SMALL ANGLE NEUTRON SCATTERING OF *ESCHERICHIA COLI* BF_1-ATPase

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1. Introduction

In *Escherichia coli*, the energy transducing ATPase is a complex enzyme system involved in the reversible synthesis of ATP during oxidative phosphorylation. The soluble part of the complex, the BF_1 factor, possesses the ATPase activity itself and shows remarkable similarities to F_1-ATPases from a variety of sources including mitochondria, chloroplasts and other bacteria [1,2]. *E. coli* BF_1 preparations consist of 5 distinct subunits as seen on SDS–polyacrylamide gel electrophoresis, with mol. wt 56 000 (α), 52 000 (β), 32 000 (γ), 20 000 (δ) and 12 000 (ϵ) [3]. The molecular weight of isolated BF_1 has been measured for several preparations using different techniques such as gel filtration, pore gradient electrophoresis and ultracentrifugation. The reported values range from 250 000–400 000 [4–6]. The oligomeric structure of the BF_1-ATPase also remains controversial and subunit stoichiometries of the type $\alpha_3\beta_3\gamma\delta\epsilon$ [7] or $\alpha_2\beta_2\gamma_2\delta_2\epsilon_2$ [3] are generally proposed.

This report presents the results of an initial study of *E. coli* BF_1-ATPase in H_2O and D_2O solutions by neutron small-angle scattering. The enzyme in H_2O buffers is shown to have a radius of gyration of 46 ± 0.7 Å and mol. wt 315 000 ± 25 000.

2. Materials and methods

2.1. *Purification of* E. coli *BF_1-ATPase*

E. coli K12, strain AN 180 (*arg* E3, *thi*-1) [8] was grown at 37°C in rich medium and the cell paste was stored frozen at –80°C. Membranes were prepared by disruption in a Sorvall-Ribi press. BF_1 was released from the membranes by chloroform treatment as in [9] and it was further purified by ion-exchange chromatography on a DEAE-cellulose column and gel filtration on Sepharose 6B [3]. Pure BF_1-ATPase was stored at 0°C in 50 mM Tris–HCl, 20% (v/v) methanol, 2.5 mM 2-mercaptoethanol, 2 mM EDTA, 1 mM ATP (pH 7.4) at ~10 mg protein/ml. Its specific activity was 35–45 μmol ATP hydrolyzed.min^{-1}.mg $protein^{-1}$ when assayed as in [10]. Our preparations completely lacked the δ subunit, which is the general case for *E. coli* K12 BF_1 [6].

2.2. *Protein concentration*

Protein concentration was measured by the method in [11]. Crystalline bovine serum albumin (Pentex, Kankakee, IL) was used as a standard. The concentration of standard solutions was determined from the absorption coefficient at 280 nm ϵ 1% = 6.6 [12].

2.3. *Neutron scattering experiments*

Neutron scattering experiments were done at the Institut Laüe-Langevin on the high-flux reactor using the small-angle scattering cameras D11 and D17 [13]. Neutron scattering curves were collected for 2 ranges in scattering vector Q ($Q = 4\pi \sin\Theta/\lambda$; Θ is half the scattering angle and λ is the wavelength). The first range corresponded to $0.013 \leqslant Q \leqslant 0.09$ and was measured with λ = 10 Å; the second to $0.03 \leqslant Q \leqslant 0.2$ with λ = 5 Å.

Before the neutron scattering experiments, BF_1 was applied to the top of a Sephadex G-50 (fine) column equilibrated in H_2O or D_2O solutions containing 50 mM Mops, 1 mM 2-mercaptoethanol,

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pH(D) 7.0. The excluded eluate was used for measurements in cells of 1 mm pathlength (in H_2O) or 2 mm pathlength (in D_2O).

