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Optical Properties and Small-Angle Neutron Scattering of Bovine Heart Mitochondrial Oligomycin Sensitivity Conferring Protein†

Alain Dupuis, Giuseppe Zaccai, and Michel Satre*

ABSTRACT: The mitochondrial oligomycin sensitivity conferring protein (OSCP) was isolated from beef heart mitochondria according to Senior (1979) [Senior, A. E. (1979) *Methods Enzymol. 55*, 391–397] with a supplementary step of Sephadex chromatography for more extensive purification. The ultraviolet and fluorescence spectra of OSCP were consistent with the presence of tyrosyl residues and the absence of tryptophanyl residues. From the circular dichroism spectrum of OSCP, 43% α-helical structure was calculated; the dichroism spectra of OSCP in H_2O and D_2O were identical. A molecular weight (M_r) of 22 000 for OSCP was determined by sodium dodecyl sulfate gel electrophoresis at different concentrations of the polyacrylamide gel. The radius of gyration (R_g) and the shape of OSCP in H_2O and D_2O were studied by small-angle neutron scattering. The experimentally determined R_g value of OSCP in H_2O was 24 ± 1 Å, and its M_r was 25 000 ± 3000. Comparison of the experimental R_g value with that expected for a compact globular protein of the same molecular weight (17 Å) led to the conclusion that OSCP is a considerably elongated molecule protein with an axial ratio higher than 3. In D_2O buffer, the R_g value was higher than that in H_2O, a situation in contrast with that observed for most globular hydrophilic proteins; this might be due to a preferential location of the positively charged lysine residues.

The oligomycin sensitivity conferring protein (OSCP),[1] a water-soluble, basic protein, is one of the subunits of the mitochondrial F_1–F_0 ATPase complex. As shown in reconstitution experiments, OSCP confers oligomycin sensitivity to F_1 and is essential for restoration of energy-linked functions such as the ATP–P_i exchange reaction or the ATP-driven reversal of electron transport. It has been proposed that OSCP not only has a structural role as a connecting link between the F_1 and F_0 sectors of the ATPase complex but also has a functional role, either directly conducting protons or mediating conformational changes between the two sectors of the complex (Tzagoloff et al., 1968; Mac Lennan & Tzagoloff, 1968; Tzagoloff, 1970; Senior, 1971, 1979). OSCP is absent in

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[1] Abbreviations: OSCP, oligomycin sensitivity conferring protein; F_1, soluble coupling factor 1; F_0, membrane sector of the ATPase complex; $NaDodSO_4$, sodium dodecyl sulfate; ASUA, submitochondrial particles isolated in the presence of ammonia and successively treated with Sephadex, urea, and ammonia; Tris, tris(hydroxymethyl)aminomethane.

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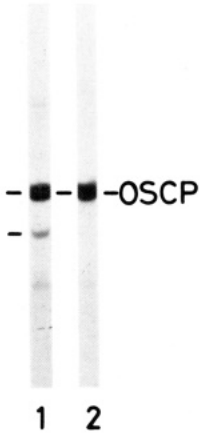

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of OSCP. Samples (30 μg of protein) before (gel 1) and after (gel 2) Sephadex G50 chromatography (see Materials and Methods) were treated with 1% (w/v) $NaDodSO_4$ and 1% (v/v) 2-mercaptoethanol and subjected to electrophoresis on 7.5% polyacrylamide gels run in 25 mM Tris–0.19 M glycine buffer, pH 8.4. Proteins were stained with Coomassie blue R250.

standard mitochondrial F_1 preparations (Knowles & Penefsky, 1972), and it has no apparent enzymatic activity. No phospholipids or bound nucleotides are associated with OSCP (Mac Lennan & Tzagoloff, 1968). By electron microscopy, negatively stained OSCP was described as a particle of dimensions 50 × 30 Å (Mac Lennan & Asai, 1968). Titration studies have shown that OSCP binds to OSCP-depleted submitochondrial particles with a high affinity and a well-defined stoichiometry with respect to F_1, namely, 2–3 mol of OSCP per mol of F_1 (Hundal & Ernster, 1979) or 1.1 ± 0.5 mol of OSCP per mol of F_1 (Dupuis et al., 1983).

