THE STRUCTURE OF THE δ-SUBUNIT FROM CHLOROPLAST COUPLING FACTOR (CF_1) IN SOLUTION

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Summary: The δ-subunit from chloroplast coupling factor (CF_1) was isolated according to Younis et al. (1977: J. Biol. Chem. 252: 1814-1818) and further purified by means of DEAE-cellulose chromatography in the absence of Mg^{2+} at pH 8.0, but in the presence of 5 % polyethylene-glycol. The homogeneous δ-protein fraction was studied by inelastic light scattering measurements, small angle X-ray scattering experiments and analytical ultracentrifugation. The hydrodynamic measurements and the small angle X-ray scattering experiments in solution support the hydrodynamic description of a prolate ellipsoid of revolution with gross dimensions of 2a = 25.0 Å, 2b = 28.0 Å and 2c = 90.0 Å, having a radius of gyration of 21.80 Å, a sedimentation coefficient of 1.70S and a translational diffusion constant of $D = 3.92 \times 10^{7}$ $cm^2 \cdot sec^{-1}$. Considering different molecular conformations, e.g. worm-like coil, ellipsoid of revolution, and flexible rod, the description of a flexible rod is the most probable one, when one considers the hydrodynamic values obtained for the δ-subunit.

There are several methods available for the purification of a soluble coupling factor ATPase from chloroplasts (1-6). The chloroplast coupling factor (CF_1) contains five subunits, α, β, γ, δ, and ε, in order of decreasing molecular weight, and it serves as a coupling factor for photophosphorylation.

Since the δ-subunit is required for the binding of CF_1 to the membrane and may represent the stalk seen in electron micrographs as a link between the protein and the membrane (7), it is of importance to know the molecular conformation of the δ-subunit in solution.

We adopted the methods of Beeckey et al. (8) and Younis et al. (9) for the purification of the δ-subunit of CF_1, and measured by means of small angle X-ray scattering experiments and inelastic light scattering measurements the radius of

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gyration, the hydrodynamic volume, the ratio of surface to volume, and the translational diffusion coefficient. Since nothing is known about the tertiary structure of the δ-subunit of CF_1, nor of the shape of this protein, we are able to deduce from the above stated measurements the first results concerning the shape and the hydrodynamic volume of the δ-subunit.

MATERIALS AND METHODS

The δ-factor from CF_1 was prepared according to Younis et al. (9). The fractions coming from a DEAE-cellulose column (1.5 x 8 cm) at 0.08-0.11 M NaCl in a tricine-urea buffer, pH 8.0 (9), were pooled, dialyzed against 0.1 M TRIS-HCl, pH 8.0, containing 0.01 M KCl and 5 mM ß-mercapto-ethanol, and thereafter lyophilized and rechromatographed on a DEAE-cellulose column in 0.1 M TRIS-HCl, pH 8.0, 0.01 M KCl and 5 % polyethylene-glycol (MW 4,000). The protein was then concentrated by dialysis against polyethylene-glycol (MW 40,000) and lyophilized. The lyophilized material was dissolved in distilled water (4°C) and dialyzed against the appropriate buffer, i) 0.1 M TRIS-HCl, pH 8.0, 0.01 M KCl and 5 mM ß-mercapto-ethanol, and ii) 0.01 M K_2HPO_4, pH 7.5 (4°C), 0.01 M KCl and 5 mM ß-mercapto-ethanol.

The partial specific volume, $\bar{\nu}_2$, of the protein in the buffer was measured using an Anton Paar (Graz, Austia) DMA-02$_C$ precision densitometer and was found to be 0.740 ± 0.002 $ml \cdot g^{-1}$, slightly dependent on buffer conditions (ionic strength) and protein concentration (aggregation) (10).

Diffraction measurements were performed using a Kratky camera with a resolution of 1000 Å. The X-ray source was a rotating anode X-ray generator (GX 13) from Elliot-Marconi (U.K.), operating at 25 mA and 30 kV with a 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. Calculations of the theoretical scattering curves for various tri-axial bodies were performed on a CD 72 at the Institute for Theoretical Physics, Free University Berlin.

