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LETTERS

Small-Angle X-ray Scattering Studies of the Manganese Stabilizing Subunit in Photosystem II

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Small-angle X-ray scattering studies (SAXS) were used to determine the size, shape, and oligomeric composition of the manganese stabilizing protein (MSP) of photosystem II. This extrinsic protein subunit plays an important role in photosynthetic oxygen evolution. As its name implies, MSP stabilizes the tetranuclear Mn cluster of the water oxidation complex. Removal of MSP lowers activity and decreases the stability of active-site manganese. Reconstitution of MSP reverses these effects. In this study, MSP was extracted from spinach PSII membranes using $CaCl_2$ or urea. Through the use of MALDI-TOF mass spectrometry, the molecular weight of MSP was determined to be 26.53 kDa. X-ray scattering results show that both samples display a monodisperse scattering pattern; this pattern is consistent with a homogeneous protein solution. The $CaCl_2$ extracted and urea extracted MSP samples have radii of gyration of 25.9 ± 0.4 and 27.0 ± 0.01 Å, respectively. MSP is shown to be monomeric in solution. This was determined using a cytochrome *c* standard and the scattering intensity, extrapolated to zero scattering angle, which is proportional to the molecular weight. This SAXS study suggests that, in solution, MSP is a monomeric, elongated prolate ellipsoid with dimensions, $112 \times 23 \times 23$ Å^3 and an axial ratio of 4.8.

Introduction

In this report, the size, shape, and oligomeric composition of the plant manganese stabilizing protein (MSP) was investigated. MSP is an important subunit of photosystem II (PSII), which performs the light-driven reduction of plastoquinone and the oxidation of water in photosynthesis.[1] PSII is composed of multiple polypeptide subunits, many of which are membrane spanning.[2] Oxidation of water to molecular oxygen is catalyzed at the oxygen evolving complex (OEC), which contains four manganese atoms. The integrity and optimal function of the OEC depends on the presence of MSP, which is an extrinsic, nonmembrane spanning subunit.[3,4] MSP can be removed from PSII; extraction lowers oxygen evolving activity and decreases the stability of the OEC. Reconstitution of MSP to PSII reverses these effects.[4] From thermal stability and gel filtration studies, it has been proposed that MSP is a natively unfolded protein that retains secondary structure.[5] It has also been shown that MSP undergoes a substantial change in secondary structure upon reconstitution to PSII.[6]

In this communication, we investigate the solution structure of MSP using small-angle X-ray scattering (SAXS). Recent advances in X-ray scattering instrumentation[7] and the use of synchrotron radiation[8] now allow SAXS studies of proteins at relatively low concentrations. SAXS data give valuable information on the size and shape of proteins,[9] especially when higher resolution structural methods are not applicable.

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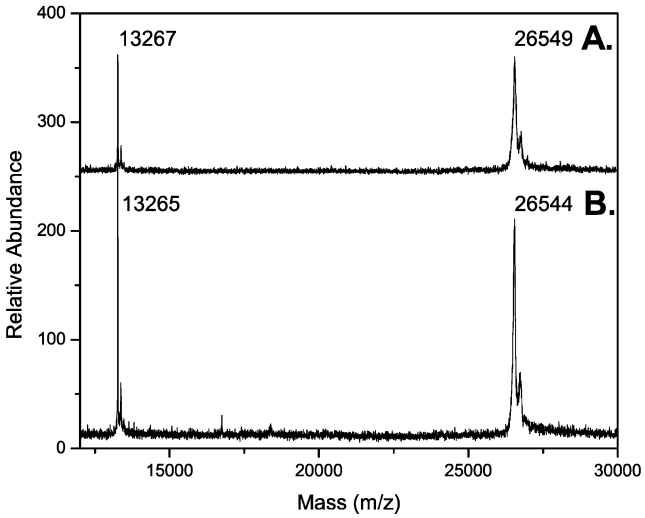

Figure 1. MALDI-TOF mass spectra for urea (A) and $CaCl_2$ extracted (B) MSP.

