DETERMINATION OF DIFFUSION COEFFICIENTS AND MOLECULAR WEIGHTS OF PARTIALLY DISSOCIATED F_1-ATPase FROM YEAST BY GEL FILTRATION COUPLED TO LASER LIGHT-SCATTERING SPECTROSCOPY

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1. Introduction

In earlier experiments we have shown that F_1-ATPase from yeast dissociates reversibly into two components, one of which was suggested to be identical with the α-subunit of the native complex [1]. To determine the hydrodynamic properties and the MW of the dissociation products, we combined quasi-elastic light scattering and gel filtration, enabling the diffusion coefficients of the components in the flowing output of a gel-filtration column to be determined. The method is based on the following reasoning.

The diffusion coefficients of globular proteins are weakly dependent on molecular weight. To determine reliable molecular weights using the Svedberg equation, the diffusion coefficient has to be known precisely. On the other hand, a mixture of particles whose diffusion coefficients differ by less than a factor of two (factor of eight in the MW for compact molecules) yield a nearly monoexponential autocorrelation function of the light scattering intensity, and it is not possible to decompose the signal into single components [2]. Furthermore, many proteins show a tendency to aggregate, and since light scattering weights a component of a solution according to its concentration and molecular weight, protein aggregates strongly distort the autocorrelation function of the monomers. These problems can be overcome if the light scattering spectrometer is used as a continuous and real time detector of the effluent of a chromatographic column which separates the components of the solution according to their molecular properties.

2. Materials and methods

All chemicals used were of research grade. Experiments were conducted with preparations of F_1-ATPase prepared by the methods given in [3]. A Sephadex G-100 gel column was equilibrated with buffer (10 mM Tris–HCl, 1 mM EDTA, 2 mM ATP, pH 7.5) at a temperature of 3°C.

F_1-ATPase was dissolved in the same buffer at a concentration of 2.6 mg/ml and preincubated for 1 h at 3°C. The sample was then passed through a thermostated gel column (0.4 × 30 cm, 3°C), followed by a membrane filter (Millipore 0.22 μm) and the flow cell of the light-scattering spectrometer, and was finally collected by a fraction collector (Uvicord II) attached to a UV absorptiometer from LKB (Sweden). The flow rate was controlled by a peristaltic pump (9.9 ml/h) located prior to the LKB-unit. The dead-time between the end of the column and the laser light beam was 2 min. A constant temperature of 3°C throughout the filtration and measurement procedure was maintained by a thermostat connected to all parts of the whole system.

The laser light-scattering experiments were performed with a modified Malvern Molecular Analyser, System 4300, equipped with a Spectra Physics 4 W Argon laser, model 165-08. The modifications are described in [4]. The system allows the measurement of the integrated scattering intensity and of the single-clipped intensity autocorrelation function.

The autocorrelation function of the electric field scattered by a suspension of macromolecules is [5,6]:

$$g^1(t) = \sum_i A_i \exp(-q^2 D_i t)$$

with D_i, A_i and q denoting the translational diffusion coefficient of component i, an intensity factor, and the magnitude of the scattering vector, respectively.

An Interdata 732 computer which was connected on line to the system received a signal from the digital correlator every 90 s and calculated the average scattering intensity and the correlation time of the autocorrelation function.

The whole system including the column was tested with a mixture of monomers and dimers of bovine serum albumin. The correlation function of the two separate components turned out to the monoexponential and the diffusion coefficients compared well with those obtained under non-flowing conditions as well as with those in the literature [7]. In samples of F_1-ATPase, a small amount of aggregated material was still present, and a two exponential analysis of the correlation function was applied by means of the non-linear fit program (Harwell Subroutine VCO 5A) modified by K. H. Müller and Th. Plesser of this Institute.