The molecular weight (M) of BF_1-ATPase in H_2O was calculated by using the formula discussed in [14]:

$$\frac{I(0)}{c} = 4\pi \times \frac{T}{1-T} \times 10^{-3} \times t \times N_A \left[\frac{\Sigma b'}{M'} - \rho_s \times \frac{\bar{v} \times 10^{24}}{N_A}\right]^2 \times M$$

where: $I(0)$ is the scattered intensity at zero angle divided by the incoherent scattering of water in the same solid angle; c, the protein concentration in mg/ml; T, the transmission of the neutron beam through the solvent; t, the pathlength through the sample in cm; N_A, Avogadro's number; $\Sigma b'$, the sum of the scattering lengths in cm of the amino acid residues for a given mass of protein M' in daltons (see table 1). A specific neutron scattering length $\Sigma b'/M' = 2.280 \times 10^{-14}$ cm/g was calculated for *E. coli* BF_1 from its amino acid composition, by using the scattering lengths in [15]; ρ_s, the neutron scattering density of water in cm/A^3; $\bar{v}$, the partial specific volume of BF_1-ATPase in cm^3/g. $\bar{v} = 0.737$ cm^3/g was estimated from the amino acid composition (see table 1) according to [16].

3. Results

3.1. *Effect of D_2O on BF_1-ATPase activity*

The ATPase activity of soluble BF_1 is progressively inhibited as the concentration of D_2O in the assay mixture is increased. Inhibition is a linear function of the D_2O content of the reaction medium and the activity is inhibited by ~50% in 96% D_2O. Figure 1 also shows the reversal of the D_2O inhibition of ATPase activity. Inhibition of the BF_1-ATPase exposed to D_2O was almost completely reversible by dilution in an H_2O assay medium.

3.2. *Radius of gyration and molecular weight*

The neutron scattering curves for the small angle region of *E. coli* BF_1-ATPase in solution in H_2O (for two concentrations) and in D_2O are shown in fig.2

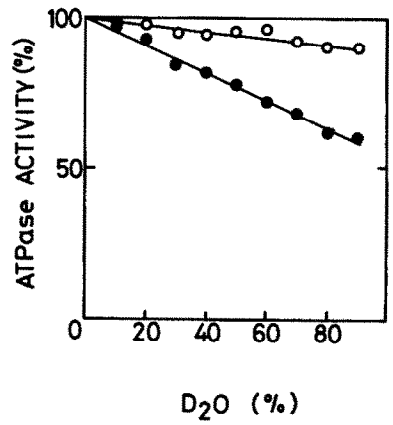

Fig.1. Effect of D_2O on the ATPase activity of *E. coli* BF_1. ATPase activity was measured at 37°C in a reaction mixture containing 100 mM Tris–SO_4, 20 mM ATP, 10 mM $MgSO_4$ and 5 μg BF_1-ATPase in 0.5 ml total vol. The concentration of D_2O was as indicated (•—•). Solutions were adjusted to pH (D) 8.5 by adding ($0.3314\,n + 0.0766\,n^2$) pH units to the observed pH meter reading, where n is the proportion of D_2O. In another experiment, BF_1-ATPase was preincubated for 15 min at 37°C in 100 mM Tris–SO_4, pH (D) 8.5 with D_2O at the indicated concentrations. ATPase activity was then assayed after a 10-fold dilution in medium containing no D_2O.

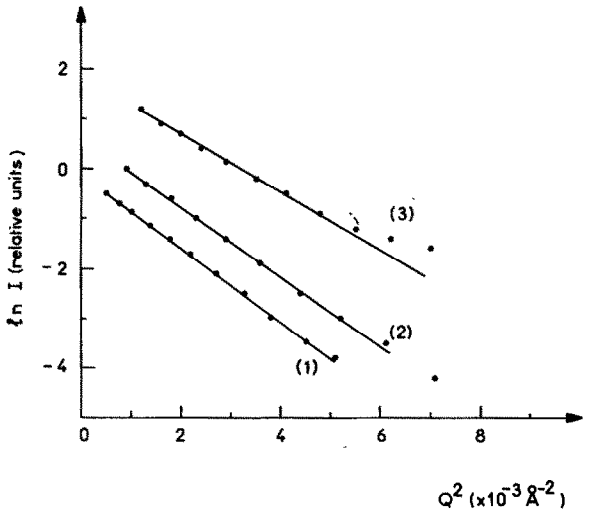

Fig.2. Guinier plots of scattering curves of *E. coli* BF_1 in H_2O and D_2O buffers. Protein concentrations, in H_2O: (1) 4.8 mg/ml, (2) 9.6 mg/ml; in 96% D_2O: (3) 5.8 mg/ml.