Materials and Methods

PSII was isolated from market spinach.[10] Purification of MSP from spinach PSII was performed through the use of ion exchange chromatography.[11] MSP was released either with $CaCl_2$ or with urea.[6] The MSP concentration was determined from the absorbance at 276 nm using an extinction coefficient of 16 mM^{-1}.[12] MSP was concentrated to 0.2−0.3 mM and exchanged into a buffer containing 5 mM MES−NaOH, pH 6.0, using a Centricon-10 filter (Millipore, Bedford, MA). MSP purity was assessed by polyacrylamide gel electrophoresis (SDS-PAGE).[13] For each MSP preparation, a single, Coomassie blue stained MSP band was observed on SDS-PAGE (data not shown).

MALDI-TOF data were acquired on a Bruker Biflex III instrument. Samples were diluted to 20−30 μM using 0.1% trifluoroacetic acid, 50% acetonitrile, and a sinapinic acid matrix. Myoglobin, cytochrome *c*, and trypsinogen were employed as mass standards.

SAXS experiments were performed using the instrument (BESSRC beamline 12-ID) at the Advanced Photon Source at Argonne National Laboratory.[8] The wavelength, λ, was 1.028 Å, and the sample-to-detector distance were set such that the detecting range was $0.004 < q < 0.3$ $Å^{-1}$. Transmission coefficients for the sample and buffer background were measured using a photodiode mounted on the beamstop. The scattering vector, q, was calibrated using silver behenate standard at $q = 1.076$ $Å^{-1}$.[14] Precautions to prevent radiation damage included the use of a flow cell and short exposure times (2 s/image). Data from 10 images were averaged for each data set. The criterion, $Rq \leq 1.3$, was used to select the upper q limit for Guinier analysis of MSP and cytochrome *c*. It has been shown that the linear Guinier region extends to this limit in globular proteins.[15,16] Curve fitting, calculations, and modeling were done with Origin 6.0 (Microcal Software, Inc., Northampton, MA) or Mathematica 4.0 (Wolfram Research, Inc., Champaign, IL). As a standard, horse heart cytochrome *c* (Sigma-Aldrich, St. Louis, MO) was used in a buffer containing 10 mM HEPES−NaOH, pH 7.2, and 200 mM NaCl. Reduced solutions of cytochrome *c* contained 20 mM dithiothreitol.

Results and Discussion

MALDI-TOF mass spectrometry was performed to measure the molecular mass of MSP (Figure 1). In both the urea and $CaCl_2$ released samples, two major ions were observed with mass-to-charge ratios (m/z) of 26544−26549 and 13265−13267. These ions can be assigned to $(M + H)^{+1}$ and $(M + 2H)^{+2}$. Two additional ions in each mass spectrum are attributable to conjugates of MSP and the matrix (Figure 1). The +2 ion shows a narrower peak and was bracketed by mass standards. Therefore, the +2 ion was used to predict a MSP molecular mass of 26.53 kDa. The result is in agreement with previous mass spectroscopic studies, which have yielded molecular masses of 26.52 kDa[17] or 26.54 kDa.[18] The mass calculated from the amino acid sequence is 26.53 kDa.[17] The good agreement between the measured mass and the calculated mass suggests that MSP is not posttranslationally modified. However, when subjected to SDS-PAGE, MSP has an apparent molecular mass of 33 kDa. This anomalous migration has been reported previously[5,6,17] and has been attributed to an extended MSP conformation under SDS-PAGE conditions.[17]

SAXS data (Figure 2A) were acquired from $CaCl_2$ and urea released MSP samples. A function was fit to a plot of scattered intensity, I, versus q^2, according to the equation:[9]

$$I(q) = I_o \exp\{-R^2q^2/3\}$$

where $q = 4\Pi \sin \theta/\lambda$, 2θ is the scattering angle, λ is the X-ray wavelength, and R is defined, in analogy with Rg in classical mechanics, as the electronic radius of gyration about the electronic center of mass. Both sets of data exhibit a linear region in a Guinier plot (Figure 2B), consistent with a