Light scattering experiments were performed using an argon ion laser (Spectra Physics Model 165) as a source of incident light (λ_o = 4579 Å). The laser beam was operated at a power level of 60-100 mW and was focused at the center of a

scattering cell of dimensions 8.5 x 4 x 10 mm, containing the sample, that had been filtered through a 0.25 μm Millipore filter. The translational diffusion coefficient and the corresponding Stokes' radius can be determined by quasi-elastic light scattering (14). For a monodisperse solution of particles, undergoing Browniam motion, the autocorrelation function decays exponentially with a correlation time, τ_c, which is given through the relation

$$\tau_c = (2D \cdot |\vec{K}|^2)^{-1}$$

with $|K| = (\frac{4\pi n}{\lambda_o}) \sin \frac{\theta}{2}$ denoting the scattering wave vector, and n the refractive index, θ = the scattering angle and λ_o = the wavelength of the incident light. The scattering angle (θ) was between 40° and 90° (15).

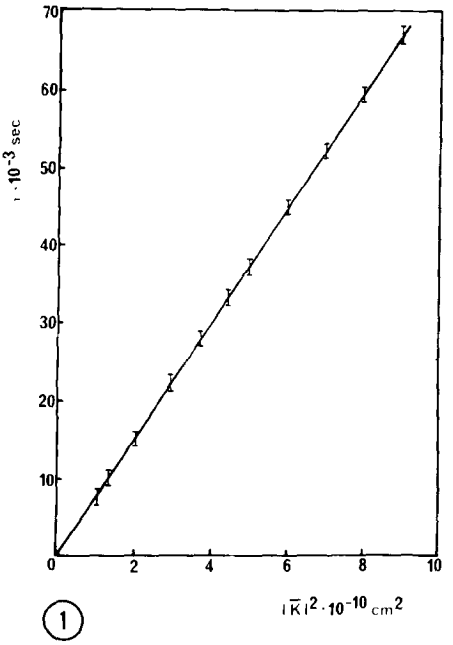
Analytical ultracentrifugation measurements were performed on a Beckman Model E instrument, equipped with an RTC-temperature control and a photoelectric scanner. Sedimentation velocity experiments and sedimentation equilibrium experiments were carried out as described previously (15, 16).

Viscosity measurements were performed in a Cannon Micro-viscosimeter at 4°C with flow times of 145 sec for water.

RESULTS

The plot of the decay rate, Γ, where $\Gamma = (\tau_c)^{-1}$, as a function of the scattering wave vector, $|\vec{K}|^2$, for quasielastic light scattering, is linear at 4°C. This finding clearly indicates that the solution of δ-subunit of CF_1 is free of aggregates and is uniform with respect to particle size. The translational diffusion coefficient, as determined from the measurements of the correlation time, τ_c, was found to be independent of δ-subunit concentration in the range of 0.5 - 5 mg/ml (Figs. 1 and 2). From these data we determined a translational diffusion coefficient of $D_{20,w} = (3.92 \pm 0.02) \times 10^7$ cm$^2 \cdot$ sec^{-1}. The mean particle diameter, determined from the Stokes-Einstein equation, was $2R_o = 51.0$ Å, with $R_o = \frac{k_B \cdot T}{6\pi h \cdot D}$ where R_o is the hydrodynamic radius, k_B is the Boltzmann constant, T is the absolute temperature, and h is the solution viscosity.

From ultracentrifugation velocity runs we obtained a Svedberg constant of 1.70S. By applying a partial specific volume of 0.740 ml $\cdot$ g^{-1} we obtain a molecular weight of 22,500, which is in excellent agreement with the value obtained by sedimentation-equilibrium measurements and small angle X-ray measurements (Tables 1 and 2). The independent value obtained