The beef heart mitochondrial OSCP and the δ-subunit from from *Escherichia coli* F_1 have closely related amino acid sequences (Walker et al., 1982; Grinkevich et al., 1982) and are probably equivalent in function (Sternweis & Smith, 1977; Walker et al., 1982). Similarly, in the ATPase complex of *Streptococcus faecalis*, the δ-subunit (also called nectin) appears to be the counterpart of mitochondrial OSCP and equivalent to the *E. coli* δ-subunit (Abrams et al., 1976a,b). The same may apply to the δ-subunit from chloroplastic F_1 (Schmidt & Paradies, 1977). The spectral and structural properties of purified OSCP from beef heart mitochondria have been investigated to provide complementary data for further comparison of OSCP with the δ-subunit of *E. coli* F_1 and chloroplast F_1. The results are summarized in the present paper.

Materials and Methods

Purification of OSCP. Mitochondria were isolated from beef heart according to Smith (1967). OSCP was prepared as described in Senior (1979), starting from 20 g of mitochondria, with, in addition, a final step of purification consisting of chromatography on a Sephadex G50 (fine) column (1.5 × 80 cm) equilibrated in 50 mM Tris–sulfate, pH 7.5, to eliminate a small molecular weight contaminant (Figure 1). Fractions containing OSCP were pooled and precipitated with ammonium sulfate (42% saturation). After 30 min at 0 °C, OSCP was collected by centrifugation for 15 min at 20000*g*. The OSCP pellet was solubilized in 50 mM Tris–sulfate, pH 7.5, to give a protein concentration of about 4 mg/mL and was stored in liquid nitrogen.

Biological Assays. ATPase activity was measured as described by Pougeois et al. (1978). OSCP activity was determined by the conferring of oligomycin sensitivity on the ATPase activity of isolated F_1 (Van de Stadt et al., 1972) in a reconstitution assay involving submitochondrial particles depleted of endogenous F_1 and OSCP. Protein was estimated by the dye binding method of Bradford (1976) or with the Folin–phenol reagent (Zak & Cohen, 1961) using bovine serum albumin as the standard. The dye Serva Blue G was obtained from Serva (Heidelberg, West Germany). Both methods gave identical results.

Polyacrylamide Gel Electrophoresis. The molecular weight of OSCP was determined from the rates of electrophoretic migration on five acrylamide gels with concentrations of acrylamide plus bis(acrylamide) ranging between 5 and 14%, with the buffer system of Weber et al. (1972). The following proteins were used for molecular weight calibration: bovine serum albumin, (M_r 66 296), ovalbumin (M_r 42 807), lactate dehydrogenase (M_r 36 457), carbonic anhydrase (M_r 28 980), soybean trypsin inhibitor (M_r 20 095), β-lactoglobulin (M_r 18 363), lysozyme (M_r 14 314), and α-lactalbumin (M_r 14 183).

Fluorescence Measurements. Fluorescence spectra were measured at 22 °C with a Perkin-Elmer MPF-2A spectrofluorometer. In the experiment of fluorescence quenching by iodide, a stock solution of 1 M KI, with 0.1 mM $Na_2S_2O_3$ to prevent I^{3-} formation, was prepared freshly prior to the titrations, and aliquots were added to the cuvette containing OSCP in 5 mM Tris–sulfate, pH 7.5.

Small-Angle Neutron Scattering. OSCP (1–4 mg of protein/mL) was dialyzed 18 h at 0–4 °C against 100 volumes of 50 mM Tris–sulfate, pH 7.5, either in H_2O or in 95% D_2O (no pH correction was made for D_2O solutions). OSCP and the dialysis buffer were then used for neutron-scattering measurements on the D11 instrument at the Institute Laue-Langevin, Grenoble. The incident neutron wavelength, λ, was 7 Å. The two-dimensional multidetector was placed at a distance of 2.66 or 4.84 m from the sample. This setup allowed measurements over a range of the scattering vector (**Q**) from approximately 0.013 to 0.12. **Q** is equal to $(4\pi \sin \theta)/\lambda$, 2θ being the total scattering angle. Scattering intensities were obtained from the neutron counts by subtraction of scattering from the buffer and normalization to the incoherent scattering from water. the radius of gyration (R_g) and the scattered intensity at zero angle [$I(0)$] were obtained through linear least-squares fits of the scattering curves according to the Guinier law (Jacrot, 1976). The relative molecular weight (M_r) was calculated as described in Jacrot & Zaccai (1981).