as Guinier plots [18]. During measurements, BF_1 showed no significant dissociation into subunits or intermolecular aggregation.

Guinier plots are linear over a very wide range and the values of R_G and $I(0)/c$ obtained are independent of concentration. Straight lines were fitted to $0.0004 < Q^2 < 0.004$ to give a radius of gyration (R_G) of 46.3 ± 0.7 Å in H_2O and 42.6 ± 0.3 Å in 96% D_2O buffer. No change in radius of gyration was observed in the presence of 2 mM ATP or 2 mM AMPPNP, a non-hydrolyzable ATP analog.

From the intensities extrapolated to zero angle, $I(0)$, in H_2O and D_2O, we find a match point of 42% D_2O for the protein, which is the same value as for other soluble proteins [15]. The molecular weight of *E. coli* BF_1-ATPase was calculated from $I(0)$ in H_2O with $\Sigma b'$ calculated from the amino acid composition (table 1). The molecular weight was found to be 315 000 ± 25 000.

3.3. *Scattering curves of the enzyme*

Neutron scattering curves obtained up to Q values of 0.2 for *E. coli* BF_1-ATPase in H_2O and D_2O medium are shown in fig.3. The scattering intensities were plotted as log $I/I(0)$, where $I(0)$ is the intensity

Table 1
Amino acid composition of *E. coli* BF_1-ATPase

Amino acid	mole %
Aspartic acid	9.5
Threonine	5.0
Serine	5.8
Glutamic acid	11.2
Proline	3.9
Glycine	9.8
Alanine	9.4
Valine	8.0
Methionine	2.3
Cysteine	1.5
Isoleucine	5.9
Leucine	9.7
Tyrosine	3.1
Phenylalanine	2.8
Tryptophan	0.7
Lysine	4.9
Histidine	1.5
Arginine	5.0

Each value is the average of 2 determinations

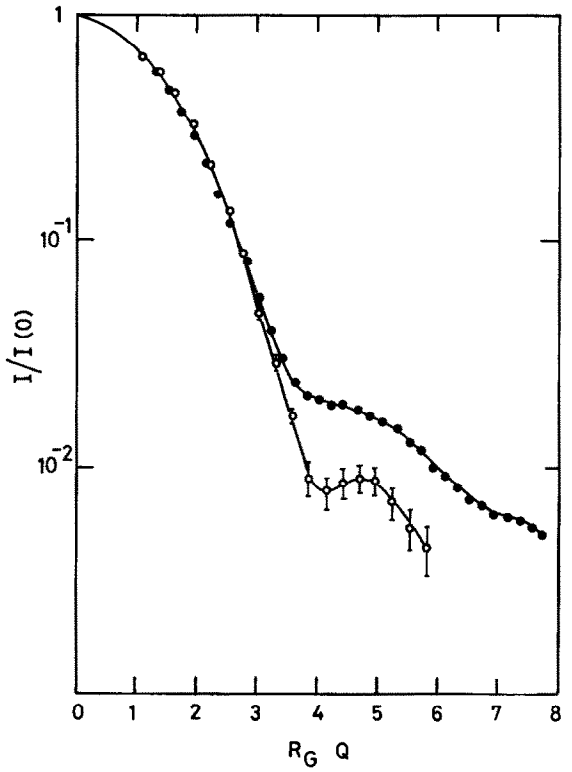

Fig.3. Experimental neutron-scattering profiles from *E. coli* BF_1 at 9.6 mg/ml in H_2O (o—o) and at 5.8 mg/ml in 96% D_2O (•—•).

at zero angle, as a function of $R_G \times Q$. The neutron scattering profiles of ATPase in H_2O and D_2O solvents are different. The side maximum observed in both curves at $R_G \times Q = \sim 4.5–5.0$ is a factor of 2 more intense in D_2O with some hint that it might be followed by a second maximum.

