The structure of the ε-subunit from the chloroplast coupling factor (CF_1) studied by means of small angle X-ray scattering and inelastic light scattering

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Summary: The ε-subunit from the chloroplast coupling factor (CF_1) was purified on a BioGel A 0.5 m column, and the size, shape and radius of gyration were determined. The diffusion coefficient, $D_{20,w}$, was determined by means of inelastic light scattering and was found to be (11.3 ± 0.05) x 10^{-7} $cm^2 \cdot sec^{-1}$ and independent of pH and ionic strength. From the sedimentation coefficient (1.70S) and $D^{\circ}_{20,w}$ we obtained a molecular weight of 11,900 with a partial specific volume of 0.740 ± 0.003 $ml \cdot g^{-1}$. The radius of gyration of ε in 0.05 M TRIS-HCl, pH 7.0, was found to be 11.8 ± 0.04 Å, the volume 17.0 x 10^3 ($Å^3$) and the specific inner surface 0.19 $Å^{-1}$, indicating a spherical molecule with overall dimensions of D = 31.8 Å with a given axial ratio of 1:1:2.4. The description of the ε-subunit in solution as a prolate ellipsoid of revolution with half axes a = b = 12.7 Å and c = 25.4 Å was obtained from a comparison of the theoretical and the experimental scattering curves. The degree of hydration was determined to be 0.15 g H_2O/g protein.

The chloroplast coupling factor (CF_1) contains five subunits, α, β, γ, δ, and ε, with molecular weight of 56,000, 54,000, 32,000, 20,000 and 13,500 (1, 2), respectively, and serves as a coupling factor for phosphorylation. Furthermore, electron microscopy studies showed a spherically shaped particle of solubilized CF_1 with a diameter of approximately 90 Å. A derivative of CF_1, containing only the α, β, and γ subunits, has ATPase activity that can be inhibited by the ε-subunit, but it does not bind to the membrane (3, 4). Since most of the biochemical information concerning the individual subunits of CF_1 has come from immunological studies (5) and investigations of the specific binding of different ligands

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to the purified CF_1 (6, 7), nothing is known about the shape and hydrodynamic behavior of the individual subunits. A chemical approach in the study of the extent of the subunits within the organization of the CF_1 enzyme is chemical cross-linking with reagents of different molecular lengths (8). In this paper we report the successful determination of the shape and size of the ε-subunit of CF_1 in 0.05 M TRIS-HCl, pH 7.0, as well as its hydrodynamic properties, determined by means of diffusion coefficient measurements. From these investigations we can show that the ε-subunit is an almost spherical protein in solution.

MATERIALS AND METHODS

CF_1 was prepared from spinach according to Lien and Racker (9). The purified enzyme has a specific ATPase activity of 15 μg/ml · min under the following assay conditions: 0.8 mM disodium ATP, 10 mM $CaCl_2$, and approximately 10-15 μg/ml of CF_1, pH 8.0, 25°C, in 0.05 M TRIS-HCl, using the pH-stat technique. The ε-subunit was prepared at 4°C in 0.05 M TRIS-HCl, pH 7.0, containing 10 mM β-mercapto-ethanol and 5 mM $MgCl_2$, by dissociating the multimeric complex through continous freezing and thawing in salt solutions with ionic strengths ranging from μ = 0.1 - 0.2 M ($MgCl_2$). The purification of the ε-subunit on a DEAE-cellulose column and by chromatography on a BioGel A-0.5 m column in 0.05 M TRIS-HCl, pH 7.0, containing 0.05 M $MgCl_2$ at 4°C, yields about 0.200 mg ε-subunit, if one starts with 25 mg CF_1. The purification on a large scale will be described in detail elsewhere. Sodium-dodecyl sulfate polyacrylamide gel electrophoresis (10) in 7.5% gels, containing 1% detergent, showed one single band of a molecular weight of 13,000.

Small angle X-ray scattering experiments were performed using a Kratky camera with divergent beam geometry. The X-ray source was a rotating anode (GX 13, Elliot, U.K.), operating at 25 mA and 30 kV with fine focus setting of 0.1 x 1 mm^2. The entrance slit width was 150 μ with a corresponding counter slit width of 250 μ, resulting in a resolution of approximately 1000 Å at a sample-to-detector distance of 220 mm. The experimental setup, e.g. step scanning device and monochromator, is described in (11, 12). Normally, 90 steps were scanned for each scattering curve by counting 10^5 pulses for each measurement, which corresponds to a statistical error of 1.5%. The measured intensity was first corrected for absorption and thickness, then the excess intensity was calculated and plotted against the scattering angle. The constant scattering background, coming from fluctuations in the electron density within the scattering particles, was eliminated according to Luzzati (13). The values obtained were then converted for collimation error (14).

