33 kDa extrinsic protein contains a relatively large proportion of β-sheet structure (36%) and a sizable amount of α-helical structure (27%). The secondary structure analysis obtained by FTIR were also compared with those of other results predicted from either unconstrained Chou-Fasman analysis [12] or from the methods of Biou et al. [33] as implemented by Beauregard for the 33 kDa extrinsic protein [34]. Neither computational approach provides a satisfactory description of the protein secondary structural contents. Chou-Fasman estimates α-helices to represent 28% of the protein domains, which is similar to our method, however it seriously underestimates the amounts of β-sheet and turn conformations. The analysis of Beauregard [34] seriously underestimated β-sheet (9%) and α-helix (13%) contents of this protein and besides, it could not predict the amount of turn or antiparallel structure in the polypeptide.

The secondary structure fractions recently obtained from the circular dichroism method [13] yields fairly reliable appraisal of the amount of β-structure (38%) but the amount of α-helix (9%) and turns (17%) were seriously underestimated (Table I).

The present infrared spectroscopic study shows that the main component of the secondary structures of the 33 kDa polypeptide is β-sheet. Qualitatively, this is in agreement with the results obtained from circular dichroism [13]. However, quantitative measurements of the protein secondary structure obtained from the analysis of the infrared and circular dichroism data are considerably different (Table 1). The reasons for these discrepancies are not fully clear. The source of potential error in these two approaches are different. The circular dichroism spectrum of protein is affected by interfering absorption and

Table 1
Secondary structure analysis of the 33 kDa extrinsic polypeptide

Amide I components	Circular dichroism[a]	Chou-Fasma[b]	FTIR[c]
α-helix	9%	28%	27%
β-sheet	38%	25%	36%
Turn	17%	12%	24%
Other	35%	35%	13%

[a] From [13].
[b] From [12].
[c] This study, data calculated from Fig. 2 as described in section 2.

by light scattering. Quantitative analysis of circular dichroism spectra relies on the set of reference data [32]. These data, obtained from the spectra of aqueous solutions of globular proteins having known three-dimensional structure may be not fully applicable to membrane-bound proteins. Estimates of the secondary structure based on circular dichroism spectra are also influenced by uncertainties in protein concentration.

The approach used in this study seems to be free of most of the problems listed above. Particularly, the analysis based on infrared spectra does not depend on any transferred secondary structure data. On the other hand, a potential source of uncertainty in quantitative interpretation of infrared data arises from the ambiguity in the assignment of the component bands between 1,670 and 1,680 cm^{-1}. This problem concerns particularly those cases where the characteristic 'turn' band around 1,665 cm^{-1} is not observed in the infrared spectra. Under certain circumstances the above ambiguity may effect the accuracy of the estimate of the α-helix content. Another problem with analysis of infrared spectra arises from the unknown intrinsic absorptivities of the amide I vibrations of C = O groups in different conformational states. The present approach is based on the assumption that the various amide I bands have comparable absorptivities and, accordingly, that the total fractional areas of the bands assigned to various components of the secondary structure represent the real content of these structures. The good correlations that were found between the secondary structure estimation obtained from X-ray data and infrared analysis for more than 20 proteins [23] strongly suggest that the infrared method provides a realistic approximation, which will not lead to systematic errors.

The quantitative interpretation of the infrared spectra obtained from the isolated 33 kDa polypeptide must be taken with caution because the extrinsic protein may undergo some minor conformational changes during its removal from the PSII membranes. We believe that the present analysis of the secondary structure, and particularly the relatively high content of β-type conformation, represents a real structural property of the polypeptide in solution but some rearrangements may occur upon binding to the PSII core.