Correlation functions were obtained from $I(Q)$ by Fourier transformations of the intensity, $\gamma(r) = \int Q^2 I(Q) (\sin Qr/Qr)\, dQ$ (fig.4). Except for their similar crossover points, the correlation functions in H_2O and D_2O are quite different. The maximum dimension of the enzyme in H_2O and D_2O solutions was determined from the plot to be 130 ± 15 Å.

The entire scattering curves cannot be approximated by the scattering of simple homogeneous shapes. The subsidiary maxima indicate major fluctuations in scattering density within the enzyme molecule, which are different in H_2O and D_2O.

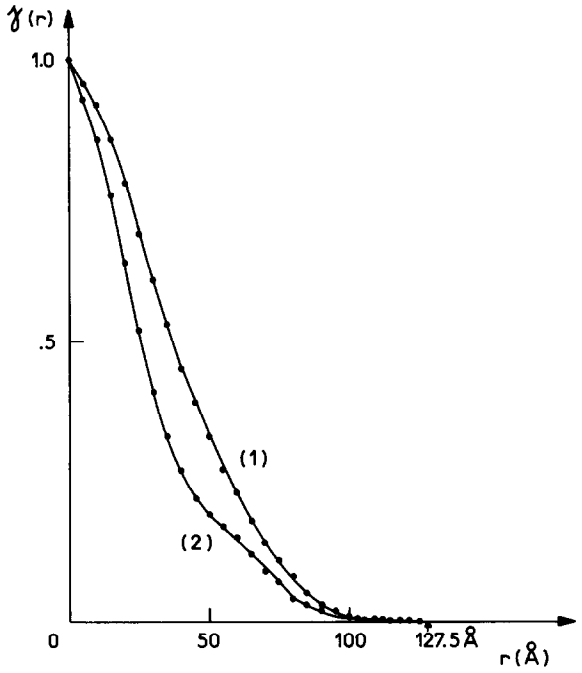

Fig.4. Fourier transform of the experimental neutron scattering profiles of *E. coli* BF_1 (1) in H_2O at 9.6 mg/ml and (2) in 96% D_2O at 5.8 mg/ml.

4. Discussion

D_2O solvents were found to inhibit reversibly *E. coli* BF_1-ATPase activity. The full extent of reversibility suggest that large changes in the conformation of the enzyme are not likely to occur in D_2O. Water has a direct role in the ATPase reaction and its replacement by D_2O is expected to influence the course of ATP hydrolysis. The effect of D_2O on mitochondrial F_1-ATPase activity was reported in [19].

Small-angle neutron scattering measurements of *E. coli* BF_1 in H_2O give $R_G = 46.3 \pm 0.7$ Å, which is decreased to $R_G = 42.6 \pm 0.3$ Å in D_2O buffer. This decrease in R_G in D_2O buffer can be interpreted in the following way. The contrast for hydrophilic groups in D_2O is much lower than for hydrophobic groups. Since the latter are usually closer to the centre of mass of the protein, the particle appears 'denser' at smaller radii, leading to the observed decrease in R_G [17].

The molecular weight of BF_1 calculated from the neutron data in H_2O is 315 000 ± 25 000. This figure should be compared to the sum of the molecular weights of the subunits. A subunit stoichiometry of $\alpha_3\beta_3\gamma\epsilon$ leads to a mol. wt 368 000 and of $\alpha_2\beta_2\gamma_2\epsilon_2$ to 304 000. Our molecular weight value calculated from the neutron data, therefore, is more in favor of an $\alpha_2\beta_2$ type of stoichiometry in isolated BF_1-ATPase.

