EFFECT OF ATP ON THE TRANSLATIONAL DIFFUSION COEFFICIENT OF THE α-SUBUNIT OF *ESCHERICHIA COLI* F_1-ATPase

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Received 22 September 1980

1. Introduction

Coupling factors (F_1) have been isolated from a variety of membranes in which oxidative phosphorylation or photophosphorylation take place [1,2]. The ATP synthetase complex ($F_1 \cdot F_o$) is composed of two multisubunit parts:

(1) One portion, namely the coupling factor (F_1), appears to be peripheral to the membrane without disruption of the phospholipid bilayer, having mol. wt 325 000–370 000 (CF_1, ECF_1, MF_1 and TF_1) [3–6];

(ii) The remaining portion of the complex (F_o) is an integral part of the membrane and mediates proton translocation. This part of the complex (F_o) has mol. wt of an order of 90 000 [7,8], e.g., for $CF_1 \cdot F_o$, and 110 000 for $ECF_1 \cdot F_o$ in detergent solution.

Reconstruction experiments for ECF_1 have been performed revealing that α, β and γ subunits are required for ATPase activity [9,10]. Moreover, ATPase activities were reconstituted from complexes containing different subunit proportions of *E. coli* ATPase [11] as well as from purified individual subunits of the ATPase of a thermophilic bacterium (TF_1) [12].

An important finding obtained through the study of isolated subunits is that α binds ATP with a K_d value of 0.9 μM, and 0.1 μM for ADP [13]. This paper describes the hydrodynamic property of the α-subunit of ECF_1 in the presence and absence of ATP by means of inelastic light scattering, indicating a 15% change in the translational diffusion coefficient, a decrease of a frictional actual ratio, f/f_o, from 1.47–1.29.

Abbreviations: F_1, the water soluble portion of the coupling factor or ATPase complex; CF_1, chloroplast coupling factor; MF_1, mitochondrial coupling factor; ECF_1, *E. coli* coupling factor; α, β, γ, δ, ε, the subunits of the coupling factors in order of decreasing molecular weight

2. Materials and methods

ECF_1 was prepared as in [4] from *E. coli* strain K-12 (λ). The α-subunit was isolated by the hydroxyapatite DEAE–Sepharose method in [13]. The buffer used for studying α under native conditions consisted of 0.05 M Tris–HCl (pH 8.0), 0.1 M NaCl, 0.1 mM EDTA and 0.1 mM dithiothreitol (4°C). ATP (Sigma, St Louis, MO) was added to 20 μM final conc. Excess of ATP was removed by Sephadex G-25 column chromatography.

Elastic and inelastic light-scattering experiments were done at 4 ± 0.05°C in a thermostatted cell as in [14,15]. A photo-counting correlator was used to analyze the signal [16], and a 5 mg/ml α (or α-ATP) solution normally yielded 12 900 photons/s. The results were reproducible within 1%. Each point here represents the average of ⩾6 runs with $\sim 5 \times 10^5$ counts in the correlation function. The data for the individual runs for α and α-ATP were averaged for 0.5–10 min, depending on the protein concentration.

3. Results

From inelastic light-scattering experiments the translational diffusion coefficient was obtained, whereas from elastic light-scattering experiments a weight-av. mol. wt 58 750 ± 2100 was determined, $(\partial n/\partial c)_{\mu\ =\ \text{const.}} = 0.1841$ ml/g obtained from measurements of the refractive index [14]. The molecular

