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Molecular Properties of the ATP Synthetase from Escherichia coli

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Summary: The molecular weight, radius of gyration and volume of the ATP synthetase from Escherichia coli were determined by small angle X-ray scattering in solution. A weight average molecular weight of 448,000 ± 23,000 for the $F_1 \cdot F_o$-complex was determined by small angle X-ray and laser light scattering at pH 7.8 at 4°C and a molecular weight of 930,000 ± 13,500 was found at 20°C. The maximum cord length of the $F_1 \cdot F_o$-complex at 4°C was determined to 17.5 ± 1.0 nm and 35.0 ± 2.0 nm at 20°C for the dimer, indicating a linear arrangement of the two $F_1 \cdot F_o$ monomers within the dimer. A possible hydrodynamic description of the $F_1 \cdot F_o$ monomer is that of a spherical cone with dimensions of H = 17.0 nm in height, a larger spherical radius of R_1 = 8.4 nm and a smaller one of R_2 = 5.9 nm, which match the experimental scattering curve satisfactorily.

Introduction

The protonophoric action of the ATP synthetase $F_1 \cdot F_o$ (E.C. 3.6.1.3, H^+-ATPase) is essential for oxidative and photophorphorylation in energy transducing reactions [1-3].

Abbreviations used:

DCCD = dicyclohexylcarbodiimide; F_1 = extrinsic ATPase sector of H^+-translocating ATPase complex; F_o = intrinsic membrane sector of the H^+-ATPase complex; $F_1 \cdot F_o$ = complete translocating ATPase complex; $CF_1 \cdot F_o$ = the H^+-translocating ATPase from spinach chloroplasts" TDC = taurodexycholate.

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In Escherichia coli as well as in mitochondria, chloroplasts and other bacteria the H^+-ATPase is composed of a soluble portion, F_1, and of a membrane-integral part, F_o, which renders DCCD-sensitivity [4, 5] that plays a crucial role in the translocation of protons [5, 6].

Whereas the F_1-portions of the H^+-ATPases from various sources show remarkable similarities, e.g. from spinach chloroplast [7], mitochondria [8] and E. coli [9, 10], in molecular weights and tertiary structure [11], no structural data of the entire complex, $F_1 \cdot F_o$, e.g. molecular weight and shape, are reported. This report presents the results of an initial study of E. coli H^+-ATPase in solution by means of small angle X-ray scattering. The enzyme solution $F_1 \cdot F_o$ in 10 mM TDC, 50 mM TRIS-HCl, pH 8.0, containing 1 mM $MgCl_2$, 0.2 mM DTT, 0.2 mM EDTA, 0.1 mM PMSF and 20% (v/v) methanol, is shown to have a radius of gyration of $R_g = 5.8 \pm 0.25$ nm and a molecular weight of 448,000 $\pm$ 21,500, determined by light scattering esperiments, also.

Materials and Methods

ATP synthetase complex $F_1 \cdot F_o$ was prepared according to Friedl et al. [12] with slight modifications as described in [13]. Protein concentrations were determined according to Lowry et al. [14] with the modification of Dulley and Grieve [15].

Densimetry measurements were performed as described in [16, 17] and the isopotential partial specific volume (ϕ) was calculated from a least squares fit from the slope of the straight line drawn between the density of the solvent medium

and that of a solution at some finite concentration of the protein [18].

Small angle X-ray scattering experiments were conducted at 4°C and 20°C in a setup as described recently [17], including data collection and correction, as well as the morphological parameters, e.g. radius of gyration, volume and molecular weight. Light scattering experiments were performed using a small angle laser light scattering apparatus as described in [17], including the determination of the refractive index increment $(\partial n/\partial c)_{\mu=const}$.