There is no simple homogeneous body for which the theoretical scattering curve corresponds satisfactorily over the entire experimental scattering profile of BF_1. The inner portions of the profiles in H_2O are best approximated by a prolate ellipsoid (2:1, axial ratio) or a flat cylinder (2:1 diameter to height). The intense subsidiary maxima on the other hand, have exactly the same position as for a sphere. Their heights are consistent with solvent volumes, for example a cavity, within the protein, possibly related to the packing of the different subunits in the active enzyme. The fact that the maximum is higher in D_2O than in H_2O would arise from an apparently larger cavity in D_2O. This can be understood in terms of the hydrophilic groups which would line such a cavity and which have a lower contrast in D_2O than in H_2O.

Information on structural aspects of ATPases have been obtained by small angle X-ray scattering. There is a large disagreement on the structure parameters of the CF_1-ATPases from spinach [20] and *Vicia faba* chloroplasts [21], although both groups agree that a cavity exists within the CF_1-protein. The small-angle neutron scattering analysis of *E. coli* BF_1 is compatible with such a cavity within the protein. Specific deuteration combined with neutron scattering is a powerful technique in the study of multisubunit enzymes [22]. It is an approach we are now applying to the *E. coli* BF_1 enzyme and which should provide much more structural information.

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References

[1] Simoni, R. P. and Postma, P. W. (1975) Ann. Rev. Biochem. 44, 523–554.
[2] Harold, F. M. (1977) Current Top. Bioenerget. 6, 83–149.
[3] Vogel, G. and Steinhart, R. (1976) Biochemistry 15, 208–216.
[4] Kobayashi, H. and Anraku, Y. (1972) J. Biochem. 71, 387–399.
[5] Hanson, J. B. and Kennedy, E. P. (1973) J. Bacteriol. 114, 772–781.
[6] Futai, M., Sternweis, P. C. and Heppel, L. A. (1974) Proc. Natl. Acad. Sci. USA 2725–2729.
[7] Bragg, P. D. and Hou, C. (1976) Arch. Biochem. Biophys. 174, 553–561.
[8] Butlin, J. D., Cox, G. B. and Gibson, F. (1971) Biochem. J. 124, 75–81.
[9] Beechey, R. B., Hubbard, S. A., Linnett, P. E., Mitchell, A. D. and Munn, E. A. (1975) Biochem. J. 148, 533–537.
[10] Satre, M., Klein, G. and Vignais, P. V. (1978) J. Bacteriol. 134, 17–23.
[11] Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.
[12] Tanford, C. and Roberts, G. L. jr (1952) J. Am. Chem. Soc. 74, 2509–2515.
[13] Ibel, K. (1976) J. Appl. Cryst. 9, 630–643.
[14] Jacrot, B. and Zaccaï, G. (1979) submitted.
[15] Jacrot, B. (1976) Rep. Prog. Phys. 39, 911–953.
[16] Cohn, E. J. and Edsall, J. T. (1943) in: Protein, Amino-acids and Peptides, pp. 370–380, Rheinhold, New York.
[17] Ibel, K. and Stuhrmann, H. B. (1975) J. Mol. Biol. 93, 255–265.
[18] Guinier, A. and Fournet, G. (1955) Small angle Scattering of X-rays, Wiley, New York.
[19] Tuena de Gómez-Puyou, M., Gómez-Puyou, A. and Cerbon, J. (1978) Arch. Biochem. Biophys. 187, 72–77.
[20] Paradies, H. H., Zimmerman, J. and Schmidt, U. D. (1978) J. Biol. Chem. 253, 8972–8979.
[21] Süss, K. H., Damaschun, H., Damaschun, G. and Zirwer, D. (1978) FEBS Lett. 87, 265–268.
[22] Stöckel, P., May, R., Cejka, C., Strell, I., Hoppe, W., Heumann, H., Zillig, W., Crespi, H. L., Katz, J. J. and Ibel, K. (1979) J. Appl. Cryst. in press.