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References

[1] Vermass, W.F.J. and Ikeuchi, M. (1991) in: The Photosynthetic Apparatus, Molecular Biology and Operation (Bogorad, L. and Vasil, I.K., Eds.) pp. 26–112, Academic Press, San Diego.
[2] Miyao, M. and Murata, N. (1989) Biochim. Biophys. Acta 977, 315–321.
[3] Murata, N., Miyao, M. and Kuwabara, T. (1983) in: Oxygen Evolving System of Photosynthesis (Inoue, Y., Croft, A.R., Govindje, Murata, N., Renger, G., and Satoh, K., Eds.) pp. 213–222, Academic Press, Tokyo.
[4] Abramowiz, D.A. and Dismukes, G.G. (1984) Biochim. Biophys. Acta 765, 309–318.
[5] Franzéin, L.G. and Andréasson, L.E. (1984) Biochim. Biophys. Acta 765, 166–170.
[6] Miyao, M. and Murata, N. (1984) FEBS Lett. 170, 350–354.
[7] Ono, T. and Inoue, Y. (1983) FEBS Lett. 164, 255–260.
[8] Cole, I.L., Yachandra, V.K., McDermott, A.E., Guiles, R.D., Britt, R.D., Dexheimer, S.L., Sauer, K. and Klein, M.P. (1987) Biochemistry 26, 5967–5973.
[9] Styring, S. and Rutherford, A.W. (1987) Biochemistry 26, 2401–2405.
[10] Andersson, B. and Akerlund, H.E. (1987) in: Topic in Photosynthesis (Barber, J., Ed.) Vol. 8, pp. 379–420, Elsevier, Amsterdam.
[11] Bricker, T.M. (1992) Biochemistry 31, 4623–4628.
[12] Chou, P.Y. and Fasman, G.D. (1978) J. Adv. Enzymol. 47, 45–148.
[13] Xu, Q., Nelson, J. and Bricker, T.M. (1994) Biochim. Biophys. Acta, in press.
[14] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–236.
[15] Rashid, A. and Carpentier, R. (1989) FEBS Lett. 258, 331–334.
[16] Xu, Q. and Bricker, T.M. (1992) J. Biol. Chem. 267, 25816–25821.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Byler, D.M. and Susi, H. (1986) Biopolymers 25, 469–487.
[19] Kauppinen, J.K., Moffat, D.J., Cameron, D.G. and Mantsch, H.H. (1987) Appl. Opt. 20, 1866–1879.
[20] Surewicz, W.K., Moscarello, M.A. and Mantsch, H.H. (1987) J. Biol. Chem. 262, 8598–8602.
[21] He, W.Z., Newell, W.R., Harris, P.I., Chapman, D. and Barber, J. (1991) Biochemistry 30, 4552–4559.
[22] Krimm, S. and Bandekar, J. (1986) Adv. Protein Chem. 38, 181–364.
[23] Goormaghtigh, E., Cabiaux, V. and Ruysschaert, J.M. (1990) Eur. J. Biochem. 193, 409–420.
[24] Susi, H., Timasheff, S.N. and Steven, L. (1987) J. Biol. Chem. 242, 5460–5466.
[25] Parker, F.S. (1983) Application of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry, Plenum Press, New York.
[26] Susi, H. and Byler, D.M. (1983) Biochem. Biophys. Res. Commun. 115, 391–397.
[27] Susi, H. and Byler, D.M. (1986) Methods Enzymol. 130, 290–311.
[28] Yang, P.W., Mantsch, H.H., Arrondo, J.L.R., Saint-Girons, I., Guillou, Y., Cohen, G.N. and Baruz, O. (1987) Biochemistry 26, 2706–2711.
[29] Yang, W.J., Griffith, P.R., Byler, D.M. and Susi, H. (1985) Appl. Spectrosc. 29, 282–287.
[30] Harris, P.I., Lee, D.C. and Chapman, D. (1986) Biochem. Biophys. Acta 874, 255–265.
[31] Mao, D. and Wallace, B.A. (1984) Biophys, J. 45, 382a.
[32] Chang, C.T., Wu, C.S.C. and Wang, J.T. (1978) Anal. Biochem. 91, 13–31.
[33] Biou, V., Girrat, J.F., Levin, J. and Garnier, J. (1988) Protein. Eng. 2, 185–191.
[34] Beauregard, M. (1992) Envir. Exp. Bot. 32, 411–429.