Results

Molecular Weight Determination of OSCP by Gel Electrophoresis. Previous estimates of the molecular weight of OSCP ranged between 17 900 and 24 000 (Mac Lennan & Tzagoloff, 1968; Senior, 1971; Van de Stadt et al., 1972; Vadineanu et al., 1976; Hundal & Ernster, 1979; Glaser et al., 1980; Ludwig et al., 1980). For more accurate determination, the molecular weight was measured by $NaDodSO_4$–polyacrylamide gel electrophoresis at different acrylamide plus bis(acrylamide) (total acrylamide) concentrations. Figure 2 shows the plot of the logarithm of the molecular weight vs. the migration distance (R_F) for marker proteins. The apparent molecular weight of OSCP estimated from these plots was 22 000 ± 900. No systematic change in molecular weight was observed as a function of total acrylamide concentration. Ferguson plots of log R_F as a function of total acrylamide concentration were drawn from the data shown in Figure 2A. They were linear for all marker proteins and OSCP, in agreement with the equation $\log R_F = \log Y_0 - K_R[A]$, where K_R is the retardation coefficient, Y_0 the free electrophoretic mobility, and [A] the total acrylamide concentration expressed

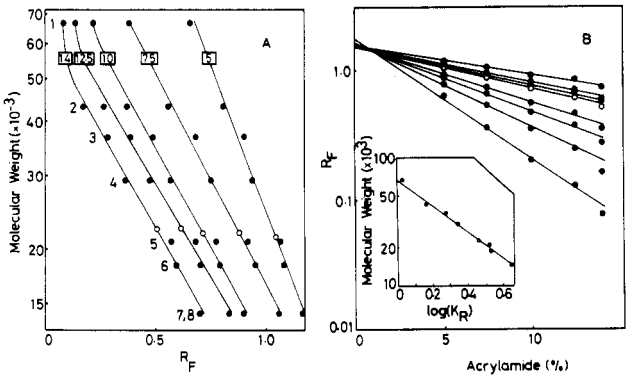

FIGURE 2: (A) Molecular weight on a semilogarithmic scale is plotted vs. the mobility (R_F) relative to the marker dye Bromophenol Blue for marker proteins (●) and OSCP (○). The concentrations in percent total acrylamide [acrylamide + bis(acrylamide)] are indicated in the figure. The marker proteins are designated by numbers as follows: (1) bovine serum albumin; (2) ovalbumin; (3) lactate dehydrogenase; (4) carbonic anhydrase; (5) soybean trypsin inhibitor; (6) β-lactoglobulin; (7) lysozyme; (8) α-lactalbumin. (B) Ferguson plot of the relative mobility (R_F) for marker proteins (●) and OSCP (○) vs. the total acrylamide concentration [acrylamide + bis(acrylamide)]. The retardation coefficient (K_R) was calculated from the slopes of the lines. Insert: Double-logarithmic plot of the molecular weight vs. the retardation coefficient (K_R).

Table I: Amino Acid Composition of OSCP[a]

amino acid	mol/100 mol	
	this work	Senior (1971)
Asp	5.35	4.6
Thr[b]	6.54	5.6
Ser[b]	9.52	6.7
Glu	10.12	12.4
Pro	4.16	4.8
Gly	4.16	6.3
Ala[c]	8.93	9.6
Cys[d]	0.20	0.6
Val[c]	8.33	9.8
Met	3.57	3.3
Ile[c]	5.95	5.9
Leu[c]	11.90	12.5
Tyr	2.38	2.25
Phe	2.38	2.2
Lys[c]	10.71	8.9
His	0.59	0.7
Arg	4.76	4.5
Trp[e]	0	

[a] Values given are means obtained from five analyses. [b] Extrapolated back to zero time of hydrolysis of OSCP samples. [c] Obtained after 72 h of hydrolysis. [d] Measured as *S*-(carboxymethyl)cysteine after reduction and alkylation. [e] Determined according to Beaven & Holiday (1952).