An experimental correlation function, a single and a two exponential fit obtained from an F_1-ATPase sample are shown in fig.1. The intensity of the large aggregated component varied between 10 and 20% of the total scattering intensity. The correlation time of the main component, 80% of the total scattering intensity, varied by about 2%. The direction of the flow was parallel to the wave vector so that it did not influence the autocorrelation function: the correlation time remained unchanged when the flow was stopped. The dissociation process of F_1-ATPase at 3°C was first analyzed during the preincubation time by recording the time-dependent decrease of the integrated light-scattering intensity in samples taken from the pre-incubated lot and measured directly. From these experiments a half time for dissociation of about 120 min was calculated, yielding the preincubation time used in the combined filtration and measurement system under flowing conditions and allowing an optimal distribution of dissociated products. The first component arrived in the cell of the light scattering photometer after 40 min.

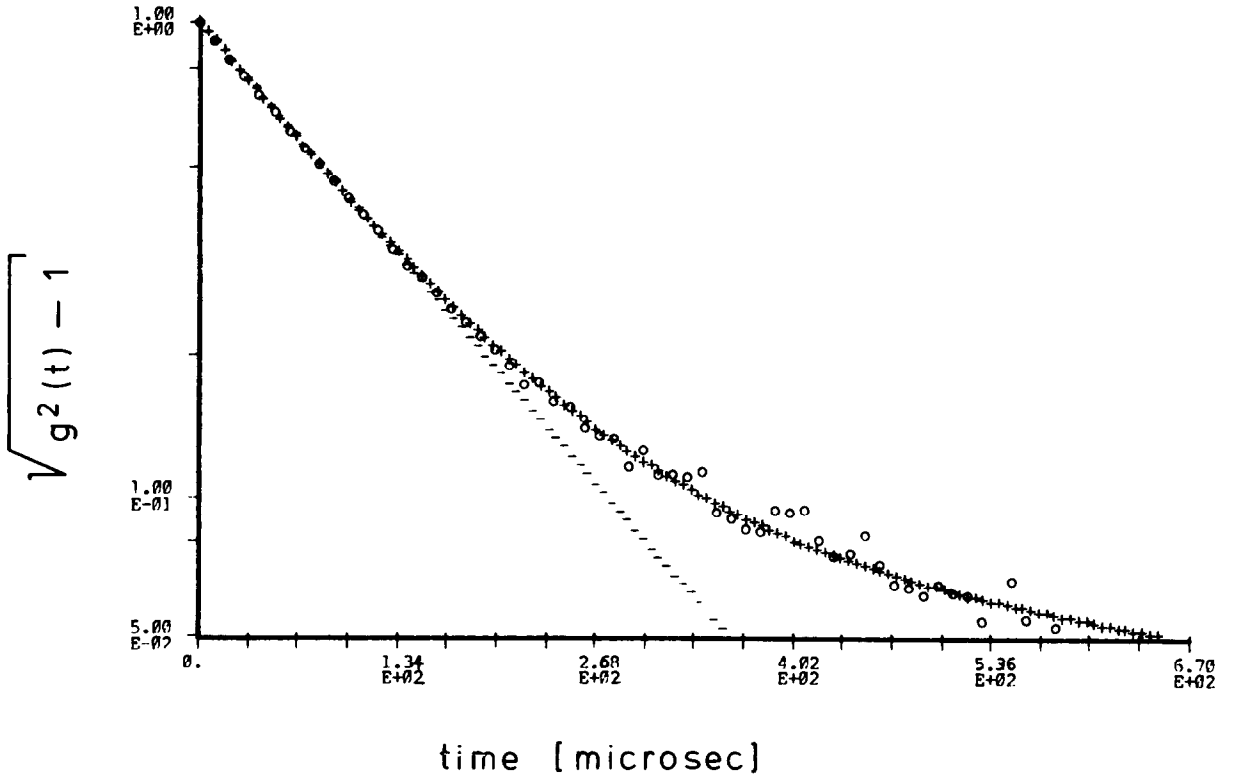

Fig.1. Square root of the normalized correlation function of component II (o) and a one (–) and two (x) exponential fits. The deviation from linearity occurs where the signal still has 4% of its original value. $T = 3°C$; $\theta = 90°$, $A_1 = 0.905$, $\tau_1 = 87$ μs, $A_2 = 0.1$, $\tau_2 = 970$ μs.