Light scattering experiments were performed by means of

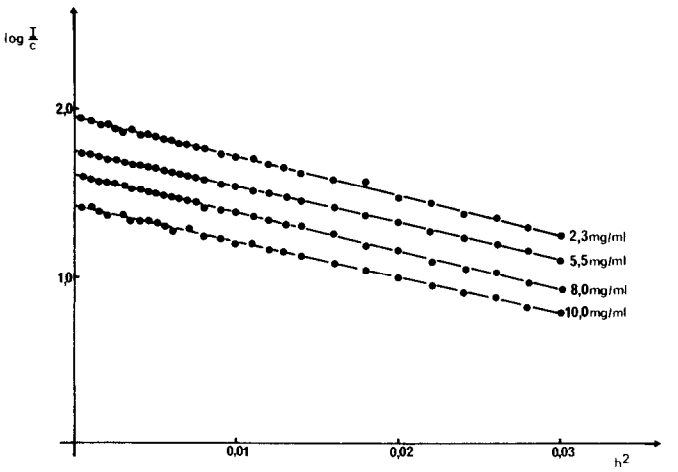


Fig. 1. Guinier plot (log I/c) versus h^2 at smallest angles for various concentrations, c, of the ε-subunit of CF_1 in 0.05 M TRIS-HCl, pH 7.0.

the homodyne method, using a 50 mWatt He-Ne-laser as a source of incident monochromatic light. The 6328 Å laser light was passed through appropriate density filters that focuss the light into the sample cell which was located in a thermostatically controlled chamber (20 ± 0.05°C). The single exponential decay autocorrelation function, calculated with a Saicor Analyzer Model 43 A (400 channels), in the clip mode, gives a homodyne decay constant, $1/\tau$ (15), which yields the diffusion coefficient (D) according to the equation

$$1/\tau = 2D(4\pi n/\lambda_o)^2 \sin^2(\theta/2).$$

The scattering angle (θ) was between 40° and 90°. λ_o = the wavelength in vacuum, and n = the refractive indes. The whole experimental setup is described in (15).

RESULTS

The scattering curve of the ε-subunit at smallest angles, according to Guinier (16), is shown in figure 1. From the slope of the innermost portion of the scattering curve we obtained a radius of gyration of 11.80 ± 0.04 Å in 0.05 M TRIS-HCl, pH 7.0, and 5 mM $MgCl_2$, and a radius of gyration of 11.81 ± 0.04 Å for the protein in the same buffer, but containing 1 M sucrose. All other values obtained from the small angle X-ray scattering experiments are listed in table I.

Table I. Gross morphological parameters of the ε-subunit from coupling factor (CF_1) obtained from small-angle X-ray scattering experiments in 0.05 M TRIS-HCl, pH 7.0, containing 10 mM $MgCl_2$ (20°C).

Unit	Value
Radius of gyration, R (Å)	11.80 ± 0.07
Molecular weight, x 10^{-4}	1.14 ± 0.05
Volume, x 10^3 (Å^3)	17.05
Surface, x 10^3 (Å^2)	3.22
α = S/V, (A^{-1})	0.19
r_g (Å)	15.32
r_v (Å)	15.97
r_s (Å)	16.05
$\bar{\nu}$ (ml · g^{-1})	0.740 ± 0.003
Degree of hydration	0.15
$\bar{\rho}_i$ (e A^{-3})	0.4205
$\bar{\rho}_i V$ (10^3 · e)	7.169
a/b from ($3V/4\ R^3$)	2.47
a/b from ($\frac{S}{V}$ · R)	2.26
u_i (10^3 · e)	6.209
Dimensions (Å)+ a(Å)	25.4
b(Å)	12.7

+Assuming prolate ellipsoid of revolution with $R = b\sqrt{(2+a^2)/5}$ and $V = \frac{4}{3}\pi b^3 \cdot a$ (see text).