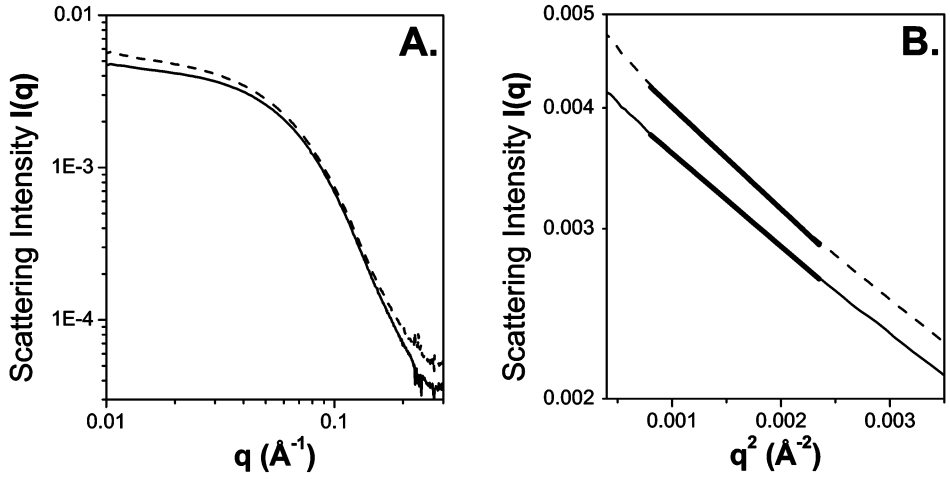

Figure 2. X-ray scattering data (A) and Guinier plot (B) for $CaCl_2$ (solid line) and urea extracted (dotted line) MSP.

monodisperse and homogeneous protein sample. MSP has been reported to sample a variety of conformations in solution.[5,6] The SAXS data suggest that this putative conformational sampling does not dramatically alter the shape of the protein. Fits to the $I(q)$ versus q^2 plots gave $R = 25.9 \pm 0.4$ Å (average of 6) for the $CaCl_2$ extracted sample. For urea extracted samples, a similar value, $R = 27.0 \pm 0.01$ Å (average of 2), was obtained. For both oxidized and reduced cytochrome *c* standards, a value of $R = 13.5 \pm 0.3$ Å (average of 18) was determined, which is in good agreement with previous measurements.[19,20]

From the Guinier plot (Figure 2B), the extrapolated intensity at zero scattering angle, I_o, can also be determined. The relationship between I_o and the molecular mass is described by the equation,

$$I_o = np^2M_w{}^2C$$

where p is an instrument dependent contrast factor, M_w is the protein molecular mass, n is the aggregation number, and C is the protein concentration. Using a standard curve of I_o vs C, the aggregation number for MSP in solution can be calculated, if the contrast factor p^2 is assumed to be invariant and a protein standard of known molecular mass is used. Recently, it has been suggested that MSP is a mixture of monomers and dimers in solution.[18] A mixture of oligomeric MSP forms can be detected by this approach.

Accordingly, cytochrome *c* was used as a standard in a concentration range from 0.05 to 0.2 mM, where a plot of X-ray scattering versus C was linear. Data from eight MSP samples were averaged, and the results were found to be invariant in a MSP concentration range from 0.06 to 0.5 mM. Using extrapolated values of I_o from the Guinier plots (Figure 2B) and $M_w =$ 26.53 kDa (Figure 1), the MSP aggregation number, n, was calculated to be 1.08 ± 0.06. The small deviation from $n =$ 1.00 may be caused by a contribution from ordered water in the MSP hydration layer. Therefore, these SAXS experiments suggest a primarily monomeric state of MSP in solution.

Using the experimentally determined values of R and M_w, a geometric shape for MSP can be determined. The volume per particle was calculated from the partial specific volume, 0.73 mL/g,[21] and the measured molecular mass, 26.53 kDa (Figure 1). An average value of $R = 26.2$ Å was derived from the SAXS data presented above and employed in this calculation. An ellipsoid was used to represent the shape of MSP. Other geometric shapes could be employed. In the future, data at wider scattering angle, which provide higher resolution information,[22] can be used to distinguish between different geometrical models.

