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INELASTIC LIGHT SCATTERING STUDIES OF SOLUTIONS OF THE CHLOROPLAST COUPLING FACTOR (CF_1) AT HIGH AND LOW IONIC STRENGTH

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Summary: The translational diffusion coefficient of CF_1 at low and high protein concentration as well as at different ionic strength (0.05-1.65 M) wsa determined by means of quasi-elastic light scattering experiments. The diffusion coefficient changes from $D^0_{20,w} = 3.12 \times 10^{-7}$ cm^2 · sec^{-1} at 0.05 M, pH 7.8, 20°C, to $D^0_{20,w} = 3.52 \times 10^{-7}$ cm^2 · sec^{-1} at 1.6 M, pH 7.8, 20°C. At high enzyme concentration (20 mg/ml) and under crystallization conditions (Paradies, BBRC 91: 685, 1979) CF_1 behaves as a solution of "true" hard spheres, whereas at low salt concentration the ionic atmosphere has a larger spatial extent, resulting in a higher effective hydrodynamic radius (R_H = 65 Å).

INTRODUCTION

The chloroplast coupling factor (CF_1) is built up of a complex of five subunits, α, β, γ, δ and ε with molecular weights of 58,500, 54,500, 32,000, 20,000 and 13,000, respectively (1-4). The molecular weight of CF_1 was found to be 335,000 (5, 6), and recent crystallization studies of CF_1 (7, 8) yielded single crystals having the space group P422 with half a molecule in the asymmetric unit cell (7). However, crystallization studies of CF_1 in the absence of ATP revealed a total length of CF_1 of 120 Å, inferred from the pair distribution function from X-ray scattering studies (5). This surprising result prompted us to investigate the hydrodynamic properties of CF_1 at various pH, ionic strength as well as in the presence and absence of ATP by means of measuring the diffusion coefficients of CF_1 by inelastic light scattering.

Abbreviations used are: CF_1 = chloroplast coupling factor; α, β, γ, δ, ε = subunits of the CF_1 ATPases in order of decreasing molecular weight.

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MATERIALS AND METHODS

Chloroplast coupling factor 1 (CF_1) was prepared as described in (9, 10) according to a modified procedure of Lien and Racker (9). CF_1 depletion of nucleotides was achieved by passing CF_1 through a Sephadex G-25 column, equilibrated with 0.05 M TRIS-SO_4, pH 7.8, containing 50 % (v/v) glycerol and 0.01 M NaCl (20°C). The enzyme eluted in the void volume was dialyzed against 0.05 M TRIS-SO_4, pH 7.8 (20°C), containing 0.005 M NaCl. 10 % SDS polyacrylamide gel electrophoresis (5) showed all five principal bands, and the specific activity of CF_1 was 34 μmole P_i/mg protein (units) (5). Total phosphorus per A 278 unit (CF_1) was determined according to Ames (11) for testing the depletion of nucleotides (ATP, mainly) after the Sephadex G-20 column.

Inelastic light scattering. Measurements of the translational diffusion coefficient was performed as described in (5). The resulting spectrum was analyzed by fitting a single Lorenzian by a nonlinear least squares procedure to obtain the half width from which the diffusion coefficient can be directly calculated. The accuracy of the instrument was examined by measuring the homodyne spectrum from a sample of polystyrene latex spheres of known diameter. The extimation with respect to accuracy of the measurements was better than 2.5 %.

RESULTS

Figure 1 shows the angular dependence of the decay rate of the correlation function for a dilute solution of CF_1 (5 mg/ml) in the presence and absence of ATP at pH 7.8 (μ = 0.1 M) at 20°C. The scattering vector, $K = \frac{4\pi n}{\lambda} \sin\theta$ was varied both by altering the scattering angle,

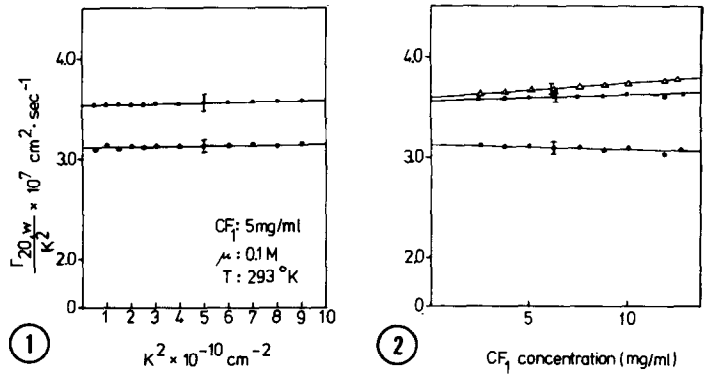

FIG. 1. Angular dependence of the decay rate of the correlation function for a dilute solution of CF_1 (20°C).