Results

The molecular weight was determined by extrapolation to zero scattering angle, where the experimental intensity was put on a particle scale by means of calibrated nickel filters (Dupont, Del., USA) according to previously published methods [19]. A molecular weight of 448,000 ± 23,000 for $F_1 \cdot F_o$ was obtained for the preparation according to Friedl et al. [12, 13], a value of 448,000 ± 22,000 for a preparation following the procedure of Foster and Fillingame [20], and a value of 460,000 ± 21,000 for the same preparation, but in the presence of 10% glycerol instead of methanol [21]. An isopotential partial specific volume of ϕ = 0.8015 ± 0.0003 $ml \cdot g^{-1}$ was applied for the calculation of the molecular weight, which was obtained by densimetry measurements. From a Guinier plot (Fig. 1) we obtained the apparent radius of gyration which was extrapolated to zero enzyme concentration, yielding a value of R_g = 5.80 ± 0.25 nm (4°C) (Fig. 1). Similar results were obtained from small angle light scattering measurements performed at various concentrations of $F_1 \cdot F_o$, as well (Table 1).

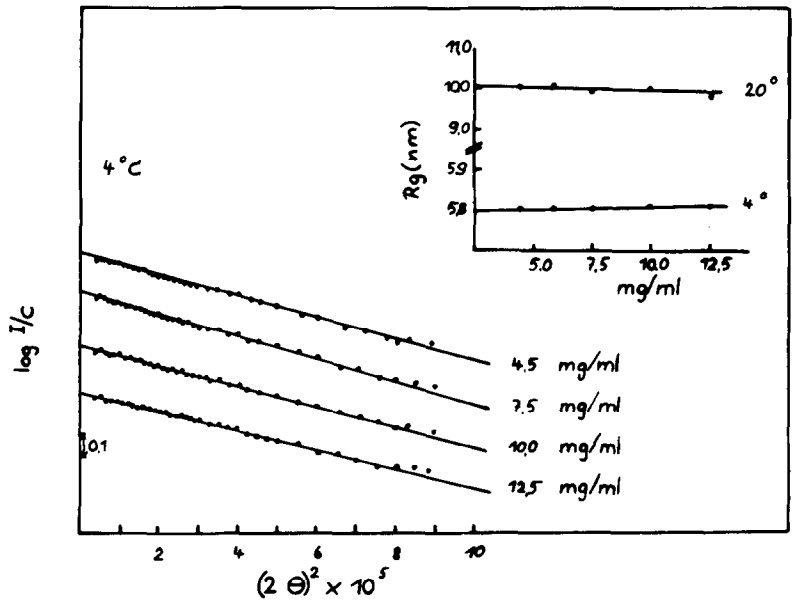

Figure 1. Guinier plot of the innermost portion of the scattering curve for $F_1 \cdot F_o$ at pH 8.0, 4°C, at various protein concentrations. $h = \frac{4\pi}{\lambda} \sin \theta$ with $\lambda = 0.154$ nm and θ = the scattering angle. Insert: The radius of gyration of the $F_1 \cdot F_o$ complex, pH 8.0, as a function of concentration, c, at 4°C and at 20° C.

$(\partial n/\partial c)_{\mu=const}$ was found to be 0.179 ± 0.003 ml·g^{-1} and independent of the protein concentration.

The possibility was investigated if undetectable aggregates, mostly dimers, may contribute to the data at larger scattering angles; model studies were carried out up to a degree of residual dimerization of 10%. These studies show that this would cause a distortion of the shape of the scattering curve for values of $\theta > 0.05$, only, but no differences were observed within the statistical error. The external surface area and the volume of the $F_1 \cdot F_o$ complex can

Table 1.

Physical parameters for the $F_1 \cdot F_o$ complex from *E. coli*, pH 8.0, at 4°C and 20°C (μ = 0.1 M).