weight of the α-subunit, measured in the presence of ATP, remains essentially the same (58 700 ± 2100), indicating that α remains in the monomeric state in the presence or absence of ATP. From the logarithmic plot of the autocorrelation function vs delay time the decay constant of $\tau = 29.6$ μs was obtained for the α-subunit, and $\tau = 23.2$ μs for α-ATP (fig.1). However, from the plot of the slopes against the square of the scattering vector, $K^2 = ((4\pi n/\lambda) \sin \theta/2)^2$, the diffusion coefficient for the α-subunit was determined, extrapolated to zero protein concentration to be $(5.64 \pm 0.02) \times 10^{-7}$ cm²/s. The corresponding value for α-ATP was determined to be $(6.45 \pm 0.02) \times 10^{-7}$ cm²/s (fig.2). Furthermore, the photo-current autocorrelation function can be described by a single exponential without any contribution from higher molecular forms of α or α-ATP, respectively, which is indicative of a highly homogeneous sample, at least in the concentration range shown in fig.1. Moreover, there was no angular dependence for D, indicating that the single particle-scattering factors of the individual components were all very similar. The apparent diffusion coefficient is:

$$D = D^{o}_{20,w} [1 + (B_2 - B^1)c]$$

$$= D_o [1 + (0.45\bar{\nu}_2 + \frac{10^3 \bar{\nu}_1 z^2}{2 \mu M})c + \ldots]$$

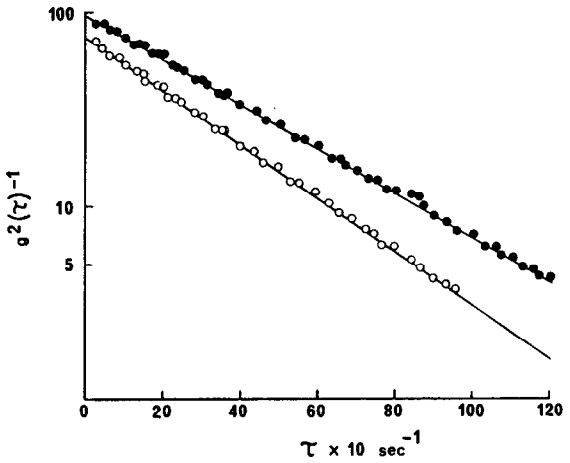

Fig.1. Clipped correlation function for scattered light for α (○——○) and α-ATP (●——●) in a buffer containing 0.05 M Tris–HCl (pH 8.0), 0.01 mM DTT, 0.1 mM EDTA, at 20°C. Least-squares fit yields $\tau_c = 29.6$ μs for the α-subunit, and 23.2 μs for α-ATP, corresponding to translational diffusion coefficients of $(5.64 \pm 0.02) \times 10^{-7}$ cm²/s and $(6.45 \pm 0.02) \times 10^{-7}$ cm²/s, respectively.

with: D_o, the diffusion coefficient at zero protein concentration [11]; $\bar{\nu}_2$, the partial specific volume of the α-subunit (0.7281 ml/g; H. P., unpublished); μ, the ionic strength; z, the average number of electrons on the protein; M, the weight-average molecular weight;

