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Laser light-scattering characterization of mitochondrial complex III-Triton X-100-phospholipid mixed micelles

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Bovine-heart mitochondrial complex III was purified in the presence of Triton X-100, and the size and shape of the resulting protein-surfactant-phospholipid mixed micelles were investigated by laser light-scattering. The protein appears to be present in the form of a dimer, irrespective of temperature (between 25 and 40°C) and protein concentration (between 0.5 and 5 mg/ml). The molecular weight of the micelle increases with temperature from 600000 (25°C) to 692000 (40°C). The variation of the solvent second virial coefficient in this temperature range suggests that, with increasing temperature, some of the free surfactant molecules become integrated in the mixed micelles. The average quadratic radius of gyration of these is of 42 ± 5 nm, corresponding in our case to an ellipsoidal shape.

Mitochondrial electron transport from ubiquinol to cytochrome *c* is catalyzed by a multienzyme complex, ubiquinol : cytochrome *c* oxidoreductase, also called bc_1 complex or complex III. It is known to contain two *b* and one c_1 cytochromes, one Fe-S protein and at least five other polypeptides [1]. Complex III was first isolated by Hatefi et al. [2] using cholate as surfactant; ultracentrifuge and light scattering measurements showed that the protein existed as a monomer in detergent suspensions [1,3], Later, other authors devised purification procedures for complex III in Triton X-100 [4,5]. This method is widely used now. Protein-lipid-surfactant mixed micelles obtained in Triton X-100 have been characterized by analytical centrifugation [5,6]. Opposite to what was found for the cholate preparations, the enzyme appeared to be in the form of dimers in the presence of Triton X-100. This enzyme preparation has not been studied by light scattering up to now, yet this technique provides a convenient and sensitive means to characterize the size and shape of micelles in solution. Therefore, we decided to investigate the structural parameters of mitochondrial complex III-Triton X-100-phospholipid mixed micelles by an improved light scattering procedure, that uses a laser beam of incident light. Our results are summarized in the present paper.

Complex III was isolated from bovine-heart mitochondria according to Engel et al. [5]. Seven bands could be separated by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate [7], of molecular weight 56000, 49000, 30000, 26000, 13000, 10000 y 6000. Aliquots of the enzyme preparation were diluted with 20 mM Mops (pH 7.5) in order to obtain final protein concentrations between 0.5 and 5 mg/ml. In order

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Abbreviations: Mops, 4-morpholinepropanesulphonic acid; A_2, the second virial coefficient; R_G^2, average quadratic radius of gyration; *Z*, intrinsic dissymmetry ($R_{45°}/R_{135°}$).

to free the samples from contaminating powder, they were filtered through Millipore filters, 0.45 μm pore diameter; the buffer had been filtered, prior to dilution, through Millipore filters 0.1 μm pore diameter. Refractive index increments were measured at 632 nm in the temperature range 25–40°C, in a Brice-Phoenix differential refractometer using as a light source a He-Ne laser (Spectra Physics, model 156) of 1 mW emission power. Laser light-scattering measurements were carried at 632 nm in the same temperature interval with a modified FICA 42000 light scattering photometer, where both light source and optical block of the incident beam were replaced by a He-Ne laser (Spectra Physics, model 157, 3mV emission power). The photogoniodiffusometer was calibrated with benzene using natural light and taking the Rayleigh ratio as $R_B = 8.96 \cdot 10^{-6}$ cm^{-1} [8]. Protein was determined according to Lowry et al. [9], and lipid phosphorus, by the method of Bartlett [10]. Lipid extractions were performed as described by Santiago et al. [11]. Triton X-100 was measured by isotopic dilution of ^{3}H-labeled surfactant (New England Nuclear).

Our preparations contain 0.238 mg Triton X-100 (average of two purifications in the presence of ^{3}H-labeled surfactant) and 0.223 mg phospholipid (average of ten determinations) per mg protein. Average molecular weights of 645 and 750 are assumed in these calculations for Triton X-100 and phospholipid, respectively. The molecular weight of the whole protein has been estimated, from electrophoresis measurements [12], to be of 270000. Therefore, our system contains, on the average, protein, phospholipid and Triton X-100 in a 1 : 80 : 100 molar ratio. This is in agreement with the estimates of Weiss and Kolb [4] and Von Jagow et al. [6] for surfactant/protein ratios in similar preparations. However, it does not automatically imply that the above molar ratios correspond to those in the mixed micelles, because a significant proportion of Triton X-100 may exist free in monomer solution. Our preparations contain between 0.12 and 1.2 mg Triton X-100 per ml, according to their respective protein concentrations; these values are near the critical micellar concentration of the surfactant, about 0.20 mg/ml at 25°C [13]. Consequently, even if the free monomer concentration of detergent will be smaller than the critical micellar concentration in the presence of phospholipid and protein [14], part of it may exist in monomer solution, and may be responsible for some phenomena described below. The presence of some free Triton X-100 prevents an exact evaluation of the maximum micelle molecular weight; it would be of about 333000, were all the surfactant molecules in micellar form. In practice, this value may be significantly lower, at least at 25°C.