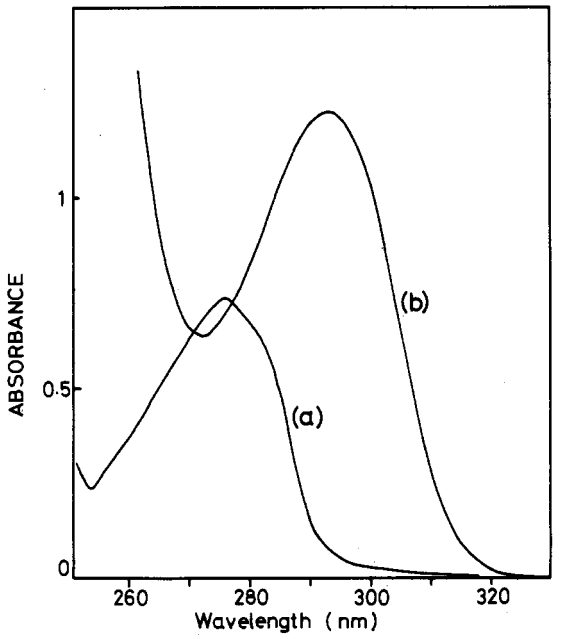

FIGURE 3: UV absorption spectra of beef heart mitochondrial OSCP. The absorption spectra of OSCP (2.45 mg/mL) were measured in (a) 50 mM Tris–sulfate, pH 7.5, and (b) 0.1 N NaOH. Spectra were obtained in a Cary Model 219 spectrophotometer. The cell path length was 1 cm.

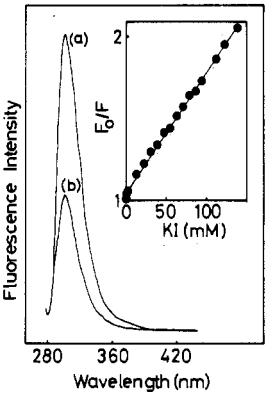

FIGURE 4: Fluorescence emission spectra of OSCP. The spectra of OSCP (0.18 mg of protein/mL) were measured in (a) 5 mM Tris–sulfate, pH 7.5, and (b) 5 mM Tris–sulfate–125 mM KI, pH 7.5. The samples were excited at 277 nm. Other experimental details are given under Materials and Methods. Insert: Stern–Volmer plot showing the quenching of OSCP fluorescence by KI.

in percent (w/v). All of the marker proteins have a nearly identical free mobility (Y_0), indicating no systematic bias in the charge densities of the marker proteins. The free mobility of OSCP was not significantly different from the average free mobility. A plot of log M_r vs. log K_R was linear. The molecular weight value for OSCP derived from this plot was 22 500 ± 700.

Optical Properties of OSCP. The UV absorption spectrum of OSCP in 50 mM Tris–sulfate, pH 7.5, exhibited a maximum at 276 nm (Figure 3). This is typical of proteins having tyrosine as the dominant chromophore. The extinction coefficient of OSCP at 276 nm ($A^{1\%,1cm}_{276nm}$) was 3.0 ± 0.1. Since neutron-scattering measurements were performed in standard spectrophotometer cells, this coefficient provided a nondestructive and direct way of measuring OSCP concentrations. The absorption maximum of OSCP in 0.1 N NaOH was at 293 nm; with these alkaline conditions, the amplitude of the absorption peak was increased about 1.7-fold. On the basis of a molecular weight for OSCP of 22 000 and a molar extinction coefficient for tyrosine at 293 nm of 2380 (Fasman, 1976), a value of 4.7 tyrosine residues per molecule was calculated. The amino acid composition of OSCP is given in Table I. It agrees with that given by Senior (1971). By analysis of tryptophanyl residues according to Beaven & Holiday (1952), no tryptophan was detected. Fluorescence spectra also confirmed the absence of tryptophan (Figure 4); only one emission peak at 304 nm was observed. The quantum yield relative to that of tyrosine was 0.49 ± 0.03, a rather high value when compared to other proteins lacking tryptophan (Fasman, 1976). Fluorescence was quenched by iodide, suggesting a peripheral location of the fluorescent tyrosine residues in OSCP. The apparent Stern–Volmer constant, K_Q, obtained from the slope of the plots of F_0/F vs. [KI] was 7.7 M^{-1}.