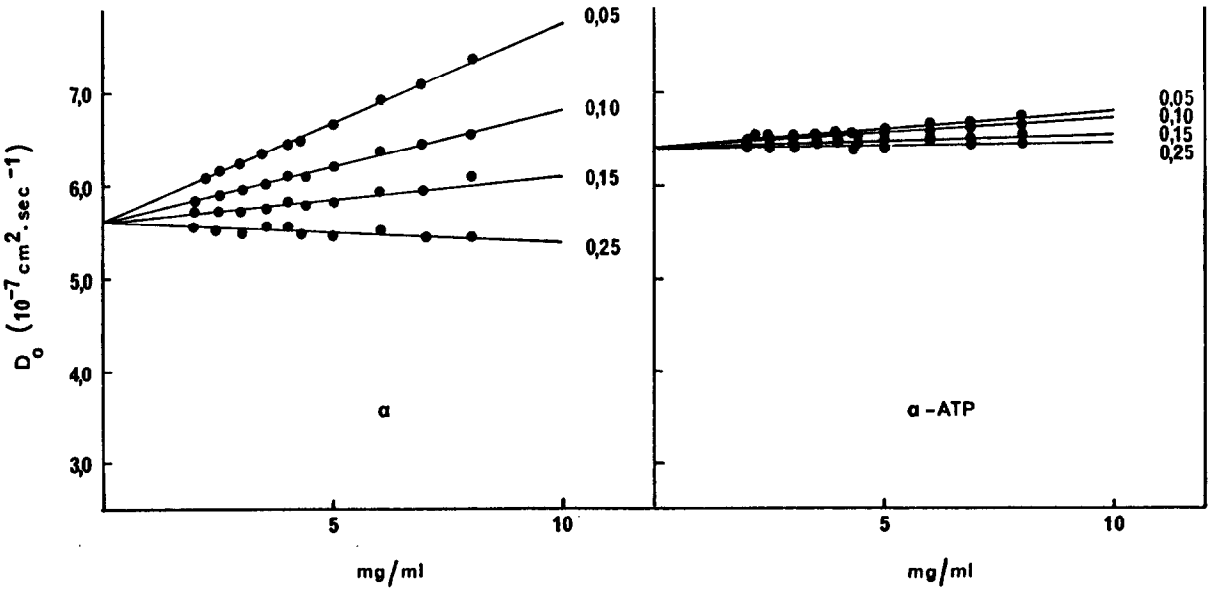

Fig.2. Dependence of the diffusion coefficient of α-subunit concentration (a) and α-ATP (b) at pH 8.0 and at different ionic strengths (0.05, 0.10 and 0.25 M).

Table 1
Effective hydrodynamic radii for α and α-ATP at pH 8, at 20°C, and at various ionic strengths

Sample	μ	(B_2-B^1) cm³/g	B_2(cm³/g)	R_H(A)[a]	R_H(A)[b]
α	0.01	13.51	18.36	38.2	37.5
α-ATP	0.01	9.08	13.93	35.0	34.2
α	0.05	14.26	19.11	38.4	38.0
α-ATP	0.05	9.28	14.12	35.2	34.3
α	0.10	14.57	19.42	38.5	38.2
α-ATP	0.10	9.35	14.20	35.5	34.4
α	0.15	15.36	20.2	38.7	38.7
α-ATP	0.15	9.45	14.3	35.6	34.5
α	0.20	16.65	21.5	38.9	39.5
α-ATP	0.20	9.83	14.67	35.8	34.8

[a] Calculated from $B_2 = (N_A/M)\ (32/3)\pi R_H^3$(ml/g)
[b] Calculated according to $D = (k_B \cdot T)/(6\pi\eta_0 \cdot R_H)$

c, the concentration in mg/ml; $\bar{v}_1$, the partial specific volume of water, and is linearly dependent on the concentration of α-subunit at increasing ionic strength, μ. This linear relationship holds from 0.01–0.25 M NaCl at 0.05 M Tris–HCl (pH 8.0). For uncharged macromolecules, B_2 depends only on the excluded volume, whereas B^1 at given pH characterizes the concentration dependence of the frictional coefficient, f, and is $B^1 \simeq 6.55 \times \nu_2$ (ml/mg), and it is assumed to be independent of ionic strength. The values for B_2-B^1, B_2 and R_{HS} at pH 8.0 and different ionic strength are listed in table 1 together with the calculated hydrodynamic radii.

The dependence of D on α-concentration was studied at ionic strengths of 0.20–0.01 M at pH 8.0. Fig.1 shows D as a function of α-concentration at various ionic strengths at pH 8.0 and the extrapolation to infinite dilution yielding a value for α of $D = (5.64 \pm 0.02) \times 10^{-7}$ cm²/s and α-ATP of $D = (6.45 \pm 0.02) \times 10^{-7}$ cm²/s. Thus the diffusion measurements yielded the same extrapolated diffusion coefficient at high and low ionic strength at given pH, and in the concentration range studied. Furthermore, at lower ionic strengths of 0.05–0.15 M, D increases with α-concentration linearly, and at any finite α-concentration D changes inversely with the ionic strength. The same is true for α-ATP at pH 8.0, but the slope is not that pronounced as for the α-subunit alone.