The radius of gyration for an ellipsoid, with radii a, b, and c, and $b = c$, can be written:[9]

$$R^2 = b^2/5\{2 + a^2/b^2\}$$

Either a prolate or oblate ellipsoid model may be applicable. To yield the two unknowns, a and b, the ellipsoid volume ($4\pi ab^2/3$) and R were solved simultaneously. By this method, the dimensions were calculated to be $112 \times 23 \times 23$ Å^3 for a prolate ellipsoid and $8.9 \times 82 \times 82$ Å^3 for an oblate ellipsoid. We consider the prolate model a more reasonable fit, because the oblate model has a thickness of only 8.9 Å, which seems unlikely. However, the oblate ellipsoid cannot be absolutely excluded. From recent hydrodynamic studies, MSP was also proposed to have either a prolate or an oblate ellipsoidal shape.[17] The proposed prolate model from that study had an axial ratio of 4.2, which is not dissimilar from the model derived here, which gives an axial ratio of 4.8.

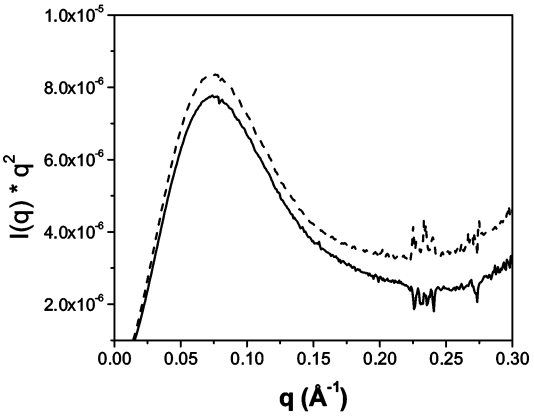

Figure 3. X-ray scattering data represented by a Kratky plot for $CaCl_2$ (solid line) and urea extracted (dotted line) MSP.

Additional structural information can be obtained from the SAXS data by examining the entire scattering pattern. A Kratky plot of the SAXS data can be used to determine whether a protein displays a folded, compact form or an unfolded, extended structure. An unfolded protein does not obey Porod's law, $I(q) \propto q^{-4}$.[23] This has previously been demonstrated for cytochrome *c* through denaturation experiments.[19,20] As shown in Figure 3, both MSP samples exhibit a Kratky plot with a distinct peak. This result is not consistent with a completely unfolded, extended protein, but rather suggests a compact molecule with a folded or an intrinsically disordered structure, in which both folded and disordered domains are present.[24]

In the 3.8 Å crystal structure of PSII, a cylindrical arrangement of β-strands have been assigned to MSP.[25] The structural assignment was based on published FTIR and CD spectroscopic data, which indicate a high content of β-sheet in some MSP samples.[4,6,26] In the crystal structure, 115 C-α carbon atoms were assigned to MSP. This would correspond to ~50% of the MSP peptide backbone, and the dimensions of this part of MSP were $35 \times 17 \times 17$ Å^3. However, only C-α carbons were assigned because the model had only 3.8 Å resolution. Also, the limitation in resolution meant that amino acid side chains could not be included. Thus, comparison of our results to the X-ray diffraction data awaits a higher resolution structural model.

Low-resolution electron microscopy data (see ref 27 and references therein) have also been used to image MSP when bound to PSII. The derived shape in some studies (but see ref 27) is different from the shape determined here and in previous hydrodynamic studies.[17] Isotope-editing and FT-IR spectroscopy have shown that conformational changes occur in the MSP upon binding.[6] Therefore, it is possible that the shape determined in solution is different from the shape of MSP when bound to PSII. It is also possible that conformational changes take place within the intrinsic PSII proteins when MSP is removed; this would complicate interpretation of the electron diffraction data, as previously discussed.[27]

In conclusion, this SAXS study suggests that, in solution, MSP is a monomeric, prolate ellipsoid with dimensions, $112 \times 23 \times 23$ Å^3.

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