The circular dichroism spectrum of OSCP is shown in Figure 5. The measured spectrum was characterized by two

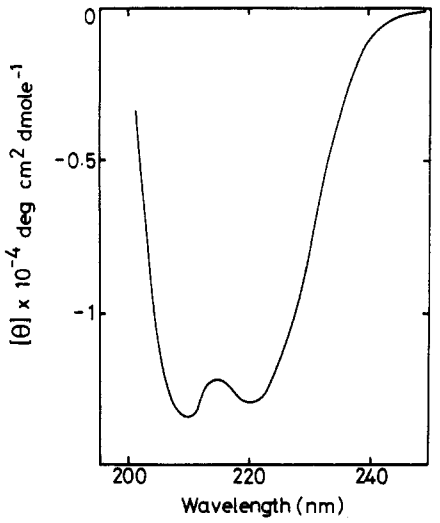

FIGURE 5: Circular dichroism spectrum of OSCP. The spectrum was measured at 20 °C in a Jouan Dichrograph III. The cell path length was 1 mm. OSCP in 10 mM 3-(*N*-morpholino)propanesulfonic acid–50 mM NaCl, pH 7.5, was at a protein concentration of 0.17 mg/mL.

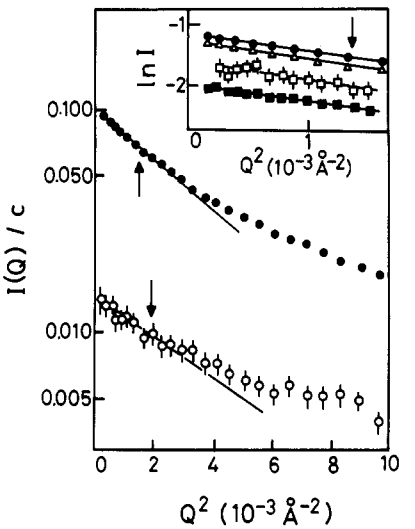

FIGURE 6: Guinier plots of scattering curves of OSCP in H_2O (○) and D_2O (●) buffer. The protein concentration in H_2O was 3.7 mg/mL (sample thickness = 1 mm) and in 95% D_2O 3.2 mg/mL (sample thickness = 2 mm). The limits ($R_gQ = 1$) of the Guinier approximation are indicated by the arrows. Insert: Guinier plot of scattering curves of OSCP in 95% D_2O buffer at various OSCP concentrations: 1.3 mg/mL (■), 1.7 mg/mL (□), 2.8 mg/mL (△), and 3.2 mg/mL (●). In all cases, the sample thickness was 2 mm.

rotational minima at 222 and 206 nm. The molar ellipticity at 222 nm was 13 000 deg cm/dmol, and thus the predicted amount of α-helical structure is about 43% (Chen et al., 1974). An identical spectrum was observed in D_2O medium, an interesting result for assessment of the neutron-scattering data to be presented below.

Size and Shape of OSCP As Determined by Neutron Scattering. The dependence of the scattering intensity, I, on the scattering vector, $\mathbf{Q}$, is illustrated in Figure 6 in the form of Guinier plots (log I vs. $\mathbf{Q}^2$) for OSCP in solution in H_2O and D_2O buffers. Two parameters were derived from the Guinier plots, the radius of gyration (R_g) of the particle and the scattered intensity extrapolated to zero angle, $I(0)$, which is related to the molecular weight (M_r).