Furthermore, by plotting the quantity $2B^1 M$ vs μ^{-1}, a surprising picture emerges (fig.3), the second virial coefficient is negative. This result reveals that a reversible association of monomeric α can occur under these conditions. However, the extrapolated value for α-ATP under the same conditions is close to zero, indicating that under these conditions there is no association of α-ATP.

4. Discussion

The α-subunit alone at pH 8.0 exists in a fairly compact form, although anisometric, whereas the

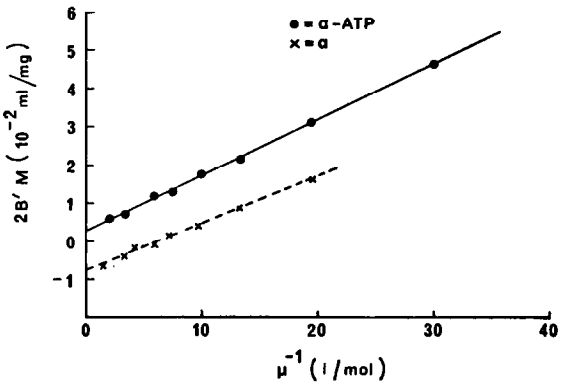

Fig.3. Effect of the ionic strength on the diffusional virial coefficient of the α-subunit (○——○) and α-ATP (●——●).

α-subunit in the presence of ATP is more isometric. The α-subunit has a relatively large positive charge at pH 8.0, and a medium ionic strength is required to shield the intraionic repulsions between different parts of the α-subunit at this pH so that a part of this positive charge can be neutralized by the strong binding of chloride anions (fig.3). Hence, a significantly higher ionic strength of the solution ($\geqslant 0.08$) is required to provide ionic shielding in these positively charged α-subunits, as deduced from diffusion coefficient measurements by means of inelastic light-scattering experiments. In the presence of salt (fig.2) the concentration dependence of D can be explained in terms of the second virial coefficient or excluded volume. Therefore, one can conclude that the moderate aggregation of α, which apparently is suppressed by addition of ATP, is due to ionic interactions because it can be decreased by adding salt. Furthermore, $D^{\circ}_{20,w}$ for α and α-ATP is dependent on ionic strength (fig.3). Thus, the apparent increase in the effective diameter according to table 1 is due to unscreened charges on the α-subunit and not due to an increased physical expansion of the molecule at low ionic strength at pH 8.0 only over the values observed at high ionic strength. Therefore, the increase in $D^{\circ}_{20,w}$ of the α-subunit in the presence of ATP at pH 8.0, as well as the changes of the overall shape of the α-subunit, are not only due to a change in surface charges, but a significant increase of the physical expansion of the α-subunit is accompanied by binding of ATP at pH 8.0, also. Moreover, it should be pointed out that the values of R_{HS} at low ionic strengths are only of qualitative significance since the net charge on the protein molecule is expected to vary with both α-concentration and ionic strength.

The 15% difference in $D^{\circ}_{20,w}$ when ATP is bound indicates a conformational change which appears to be very large for non-denaturing transitions. The increase in $D^{\circ}_{20,w}$ is due to a reduction of the frictional ratio from 1.46–1.29 due to shape. Even taking a degree of hydration of 0.2 ml H_2O/g protein into consideration, the change in $D^{\circ}_{20,w}$ cannot only account for a change in the number of water molecules associated with the α-subunit upon binding of ATP, but more likely to a change in tertiary structure. The remaining deviation from spherical behavior of α-ATP is reflected in the actual frictional ratio which is significantly >1, resulting in an apparent axial ratio of 3 and 6, respectively, applying prolate ellipsoid of revolution, and 4 and 7, respectively, for an oblate one.

Acknowledgements

The author wishes to thank Drs S. Dunn, L. A. Heppel and Y. Kagawa for valuable criticism and suggestions with respect to this work, and Dr L. A. Heppel for supplying the purified α-subunit.

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