$\bar{\rho}_i$ is the buoyant density; α is the specific inner surface; r_g, r_v, and r_s are the radii of the spheres whose radii of gyration, volume and surface are R, V and S; the deviation of these radii is an indication of the departure from a spherical shape; u_i is the number of electrons to one ε-particle; $\bar{\rho}_1 \cdot V$ is the number of electrons of one solvated particle.

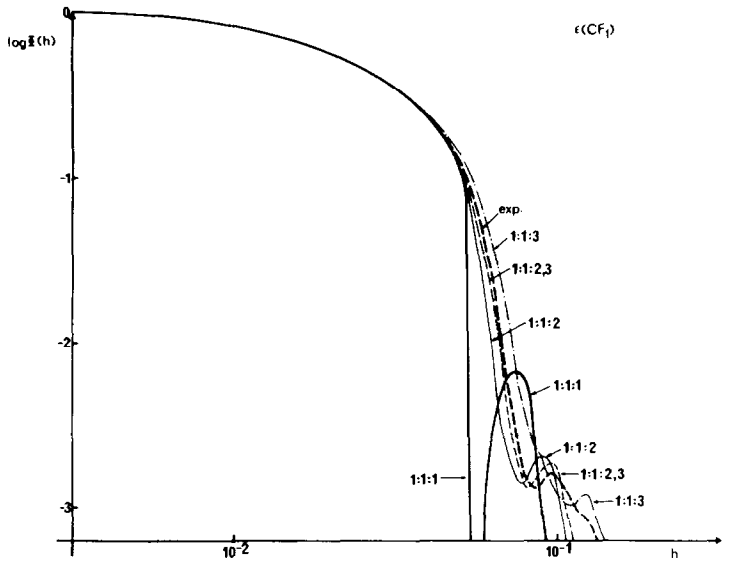


Fig. 2. Theoretical (-------) and experimental (————) scattering curves for various models of spherical particles, normalized to $\Phi(h) = 1$, with $h = 4\pi/\lambda \sin\theta$; $\lambda = 1.54$ Å and θ = the scattering angle.

The dimensions of the ε-subunit of CF_1 can be determined from R, V, M, and $\alpha = S/V$ (A^{-1}), if the partial specific volume is known. Measurements of the partial specific volume for the ε-subunit in 0.05 M TRIS-HCl, pH 7.0, yield a value of 0.740 ml · g^{-1} when using the digital density measuring device DMA-02 (A. Paar, Graz, Austria). The molecular weight, $\bar{M}$, was determined from small angle X-ray scattering experiments according to

$$M = k \cdot \frac{I_o}{P_o} \cdot \frac{a^2}{b \cdot c(z \cdot \bar{\nu} \cdot \rho)^2}$$

with I_o = zero intensity in erg/sec · cm^2, p_o = the primary energy per time unit (erg · sec^{-1}), a = distance between sample and plane of registration (220 mm), b= thickness of the sample in mm, c = concentration of the ε-subunit in g · ml^{-1}, z = number of moles of electrons per gram dissolved protein, and ν = the partial specific volume with ρ = the electron density

of the solvent in moles of electrons per ml solvent. For a spherical particle of molecular weight 11,400 and $\bar{\nu}$ = 0.740 ml · g^{-1} we calculate theoretically a radius of gyration of

$$R_{th} = (3/5)^{1/2} \cdot R_o \quad \text{with} \quad R_o = \frac{3(V + \delta \cdot \nu_o) \cdot M}{4\pi N}$$

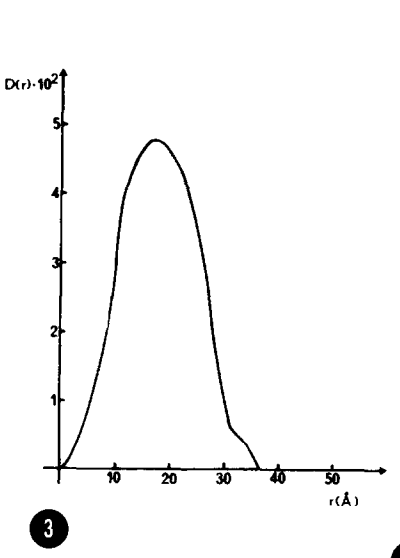
with δ = the degree of hydration and ν_o = the partial specific volume of the solvent, and we obtain values of R_o = 10.8 Å and R_{th} = 8.32 Å. Since the obtained values of R and R_o from the Guinier plot are greater than that determined for a hydrated sphere of molecular weight 11,400 and a degree of hydration of 0.2 g H_2O/g protein, the shape of the ε-subunit cannot really be spherical; it must be a little asymmetric. Considering the ε-subunit as an ellipsoid of revolution with half axes a = b, and $\nu \cdot a$, the shape can be determined by means of R and V through the relation