In H_2O medium (Figure 6), R_g determined from the slope of the scattering curve in the Guinier region was found to be 24.3 ± 1.4 Å. The molecular weight was calculated as described in Jacrot & Zaccai (1981), from the extrapolated value of the scattered intensity at $\mathbf{Q} = 0$ normalized by the incoherent scattering of H_2O. Such a calculation required knowledge of the partial specific volume ($\bar{v}$) and the neutron-scattering length per unit mass ($\Sigma b/M$) of OSCP. These two values were calculated from the individual partial specific volumes (Cohn & Edsall, 1943) and from the scattering lengths of the amino acid residues (Jacrot, 1976). For calculation, use was made of the amino acid composition of OSCP given in Table I. The partial specific volume of OSCP was 0.75 cm^3/g; as explained by Jacrot & Zaccai (1981), however, the value of $I(0)$ in H_2O is not very sensitive to $\bar{v}$. The scattering length per unit mass of OSCP was 2.08×10^{-14} cm, which is significantly lower than the average value of 2.27×10^{-14} cm per unit mass calculated for several soluble proteins (Jacrot & Zaccai, 1981). The low value of scattering length found for OSCP is related to its peculiar amino acid composition. In fact, OSCP is relatively rich in lysine, valine, methionine, isoleucine, and leucine, which all have a low scattering density in H_2O as compared to other amino acid residues; these five amino acids account for 40% of the residues of the protein. Also, OSCP is characterized by a low content in aspartic acid, which is the amino acid with the highest scattering density in H_2O (Jacrot, 1976). On the basis of these data, the calculated molecular weight value for OSCP was 25 000 ± 3000, which is in the range of values obtained by migration on $NaDodSO_4$–polyacrylamide gel electrophoresis.

The R_g values of compact globular proteins in H_2O, such as hemoglobin, myoglobin, or lysozyme, are proportional to the cubic root of their molecular weight (Serdyuk et al., 1978; Paradies & Schmidt, 1979). Thus, the calculated R_g for a compact globular protein of M_r 25 000 is 17 Å. The experimentally determined value of R_g for OSCP in H_2O, 24 ± 1 Å, is considerably larger. This indicates either an expanded structure with a loose arrangement of the polypeptide chain or an elongated compact shape.

OSCP was still active in D_2O medium. For example, for an OSCP/F_1 ratio of 4, F_1 regained 84% oligomycin sensitivity in a reconstitution assay with ASUA particles (Van de Stadt et al., 1972) entirely conducted in 95% D_2O. The accuracy of the OSCP scattering data was higher than those in H_2O because of smaller incoherent scattering from D_2O and larger contrast (Jacrot, 1976). The R_g value for OSCP in D_2O calculated from the slope of the Guinier plots was 27.3 ± 0.7 Å, using protein concentrations from 1.3 to 3.2 mg/mL. Thus, the R_g value of OSCP in D_2O buffer was significantly higher than that in H_2O buffer; most water-soluble proteins show the opposite behavior. The higher R_g value in D_2O could be related to an aggregated state of OSCP. The tendency of OSCP to form aggregates was previously noted by Senior (1979), and nonspecific aggregation is often favored in D_2O medium. At small $\mathbf{Q}^2$ (Figure 6, insert), Guinier plots are straight and show no upward curvature characteristic of aggregation. R_g and $I(0)/c$ are not significantly changed as the protein concentration (c) increases from 1.3 to 3.2 mg/mL. This indicated the absence of notable amounts of aggregates in OSCP solutions. An examination of $I(0)$ in absolute units in D_2O also favors the conclusion that OSCP was still in a monomeric state in D_2O. The molecular weight of the scattering particle can be determined from the $I(0)$ value measured in D_2O provided the match point is known, the match point being defined as the concentration of D_2O for which the scattering density of the solvent is equal to that of the protein (Jacrot, 1976). The experimental $I(0)$ values in H_2O and D_2O indicated a match point lower than 42% D_2O, the usual value of water-soluble proteins (Sturhmann, 1974). However, as