Parameter	4°C	20°C
$M_r \times 10^5$ a)	4.60 ± 0.20	9.45 ± 0.30
$M_r \times 10^5$ b)	4.48 ± 0.23	9.30 ± 0.13
A_2, ml · $g^{-2} \cdot mol^{-1}$	7.8×10^{-3}	8.2×10^{-4}
$m_r \times 10^5$ e	2.37 ± 0.12	4.92 ± 0.16
R_g, nm	5.8 ± 0.25	10.25 ± 0.10
ω, ml·g^{-1}	0.74	0.52
V, nm^3	(1.15 ± 0.02) x 10^3	(1.90 ± 0.08) x 10^3
ϕ, ml·g^{-1}	0.8015 ± 0.0003	0.7945 ± 0.0004
$(\partial n/\partial c)_{\mu=const}$	0.179 ± 0.003	0.181 ± 0.004
R_o, nm	7.53	13.32
R_v, nm	6.51	7.68
D_{max}, nm	17.5 ± 1.0	35.0 ± 2.0

R_v is the radius of a sphere with the experimental volume, V; R_o is the radius of a sphere corresponding to the radius of gyration, R_g.

a) determined by laser light scattering.

b) determined by small angle X-ray scattering.

be evaluated by a plot of $I(h)h^4$ vs. h^4 [21] under the assumption that the scattering particle has a uniform electron density inside the particle volume, so $I(h) \times h^4$ approaches a constant value at large values of $h = \frac{4\pi}{\lambda} \sin\theta$, with = 0.154 nm ($CuK_\alpha$-radiation) and θ = the scattering angle [19]. This plot has a constant slope with an almost zero slope at $\overline{\Delta\rho} = \bar{\rho}_1 - \bar{\rho}_o = 80$ e/nm^3, with $\bar{\rho}_1$ the electron density of the enzyme complex and $\bar{\rho}_o$ that of the buffer, the volume measured

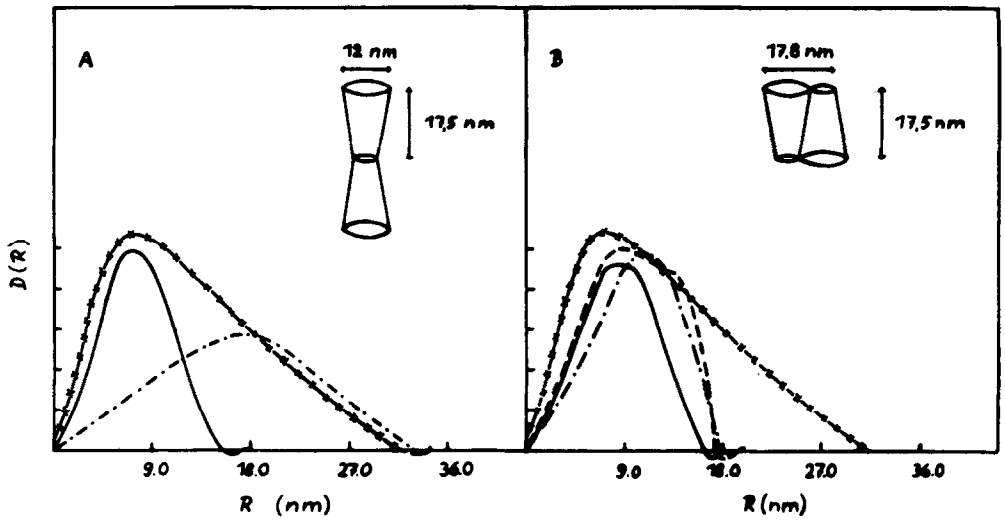

Figure 2. The distance distribution function, D(R), determined by deconvolution and Fourier-transforming the scattering data. (———) D(R) at 4°C and (×-×-×-×-×) D(R) at 20°C. The heavy solid (·-·-·-·) line is the difference between dimers and monomers. (A) linear arrangement and (B) side-to-side arrangement of two spherical cones of $F_1 \cdot F_o$.