FIG. 2. Diffusion constant of CF_1 as a function of concentration. ○——○ pH 6.5 with 2.5 mM ATP; △——△ pH 8.0 with 2.5 mM ATP; ●——● pH 7.5 without ATP. μ = 0.05 M.

θ, and the wavelength, λ. For most values of K, Γ/K^2 is constant within 1.5 %, which is the expected statistical error where $\Gamma = DK^2$ with D the translational diffusion coefficient. Figure 2 shows the results of photon correlation measurements on a concentration range for CF_1 at different pH, absence and presence of ATP. The values of D have been converted to standard conditions, 20°C, and water as solvent. At these low concentrations, < 5 mg/ml, there is no evidence of concentration dependence of D, indicating that the macromolecules are far enough separated so that the effect of interparticle interactions is negligible. Different values for $D^{\circ}_{20,w}$ for CF_1 are obtained at low and high pH, as well as in the presence and absence of ATP, and result in different values for the effective hydrodynamic radius, R_e, seen in figures 1 and 2, hence they reflect real differences in the hydrodynamic volumes of CF_1.

Figure 3 shows the results obtained from photon correlation spectroscopy experiments for CF_1 at pH 7.8 at different ionic strength. The CF_1 concentration was 20 mg/ml, the same concentration as that used for growing single crystals of the beef heart enzyme (8), liver enzyme (12), as well as CF_1 (7). Since the measurements were conducted two pH units away from the isoelectric point of CF_1, the molecule (CF_1) can be expected to carry a charge of some electron units. This

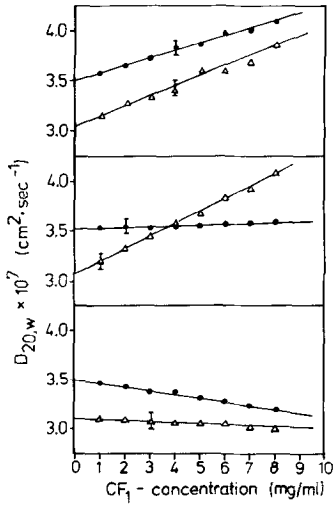

FIG. 3. Diffusion constant of CF_1 (pH 7.8) as a function of enzyme concentration and solution ionic strength. O——O with ATP; Δ——Δ without ATP.

charge will be shielded by a diffuse atmosphere of small ions of opposite charge, which depends entirely on the ionic strength of the solution. The Debye-Hückel length for 1 M NaCl is $\sim$ 3 Å, for $H_2PO_4^-$ it is 4.5 Å; thus, at this salt concentration the CF_1 solution behaves as a solution of "true" hard spheres and the usual expression for $D = D_o[1+(8-K_f)\Phi....$ can be applied, yielding values for CF_1 of $D_o = 3.45 \times 10^{-7}$ $cm^2 \cdot sec^{-1}$; $K_f = 7.15$ and Φ the volume fraction of CF_1. Furthermore, D, according to this equation, revealed a much smaller concentration dependence than for conventional light scattering or sedimentation coefficient, which is proportional to 1/f, yielding a small downward trend for D as a function of CF_1 concentration.

However, at low salt concentrations the ionic atmosphere will have a larger spatial extent, of course. For 0.15 M, the Debye-Hückel length is approximately $\sim$ 10 Å, whereas at μ = 0.01 M it is about 25 Å. Figure 3 reveals that, at a given CF_1 concentration, D increases with decreasing salt concentration, and, therefore, with increasing effective hard sphere size. Since the results shown in Figure 3 indicate the existence of coulombic interparticle interactions of CF_1 molecules, experiments were conducted at very low salt concentration and in pure water containing 2 mM EDTA, normally used for detaching CF_1 from the chloroplast membrane. The results are shown in Figure 4 where the average intensity and apparent diffusion coefficient, Γ/K^2, are plotted versus K^2. Large deviations from normal behavior can be seen, the mean intensity increases by a factor of 1.5 with increasing scattering angle. Furthermore, the apparent