Measurements of refractive index were made at 632 nm, where no protein chromophore absorbs. The results are shown in Fig. 1A, and correspond to the whole micellar suspension. Despite the corrections made by Tzagoloff et al. [3], there is little theoretical support for the idea of additive refractive indexes of protein and lipid. The observed values for dn/dc increase linearly with temperature, within the range of our study.

Laser light-scattering observations were carried

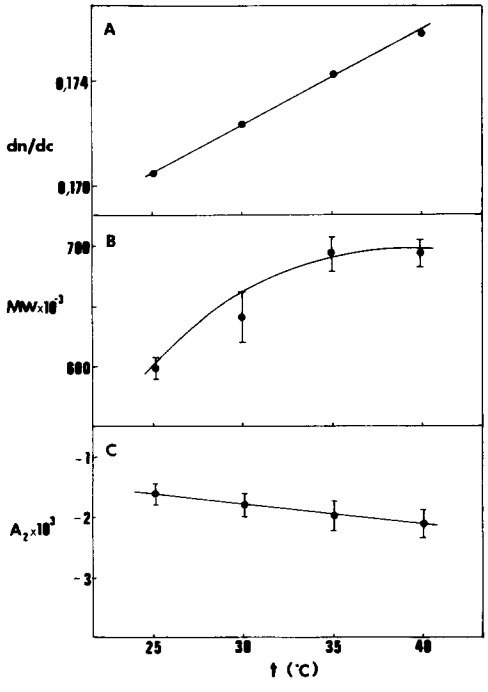

Fig. 1. Characterization of complex III-Triton X-100-phospholipid mixed micelles as a function of temperature. (A) Refractive index (dn/dc) of micellar suspensions. Average of two measurements. (B) Weight-average molecular weight (MW) of the micelles. (C) Solvent second virial coefficient. For (A) and (B) results correspond to average values of four experiments ± S.E.M.

out with suspensions containing 0.5–5 mg protein/ml. Protein concentration had no influence on Z ($R_{45°}/R_{135°}$) values. No protein absorption band occurs around 632 nm, and therefore the scattering readings need not be corrected. No time-dependent variation of light scattering was observed either. Weight-average molecular weights of the mixed micelles were calculated from refractive index and light-scattering measurements at various temperatures (Fig. 1B). Values increase from 600000 to 692000 as it goes from 25 to 40°C. This indicates that, in the presence of Triton X-100, complex III exists in the form of a dimer in the protein-surfactant-lipid mixed micelles. Other mitochondrial inner membrane proteins have also been isolated in dimeric form [15,16], and complex III has even been crystallized as a dimer [17].

In order to interpret the apparent increase in molecular weight with temperature, the second virial coefficients of the solvent were calculated by the Zimm method [18] for protein concentrations 0.5–5 mg/ml, at different temperatures. Second virial coefficients decrease with temperature (Fig. 1C); this is an indication that solute-solute are stronger that solute-solvent interactions. In our case, it may mean that surfactant molecules, existing as free monmers in solution at low temperatures, are incorporated into the mixed micelles as temperature is risen. However, only qualitative assertions can be made at this point, since there are at least two essential parameters, i.e., variation of free surfactant concentration with protein concentration and variation of critical micellar concentration with temperature, that have not been evaluated. Considering the extremely low critical micellar concentration of phospholipids [19], we can rule out any participation of these molecules in the variation of the micelle average molecular weight.

Light scattering measurements may also give information on the shape of the mixed micelles. For that purpose, the average quadratic radii of gyration R_G^2 of micelles have been calculated from Zimm's plots, and the value of 42 ± 5 nm has been found, independent of temperature or protein concentration. R_G^2 may then be used to calculate the characteristic dimension, D, of the micelle. The variation of intrinsic dissymmetry, Z, with D/λ (being λ the wavelength of the incident radiation)

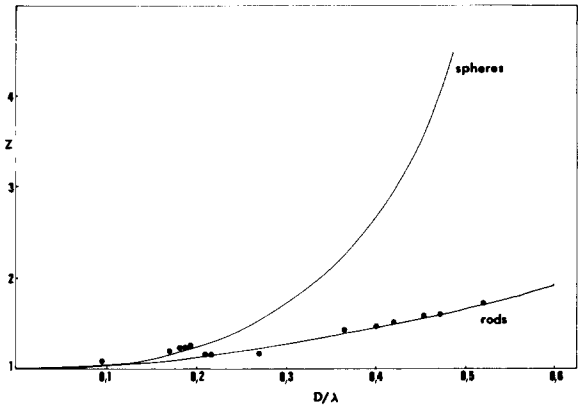

Fig. 2. Theoretical curves and experimental points relating dissymmetry coefficients (Z) with characteristic dimensions (D) and particle shapes, according to the dissymmetry method (Ref. 20).

depends on the shape of the particle [20]. Fig. 2 shows the theoretical curves for spheres and rods, together with the experimental points derived from our R_G^2 measurements. It appears that our micelles have an intermediate shape between spheres and rods, i.e., that of an ellipsoid. Von Jagow et al. [6], on the basis of frictional coefficients derived from analytical ultracentrifugation, proposed a spherical shape for the same micelles, but it is difficult, giving the uncertainties of the methods, to make any definitive assertions on this point. The fact that phospholipid contents vary depending on preparation methods implies that our data for the size and shape of mixed micelles may not be directly applicable to preparation obtained through other procedures.

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