was (1.15 ± 0.05) x 10^3 nm^3 and the external surface area was determined to (12.0 ± 0.5) x 10^2 nm^2. The main source of error in S_{ext} and V arises from the extrapolation of Ih^2 toward larger scattering angles. The measured quantity of V = (11.5 ± 0.5) x 10^2 nm^3 yields a molecular weight of 448,000 ± 23,000 when the determined isopotential partial specific volume of ϕ = 0.8015 ± 0.0003 (Table 1) is used. The scattering data (Fig. 3) of the $F_1 \cdot F_o$ complex were deconvoluted as described in [17] and were Fourier transformed to give the distance distribution function, D(R), from which structural parameters may be derived (Fig. 2). Since $4\pi D(R)dR$ is the probability of an electron pair having the separation

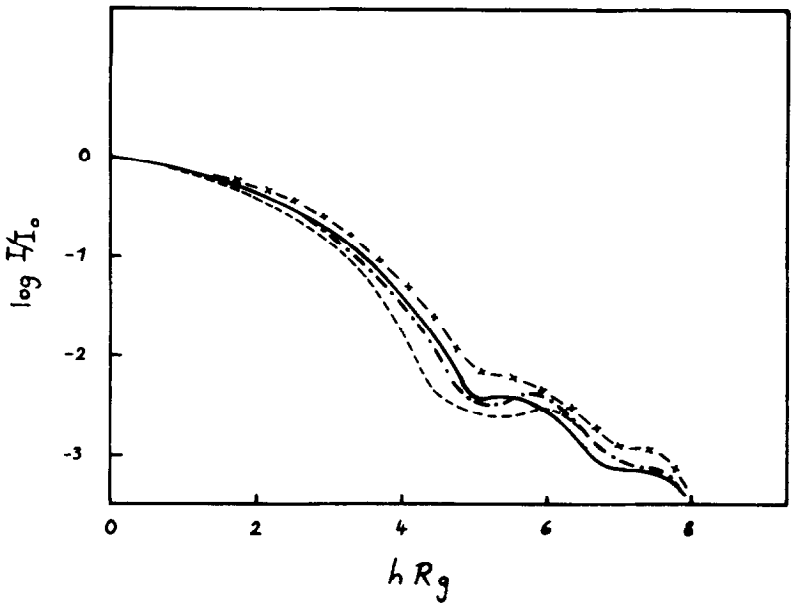

Figure 3. Comparison of the scattering from various uniform density models to the experimental data (——), all normalized to the radius of gyration of R = 5.9 nm. (–·–·–·) spherical cone with dimensions of H = 17.0 nm, R = 6.5 nm; (–×–×–) cylinder of H = 17.0 nm and R = 6.5 nm, and (– – – –) prolate ellipsoid of revolution with axial ratio 1,5 with a = 6 nm and b = 9,0 nm.

between R and R+dR, the longest expansion, D_{max}, can ideally be obtained from the R intercept. Figure 2 shows the distance distribution function for $F_1 \cdot F_o$. The small oscillation in the neighborhood of the maximum dimension is caused by residual concentration effects, hence the maximum dimension is derived to $D_{max} = 17.5 \pm 1.0$ nm ($4^{\circ}C$). The relative weakness of the oscillations and the negligible concentration effects let us conclude that the scattering pattern in all measured points is not significantly distorted, including the termination effects of the Fourier-transformation [17]. This

is consistent with the observed finding that the scattering pattern at wide angles did not change significantly over a wide range of $F_1 \cdot F_o$ concentrations from 5 mg/ml to 15 mg/ml.