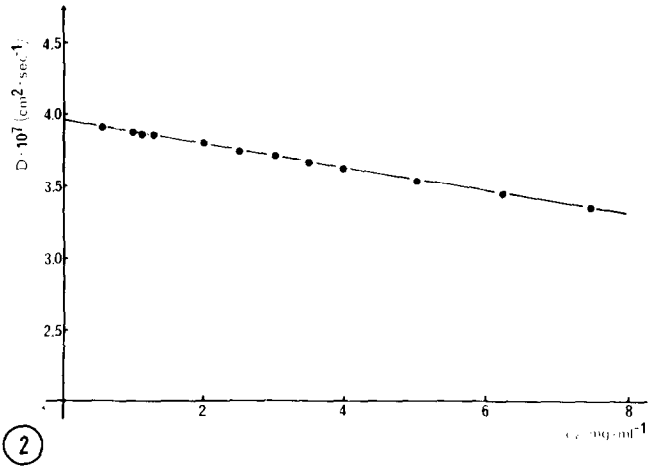


FIG. 1. Decay rate, Γ, as a function of the square of the scattering wave vector $|\vec{K}|^2$ for a solution of δ-subunit of CF_1. $|\vec{K}|^2 = 6.70 \times 10^{-10}$ cm^2.

FIG. 2. Translational diffusion coefficients of δ-subunit in 0.01 M TRIS-HCl, pH 8.0, containing 0.1 M KCl, versus protein concentration, measured at 20°C.

for $D_{20,w}$ from the Svedberg equation, by insertion of $S^o_{20,w}$ and the molecular weight, is $D_{20,w} = 3.89 \times 10^{-7}$ cm^2 · sec^{-1}.

The morphological parameters for the δ-subunit of CF_1, obtained from small angle X-ray measurements, are listed in table 2, whereas the hydrodynamic results can be seen in table 1. A Guinier plot of the data at smallest angles for four concentrations are shown in figure 3. We note, that the volume obtained from the invariant, Q, according to Porod (17), V = 3.45×10^4 Å^3, is larger than the $\nu_2 \cdot M/N$ for a dry particle of $V_{dry} = 2.75 \times 10^4$ Å^3. This swelling ratio suggests an open coil form, which disagrees with the radius of gyration and the specific inner surface. We were able to find a filled particle of uniform electron density that is equivalent in scattering. A comparison of the observed radius of gyration, R = 21.80 Å, with the radius of gyration, R = 19.5 Å, of a sphere having the observed volume, V, suggests, that the

Table 1. Hydrodynamic properties of δ-subunit of CF_1 obtained by sedimentation velocity and inelastic light scattering.

Method	Value
M_w, from sedimentation equilibrium	22,000
$S^o_{20,w}$, from sedimentation velocity	1.70S
$D^o_{20,w}$, from inelastic light scattering, x 10^{-7} $cm^2 \cdot sec^{-1}$	(3.92 ± 0.02)
$\bar{v}_2$ ml $\cdot$ g^{-1}	0.740 ± 0.002
f/f_o, from $S^o_{20,w}$	1.20
from $D^o_{20,w}$	1.19
R_o (Å), Stokes' radius, from $S^o_{20,w}$	21.5
from $D^o_{20,w}$	20.9
ß x 10^{-6} a)	2.23
Axial ratio b)	4-5
$[\eta]$ (ml $\cdot$ g^{-1})	3.5
ν c), viscosity increment	4.76

a) according to Mandelkern-Scheraga (23) with

$$ß = \frac{N \cdot S^{1/3} h}{M^{2/3} (1-\bar{v}_2\rho)} = (N/16,200\pi^2)^{1/3} \nu^{1/3}/(f/f_o)$$

b) average value, obtained from the tabulated values of f/f_o, $[\eta]$, $D^o_{20,w}$, and column chromatography on BioGel A 0.5 m, R_o.

c) Simha factor $\nu = \frac{100 \cdot [\eta]}{\bar{v}_2}$ (24).

equivalent scattering particle must be quite anisotropic. A comparison of the experimental and theoretical scattering curves of normalized intensity, that have been plotted as log Φ(h) versus h, with $h = 4\pi/\lambda \sin\theta$ denoting the scattering vector and $\lambda = 1.54$ Å the wavelength of the radiation, shown in figure 4, reveals a particle of elongated shape with an axial ratio of 1:1.3:4, applying a description of a prolate

Table 2. Morphological parameters for the δ-subunit of CF_1 obtained by small angle X-ray scattering experiments.