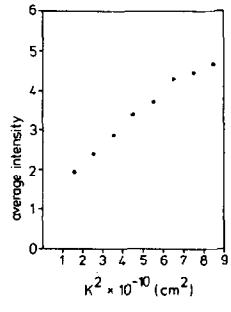

FIG. 4. Average intensity of light scattered by CF_1 (pH 7.0) as a function of scattering angle. μ = 0.001 M.

diffusion coefficient decreases, also, (Fig. 1) by a factor of 1.4, due to repulsive interparticle interactions with increasing K. This effect can be contrasted with the effect of finite particle size of CF_1, having an ionic atmosphere of the order of 70-80 Å, instead of 55-60 Å as determined at moderate salt concentrations (μ = 0.1 M), or in the presence of ATP.

DISCUSSION

The values for the effective hydrodynamic radius of CF_1 is i) dependent on the presence of ATP and ii) on the ionic strength. The results at high ionic strength reveal that CF_1 exists in a compact form, as well as at high pH, since only a small ionic strength is required to shield the intraionic repulsions between different parts of the CF_1 molecule in the pH range 7.0-9.0.

In the presence of an excess of salt the concentration dependence of D can be explained in terms of the second virial coefficient or excluded volume. Comparing of the diffusion coefficients of CF_1 at low enzyme concentration but high ionic strength, the typical values for the second virial coefficient are B_3 = 62.5 cm^3/g in the presence of ATP and B_3 = 91.5 cm^3/g in the absence of ATP, yielding a hydrodynamic radius of R = 55.5 Å and R = 67.8 Å, respectively. Thus, the diffusion coefficients for CF_1 (5 mg/ml) at pH 7.8 at high ionic strength ($\geq$ 0.1) are very close to the corresponding $D^{\circ}_{20,w}$ values in the presence of ATP at different pH, and the changes in these $D^{\circ}_{20,w}$ values reflect true changes in the hydrodynamic volume of CF_1.

At low ionic strength and pH the electrostatic repulsive energy between unscreened, charged CF_1 molecules plays an important role in the diffusive motion. Although the scattering molecule CF_1 may still be around 55 Å, its large charge can give rise to an increased effective diameter. By replacing the hydrodynamic radius by an effective hard sphere radius, this particular radius will depend only on the degree of screening produced by the salt solution, as well as only on the total charge of the enzyme molecules. So, as seen in figures 1 and 3, the effective hard sphere radius increases with increasing CF_1 charge as the ionic strength is

lowered, due to the decreasing screening effect of salt. However, the diffusion coefficient at low ionic strength, but in the presence of ATP, is very close to the corresponding value of $D^{o}_{20,w}$ of medium ionic strength (μ = 0.5), having a radius of R_e = 58 Å, and this change in $D^{o}_{20,w}$ reflects a true change in the hydrodynamic volume of CF_1.

The diffusion data of CF_1 at low and high ionic strength as well as in the presence and absence of ATP, suggest that CF_1 is probably enlarged like a cell at low ionic strength and contracts at high ionic strength and in the presence of ATP, which apparently has a significant effect on the effective hydrodynamic radius that is not so ionic strength dependent.

Information on structural aspects of coupling factors have been obtained from small angle X-ray scattering studies, also (5, 13-15), especially for CF_1 (5, 13). The data obtained are consistent with a quasispherical molecule with a cavity within the multimeric enzyme (5), but they are inconsistent with the results obtained for CF_1 purified from Vicia faba chloroplast (13). However, the data for Vicia faba CF_1 is consistent with a hydrodynamic radius of R = 75 Å, a result similar to that obtained for spinach chloroplast CF_1 at low ionic strength (μ = 0.05 M) in the absence of ATP, yielding a radius of gyration of R_g = 55.6 Å according to $R_g = k_B \cdot T/(6\pi\eta_o D^{o}_{20,w}\sqrt{2})$, consistent with the results obtained by Süss et al. (13). X-ray scattering measurements of CF_1 from spinach chloroplast seem to corroborate these findings obtained from inelastic light scattering methods, also (15).

In conclusion, the finding that $D^{o}_{20,w}$ is independent of the ionic strength when ATP is present argues strongly for an apparent decrease in the effective diameter of CF_1 and is due to a decreased physical expansion of the molecule. However, the apparent increase in the effective diameter is due to unscreened charges on CF_1 in the absence of ATP and is not due to an increased physical expansion of the enzyme at low ionic strength over the values observed at high ionic strength.

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