Measurements conducted at 20°C of the enzyme in solution yielded different values for R_g and D_{max} which were found to be 17.5 $\pm$ 1.0 nm and 35.0 $\pm$ 2.0 nm, respectively, and the weight average molecular weight of 930,000 $\pm$ 13,500 for D_{max}. The fact that the experimental points in the Guinier region [22] are lying on a straight line, from which $\overline{M_{w,c}}$ and $\overline{R_{g,c}}$ can be obtained, confirms the absence of higher aggregates. Furthermore, the absence of such higher aggregates is confirmed by the absence of an upward deviation of the data points at the lowest angles. The formation of the aggregate was analyzed quantitatively by the use of the D(R) function. The D(R) function is shown in figure 2. The data obtained at 4°C reveal a prolate ellipsoid of revolution with an axial ratio of 1:1:1.8 for the monomer. The D(R) function of the monomer indicated in any event an elongated particle with decreasing cross section toward the ends of R. The difference in distance distribution function of the lines joining the two $F_1 \cdot F_o$ monomers within the dimer are obtained from the subtraction of the distance distribution functions of the two $F_1 \cdot F_o$ monomers at 4°C from the D(R) of $(F_1 \cdot F_o)_2$, assuming a spherical cone for the monomer. The difference distribution function between a parallel or antiparallel arrangement is not compatible with the experimental D(R), but with a linear arrangement. Specifically, the whole difference distribution function with the basis of two spherical cones, is consistent with the maximum dimension of the dimer of D_{max}^{dimer} = 35.0 $\pm$ 2.0 nm, having the main peak grouped around R = 18.0 nm at $\Delta\rho$ = 75 e/nm^3.

Discussion

The present report describes for the first time the size and shape of the H^+-ATPase ($F_1 \cdot F_o$) from E. coli which is fully biochemically active.

The experimental scattering curve for $F_1 \cdot F_o$ at 4^oC is comparable with a model having the shape of a spherical cone with approximate dimensions of H = 17.0 nm, a larger spherical radius of R_1 = 8.4 nm and a smaller one of R_2 = 5.9 nm. Scattering at small angles corresponds to low spatial resolution, therefore the acceptability of a model is determined by the magnitude of the hR_g value at which its scattering curve first begins to deviate from the experimentally determined pattern. As it can be seen in figure 3, the preliminary simple model of a spherical cone is not superior to that of a prolate ellipsoid of revolution with respect to the position of the first subsidiary maximum. Of course, some very simple models appear to yield the correct overall pattern, but they have to be excluded because of their very poor fit at higher scattering angles. More detailed studies on the $F_1 \cdot F_o$ complex are underway in order to produce a low resolution model in solution, e.g. define the location of the phospholipids or detergents, as shown for $CF_1 \cdot F_o$ [23].

An interesting finding is the aggregation of the $F_1 \cdot F_o$ from E. coli to form dimers. As shown by the distance distribution function, the aggregational process proceeds linearly with a main chord of D_{max} = 35.0 nm, indicating a high degree of asymmetry.

Although the degree of purity of the $F_1 \cdot F_o$ preparations from various groups seem to vary [24], the range of the weight average molecular weight of the entire H^+-ATPase

lies in the range between 450,000 to 480,000, depending on the amount of detergent or phospholipid bound to the protein complex [23, 25]. Since the molecular weight of the peripheral part of the H^+-ATPase (F_1) is in the range of 360,000 $\pm$ 15,000 [9], the F_o-portion of the $F_1 \cdot F_o$ complex has a molecular size of 125,000. The aggregation number of the TDC under the conditions applied here is 3 $\pm$ 1 (μ = 0.1 M) and is very weakly dependent on bile salt concentration at both temperatures (4°C and 20°C) [26]. The aggregation number of TDC at μ = 0.1 M in the presence of 10% (v/v) methanol or glycerol was found to be 4 $\pm$ 1 and completely independent of bile salt, but only dependent on the concentration of the primary alcohol. Therefore, the estimate of the molecular weight of the $F_1 \cdot F_o$ complex is not significantly influenced by the detergent used.

Although information on structural aspects of the F_1-portion has been obtained by hydrodynamic methods, as well as small angle X-ray scattering [1, 9] and single crystals [27-29], there is at present no detailed structural information available for the F_o-portion of the entire $F_1 \cdot F_o$ complex. Preparation of active F_o, which is capable of assembling with the F_1-portion of the H^+-ATPase, is underway in order to compare the size and shape of this F_o with respect to the entire $F_1 \cdot F_o$ complex.

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