3. Results

The results of our experiments are shown in fig.2 where the integrated light-scattering intensity is plotted as a function of time. Three distinct components are observed (I–III). We obtained a diffusion coefficient for each experimental point from the corresponding correlation function of the scattered field. The average diffusion coefficient of each of the components is given in table 1. Component I has the same diffusion coefficient as the intact F_1-ATPase complex [3]. The diffusion coefficients of components II and III are larger. Using the sedimentation coefficients obtained from [1], a partial specific volume $\bar{v} = 0.745$ and the Svedberg equation, we calculated the MW of the components. Component I has a MW close to that of the intact F_1-ATPase complex. Components II and III are dissociation products. According to their MW, component III should be a subunit, whilst component II appears to be a complex.

4. Discussion and conclusion

The technique of quasi-elastic light scattering coupled to a gel filtration column allowed identification of two intermediates of dissociation of the native F_1-ATPase complex and determination of individual diffusion coefficients. This is in accordance with the results obtained from gel electrophoresis, where two components are also observed [1]. The smallest component with a MW of 61 000 is suggested to be identical with the α-subunit of the complex. Indeed, independent analysis strongly favoured this view [1,8]. The sedimentation coefficient of the subunit (2.6 S) is somewhat larger than that obtained in 6 M guanidinium hydrochloride (2.2 S). However, for a compact globular protein of this MW one expects a sedimentation coefficient of about 4 S. Similarly the diffusion coefficient (4.01 F) is larger than that estimated in 6 M guanidinium hydrochloride (3.3 F) [8]. Here, for interpretation it should be noted that bovine serum albumin, with a similar MW, has a diffusion coefficient of 5.78 F. From these deviations we suggest that the subunit (component III) is highly asymmetric or partially unfolded.

The MW of component II is closely similar to that of one of the cross-linked products of F_1-ATPase described in [9]. The MW of component II is based on the assumption that the sedimentation coefficient of 10.6 S is not an average of component I and II: The sedimentation peak is symmetrical which speaks for homogeneity. Furthermore, the sedimentation velocity experiments were run after 3 h incubation in the cold, and under these conditions nearly no intact F_1-ATPase complex can be detected by gel electrophoresis [1,8]. A quantitative comparison of the data suggests that component II is a complex which has lost two α-subunits of native F_1-ATPase. If one assumes that F_1-ATPase contains three α-subunits [3], the loss of two α-subunits would still leave the third α-subunit in the complex II as a residue, and a complete α-less F_1-ATPase complex would still be expected. Indeed, an undetermined cross-linked product with a MW of 227 000 was observed by Enns and Criddle [9], which might be the expected dissociation product. The problem is under investigation.

Table 1
Diffusion and sedimentation coefficients and calculated molecular weights of components shown in fig.2

	$D_{20,w}$	$s_{20,w}$	M
I	3.07 F	12.6 S[a]	390 000 D[a]
II	3.70 F	10.6 S[b]	270 000 D
III	4.01 F	2.6 S[b]	61 000 D

[a][3]; [b][1]

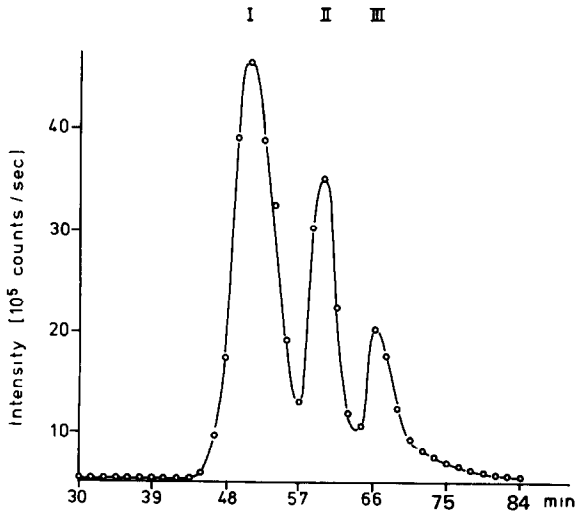

Fig.2. Photon count rate at 90°C of the light scattered by the effluent of the gel column, sampling time: 90 s.

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