$$R = a\left(\frac{2 + \nu^2}{5}\right) \text{ and } V = \frac{4}{3} a^3 \cdot \nu = \frac{4}{3}\pi \left(\frac{a}{f}\right)^3 (5f^2 - 2)^{1/2}$$

where $R = a \cdot f$. For $f = 1$, the case of a spherical protein is valid, but for $f \lesssim 1$, an oblate or prolate ellipsoid of revolution must be considered. According to the values obtained, the volume of the ε-subunit, $\alpha = S/V(A^{-1})$, only a prolate ellipsoid of revolution with an axial ratio 1:2 fulfills the requirements listed in table I. Comparisons of the theoretical and experimental scattering curves for the ε-subunit are in fairly good agreement, although the height of the first side maximum of the theoretical value is higher than the measured one. The absense of ideal symmetry is already documented by the absense of zero positions of the experimental scattering curve. The largest dimension of the ε-subunit was 51.0 Å, determined from the pair distribution function (D(r))

$$D(r) = \left(\frac{2}{\pi}\right) \int_0^\infty r \cdot hI(h) \sin(hr)\, dh$$

where D(r) is defined so that $4\pi r^2 D(r)\, dr$ is the total number of electron pairs in the molecule with a separation between r and dr. Moreover, D(r) for $r \gtrsim L$ is zero with L the greatest diameter of the scattering particle in solution. From the



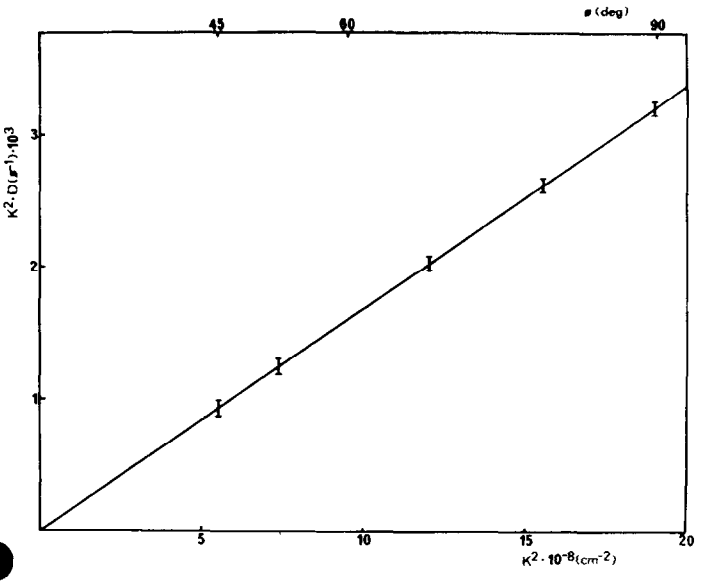


Fig. 3. Pair distribution function (D(r)) of the ε-subunit in solution.

Fig. 4. A plot of the decay constant, $K^2 \cdot D$ versus K^2 to determine the average diffusion coefficient.

pair distribution function in figure 3 we see that the maximum of D(r) is at r = 16.0 Å, indicating that this distance is rather frequent in the scattering particle.

The measured radii of gyration of the ε-subunit does not change very significantly with an increase of the solvent density. If there were an external hydration layer, containing the added sucrose, one would expect a decrease of the radii of gyration. However, while the hydration layer will have an electron density less than that of a sugar-water solvent, an adjacent polypeptide chain layer would have a density greater than that of the solvent. Moreover, a plot of $\Delta\rho\ R^2(\rho)$ versus $\Delta\rho$ is a straight line, indicating that the particles are homogeneous in density and impenetrable to sucrose, and, therefore, that their internal structure is independent of the electron density of the solvent.