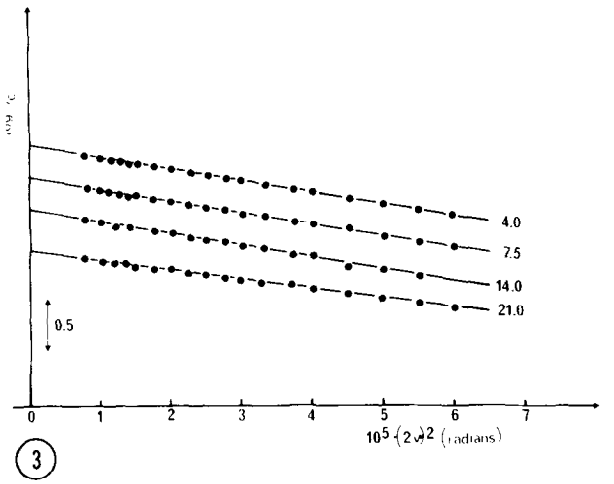
Unit	Value
Radius of gyration, R (Å)	21.80 ± 0.05
Molecular weight	21,500
$\alpha = S/V$ (A^{-1}), specific inner surface	0.27
Surface, S x 10^3 ($Å^2$)	9.15
Volume, V x 10^4 ($Å^3$)	3.29
Degree of hydration, g H_2O/g protein	0.31
r_g (Å)	28.14
r_v (Å)	19.88
r_s (Å)	27.00
$\bar{\rho}_1$ (eA^{-3})	
$\bar{\rho}_1 \cdot V$ (10 · e)	
Overall shape dimensions, 2a (Å)	25.00
2b (Å)	28.00
2c (Å)	90.0
a/b from $\frac{3V}{4\pi R^3}\left(\frac{2+(a/b)^2}{5}\right)^{1/3}$	3.8
a/b from $\frac{S}{V} \cdot R\left(\frac{2+(a/b)^2}{5}\right)^{1/3}$	4.1

$\bar{\rho}_1$ = bouyant density; corresponding to an isopycnic density of 1.15-1.16 g · cm^{-3}.

$\bar{\rho}_V$ = the number of electrons of one solvated particle of δ-subunit of CF_1.

r_g, r_V, r_S = the radii of spheres, determined from the radius of gyration, the volume and the surface, showing the departure of the radius of a spherical particle, whose volume, radius of gyration and surface is V, R, and S, respectively.

ellipsoid of revolution with half axes a, b and c. Considering the radius of gyration and the volume of the scattering particle, the best description of the δ-subunit in solution under these conditions would be that of a flattened, elongated ellipsoid



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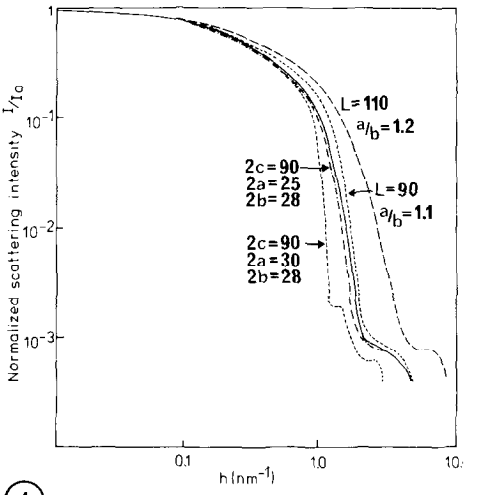

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FIG. 3. Guinier plot of the δ-subunit of CF_1 for different concentrations in 0.01 M TRIS-HCl, pH 8.0, containing 0.1 M KCl and 6 mM ß-mercapto-ethanol. $h = 4\pi/\lambda \cdot \sin\theta$, with λ = 1.54 Å and θ = half of the scattering angle.

FIG. 4. Experimental (————) and theoretical (------) scattering curves of the δ-subunit for a radius of gyration of R = 21.80 Å, ellipsoids of revolution with lengths of L= 90.0 Å and elliptic cylinders with heights of L = 110 Å and elliptical ends with half axes of a = 12.5 Å and 14.0 Å.

with half axes of a = 25.0 Å, b = 28.0 Å and c = 90.0 Å, or, without a distinct difference in scattering behavior, that of an elliptic cylinder with a length of L = 110.0 Å and an axial ratio of the half axes of the basis of 1:1.2, resulting in an axial ratio of length to diameter of five to six.