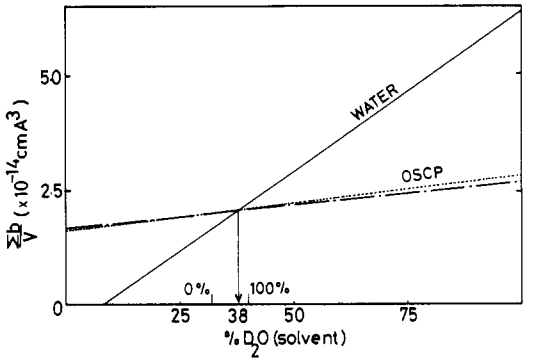

FIGURE 7: Neutron scattering density ($\Sigma b/v$) of OSCP as a function of the D_2O content of the solvent. This has been calculated by using for OSCP the amino acid composition of Table I and the individual scattering densities of amino acid residues given in Jacrot (1976). The OSCP lines were obtained by assuming that 80% of the total protons were exchangeable and amino acid compositions containing only Asn and Gln (---) or only Asp and Glu (----) were present. The match point of OSCP was obtained from the intercept with the water line. The arrow indicates the extreme match points, assuming either no exchangeable protons or 100% exchangeable protons.

shown below, a low value is not unexpected, considering the peculiar composition of OSCP. The OSCP match point was obtained from the intercept of the H_2O/D_2O and OSCP scattering density lines (Figure 7). When the two extreme cases of H–D exchange are considered, i.e., either no exchange or complete exchange, the match point of OSCP is in the range of 32–40% D_2O. It could be estimated to be 38 ± 1% in D_2O under the reasonable assumption that 80% of OSCP-labile hydrogens are exchangeable with the solvent as in a number of proteins (Jacrot & Zaccai, 1981).

In solvents where the protein scatters as a homogeneous particle, a simple shape such as an ellipsoid or a cylinder could be fitted to the scattering curve at angles higher than the Guinier range. Because of the nonhomogeneous exchange of labile H, however, extrapolation of the scattering curve to infinite contrast does not yield the scattering of the homogeneous shape of the protein, and usually the best approximation to a homogeneous particle is obtained for the protein in H_2O. This has been discussed by Zaccai & Jacrot (1983). To determine the axial ratio of models equivalent in scattering to OSCP, the experimental scattering curve for OSCP in H_2O was compared to theoretical scattering curves for simple homogeneous bodies such as cylinders or ellipsoids (Figure 8). The experimental points for the scattering of OSCP in H_2O fit well to the curves calculated either for a 3.3:1:1 prolate ellipsoid with dimensions of 90 Å × 30 Å × 30 Å or for cylinders with a height to diameter ratio of 3.3 and of height 78.5 Å and diameter 24 Å. The volume of these models equivalent in scattering behavior should be compared to the volume of OSCP. The dry volume was calculated to be $V = 31000A^3$ according to the formula $V = (M_r\bar{v})/N_A$ where M_r is the molecular weight, $\bar{v}$ the partial specific volume ($\bar{v} = 0.75$ cm^3/g), and N_A Avogadro's number. Maximum hydration of the protein was determined according to Kuntz (1971), and a value of 0.3–0.4 g of H_2O/g of protein was found. When this value is taken into account, the volume of OSCP is thus close to 42 000–48 000 Å^3. Given the experimentally determined R_g, only the prolate ellipsoid model was consistent with the calculated volume of OSCP, the volume of the cylindrical model being too small.

As a matter of interest, a simple homogeneous shape was also fitted to the D_2O data (Figure 8B) even though the scattering of the particle in D_2O is more strongly dominated by nonhomogeneous labile H–D exchange (Zaccai & Jacrot, 1983). A prolate ellipsoid of axial ratio 2.6:1:1 gave the best fit. The R_g value of water-soluble proteins is usually slightly decreased in D_2O as compared to H_2O because the hydrophilic amino acid residues are located preferentially at the surface of the protein and have a higher average scattering length density than the hydrophobic residues (Jacrot, 1976). In D_2O, therefore, the contrast of the outside of the particle is reduced, and the particle appears smaller. The OSCP protein is particularly rich in lysine residues with a low scattering density per mass unit in H_2O. With respect to neutron scattering, these residues are characterized by a large increase of contrast in D_2O. The increased R_g and the decreased axial ratio observed for OSCP in D_2O might be explained by an uneven mode of distribution of the lysine residues. These residues could possibly dominate in the equatorial region and form part of a basic ring around the central region of OSCP.