From the inelastic light scattering experiments we

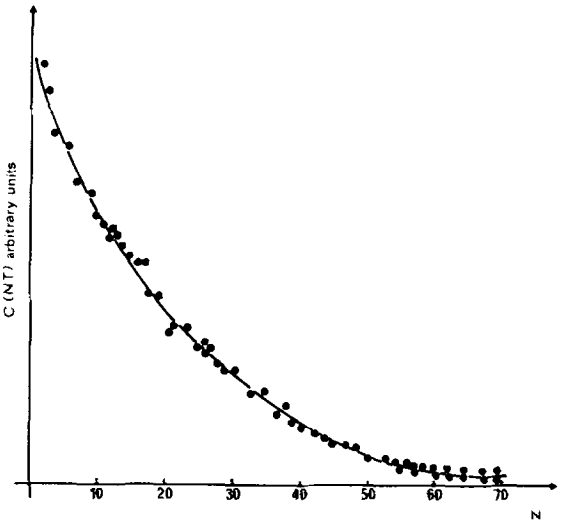


Fig. 5. Clipped photo-current correlation function C(NT) for pure homodyne scattered light from a solution of 1.5 mg/ml of ε-subunit in 0.05 M TRIS-HCl, pH 7.0, containing 5 mM $MgCl_2$, at 22°C. Scattering angle, $\theta = 90^{\circ}$, and time interval between successive points was T = 4.0 μsec. Solid line least squares curve $C(t) = \exp|-t/(8.72 \times 10^{-3})|$.

obtained the translational diffusion coefficient. From the logarithmic plot of the autocorrelation function versus delay time we obtained the decay constant, τ = 21.6 μsec, and from the plot of the slopes against K^2 ($K = 4\pi n/\lambda_o \sin\theta/2$) we got the diffusion coefficient, which was $(11.3 \pm 0.05) \cdot 10^{-7}$ $cm^2 \cdot sec^{-1}$ (Fig. 4). The effective hydrodynamic radius (R_h) was determined to be 16.1 Å, yielding a sedimentation constant of $S = 1.7 \times 10^{-13}$ sec (see Table II). The photocurrent autocorrelation function can be precisely described by a single exponential. Extrapolation to infinite dilution of the diffusion data obtained in figure 5 gives a straight line with no indication of aggregation.

Both inelastic light scattering experiments and small angle X-ray scattering measurements of the ε-subunit show that the protein does not deviate very much from a spherical particle, which is documented by the frictional ratio, f/f_o, the radius of gyration, and the Stokes' radius. Moreover according to the correlation between $D^{o}_{20,w}$ and R

Table II. Hydrodynamic parameters obtained by quasi-elastic light scattering experiments in 0.05 M TRIS-HCl, pH 7.0, containing 10 mM $MgCl_2$ (20^oC).

$D^o_{20,w}$ x 10^{-7} $cm^2 \cdot sec^{-1}$	11.40
R_S (Å)$^+$	16.1 ± 0.05
f/f_o	1.09 - 1.10
f x 10^{-8} g $\cdot$ sec^{-1}	33.58
$S^o_{20,w}$ x 10^{-3} sec	1.70
β x 10^{-6} $^{++}$ (17)	2.13
R (Å) $^{+++}$	12.1

$^+$ determined according to $D = \frac{k_B \cdot T}{6\pi\eta_o \cdot R_S}$ with k_B = Boltzmann constant, η_o = the viscosity of the solvent in poise, and T = the absolute temperature.

$^{++}$ with $\beta = [N/16{,}200\ \pi^2]^{1/3}\ (f_o/f)\ \bar{v}^{1/3}$; calculated from $\bar{v}$ and f_o/f, where $f_o = 6\pi\eta_o \cdot R_o$, with R_o = the radius of a sphere of molecular weight, M, and $6\pi\eta_o \cdot R_S$, with R_S = the Stokes' radius determined from the diffusion measurements.

$^{+++}$ according to $T = (3/5)^{1/2} \times \frac{k_B \cdot T}{D \cdot 6\pi\eta_o} = \frac{f}{6\pi\eta_o} \cdot (3/5)^{1/2}$.

$$R = (3/5)^{1/2} \times \frac{k_B \cdot T}{6\pi\eta \cdot D}$$

the ε-subunit must be almost uniform density and of spherical shape; otherwise, one would obtain deviations in R by a given $D^o_{20,w}$.

Since it has been show that the ε-subunit of CF_1 is involved in the masking of the ATPase activity (3), hydro-

dynamic studies can show how single subunits can be arranged within the CF_1 molecule with respect to their size and hydrodynamic volume. Therefore, hydrodynamic studies and small angle X-ray scattering measurements in solution can be powerful tools in evaluating the stochiometry of the subunits of CF_1.

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