DISCUSSION

To exclude the possibilities of a random-coiled or a worm-like chain conformation of the δ-subunit, we measured some hydrodynamic values, e.g. $[\eta]$ and R, of a solution of the δ-subunit in 6 M guanidinium-hydrochloride. For a random-coiled polypeptide chain of molecular weight 21,000, or 205 residues, the intrinsic viscosity would be approximately 27 ml $\cdot$ g^{-1}, according to the Mark-Houwinck equation (18), or a radius of gyration of R = 45.7 Å. In the presence of 6 M guanidinium-hydrochloride we found values of $[\eta]$ = 25 ml $\cdot$ g^{-1} and a radius

of gyration of R = 42.5 Å; both values are lower than the values calculated for a Gaussian immobilized coil. However, these values are significantly higher than those obtained for the δ-subunit of CF_1 under non-denaturing conditions and thus rule out an open coil structure. Further investigations of the folded conformation of the δ-subunit by circular dichroism measurements, diffusion experiments and intrinsic viscosity determinations (19) lead to the conclusion that the δ-subunit of the chloroplast coupling factor (CF_1) has a certain folded conformation, which obviously is very sensitive to ionic strength and to pH (10).

The high intrinsic viscosity, compared to a value of about 3.4 ml/g for spherical proteins, and the high radius of gyration, compared to the radius of gyration of a sphere of molecular weight 21,000, R = 14.5 Å, are indicative of either an asymmetric structure or an expanded flexible chain. The relation between the intrinsic viscosity, $[\eta]$, and the radius of gyration, R, for a flexible chain is given by $[\eta] = (10\pi N/3M)\xi^3 \cdot R^3$, with N = Avogadro's number, M = molecular weight, and ξ = a dimensionless quantity relating the radius of the equivalent hydrodynamic sphere to R. By applying the values of $[\eta]$ and R, we obtain ξ = 0.811, which is in the range of 0.775-0.860 found for flexible chains (18) and demonstrates, therefore, that the viscosity data are consistent with a flexible chain model. Furthermore, for an unhydrated polypeptide, $[\eta] = \bar{v} \cdot \bar{v}_2$, with v = Simha factor (19), and $\bar{v}_2$ = the partial specific volume of 0.740 ± 0.002 ml · g^{-1}, the value of Simha's constant is equivalent to a prolate ellipsoid of revolution with an axial ratio of 5-6. An oblate ellipsoid model would lead to a somewhat higher axial ratio, but this is inconsistent with the determined radius of gyration. Furthermore, the volume of the protein particle of V = 3.3 x 10^4 $Å^3$ is consistent with the calculated volume of the scattering particle, as well as that obtained by sedimentation and intrinsic viscosity measurements of V(h) = 3.55 · 10^4 $Å^3$.

Similar analysis of the constant, ξ, was carried out from sedimentation data and small angle X-ray scattering data, applying different molecular conformations, e.g. immobilized Gaussian coil and worm-like chain. We obtained a value of ξ = 0.710, which is considerably larger than the value of 0.655

for a random coil, according to Kirkwood and Riseman (20). However, we did not find, according to the Debye (21) scattering function for a random coil, a $I \sim h^{-2}$ dependence beyond the Guiner region, since a chain with finite persistence length will undergo a transition with increasing scattering angle from coil behavior, $I \sim h^{-2}$, to rod-like behavior, $I \sim h^{-1}$. This is furthermore a strong indication that the δ-subunit in solution has a distinct molecular conformation, and not an open coil structure.

The combined results of all hydrodynamic measurements, including the small angle X-ray scattering measurments and the determination of the translational diffusion coefficient, eliminate an oblate ellipsoid of revolution and an open random coil structure. In contrast to the ε-subunit of the ATPase from E. coli (22), the δ-subunit of CF_1 is most likely an asymmetrically shaped molecule under the described conditions.

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