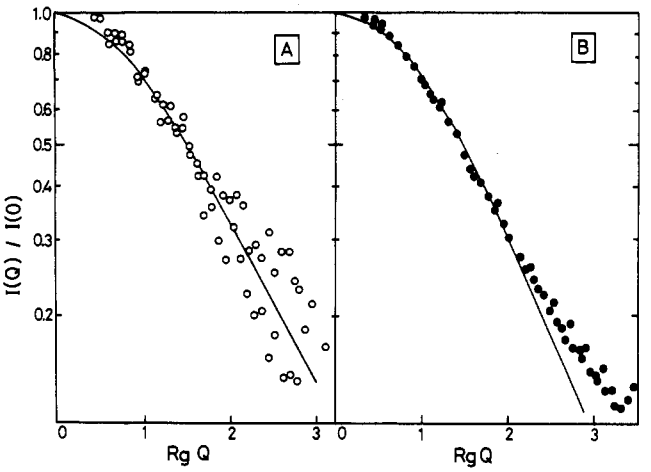

FIGURE 8: Neutron scattering profiles of mitochondrial OSCP at 3.7 mg/mL in H_2O (○) and at 3.2 mg/mL in 95% D_2O (●). The line drawn through the data points in H_2O is the calculated scattering profile for a prolate ellipsoid of dimensions 90 × 30 × 30 Å.

Discussion

In this study, a number of structural parameters of purified OSCP in solution have been characterized. The high fluorescence output of tyrosine residues in OSCP indicated the presence of tyrosine residues which are exposed to solvent and not quenched. Exposure to solvent is also in agreement with the quenching effect of added iodide, as only fluorophores close to the surface of proteins are considered to be accessible to ionic quenchers. OSCP is a basic protein of pH_i 9.3 (Senior, 1971), and this possibly contributes to its high fluorescence as carboxylic residues are efficient quenchers of tyrosine fluorescence (Lehrer & Leavis, 1978).

The molecular weight of 25 000 ± 3000 derived from neutron-scattering experiments is in fair agreement with the value 22 000 determined by $NaDodSO_4$–polyacrylamide gel electrophoresis. The large R_g value and the shape of the scattering curves were interpreted to mean that OSCP has a quite elongated structure with an axial ratio of about 3, consistent with a prolate ellipsoid of approximate dimensions 90 Å × 30 Å × 30 Å. The data for OSCP in solution in D_2O gave a larger R_g value which might suggest a preferential arrangement of the exchangeable lysine residues around the short axis of the particle.

As suggested by Walker et al. (1982), the δ-subunit from *E. coli* F_1 is homologous to the mitochondrial OSCP in that it connects the F_1 and F_0 sectors of the ATPase complex. This similarity of function is of interest since an elongated shape was already proposed for the δ-subunit of *E. coli* F_1 to explain its high apparent molecular weight derived from molecular

sieve chromatography data (Sternweis & Smith, 1977). A detailed study of the structure of the δ-subunit from chloroplastic F_1 by small-angle X-ray scattering was reported earlier by Schmidt & Paradies (1977). The δ-subunit of chloroplastic F_1 is also functionally equivalent to OSCP (Younis et al., 1977; Baird & Hammes, 1979; Andreo et al., 1982); there indeed exist striking structural analogies between OSCP and the δ-subunit, from chloroplastic F_1. Like OSCP, the δ-subunit has an R_g value of 21.8 Å and is highly asymmetrical (axial ratio = 4–5); in X-ray scattering, it is equivalent to an ellipsoid of dimensions 90 Å × 28 Å × 25 Å (Schmidt & Paradies, 1977). It is therefore probable that the bacterial or chloroplast δ-subunits and the mitochondrial OSCP fullfill equivalent functions in their respective enzymes. To what extent their elongated shapes are related to their function either as a simple link between the F_1 and F_0 sectors or as a proton conductor remains to be evaluated.

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Registry No. Oligomycin, 1404-19-9; neutron, 12586-31-1; ATPase, 9000-83-3.

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