THE UNCOUPLING PROTEIN FROM BROWN ADIPOSE TISSUE MITOCHONDRIA IS A DIMER. A HYDRODYNAMIC STUDY

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1. Introduction

In the preceding paper we described the purification of a purine nucleotide binding protein from brown adipose tissue mitochondria which appears to be the uncoupling factor, peculiar to these mitochondria [1]. The protein was obtained with more than 90% purity in relatively high yield associated with Triton X-100. The MW of its subunit was estimated by SDS gel electrophoresis to be 32 000. The solubilized protein has the same binding capacity for GDP as in mitochondria which indicates that the isolated protein has largely retained the native conformation. The number of binding sites for GDP was found to be 16 μmol/g protein, corresponding to a MW of 62 000. It was therefore suggested that the protein consists of a dimer of two subunits of 32 000 with one binding site of GDP. This case would be analogous to the ADP, ATP carrier of mitochondria which has a MW of 60 000 with two subunits of 30 000 and one binding site for carboxyatractylate [2–4].

In the present paper we will present some data on the hydrodynamic properties of the isolated uncoupling protein with the aim of establishing its MW. In view of the analogy to the ADP, ATP carrier, the solubilized uncoupling protein can be expected to exist as a mixed protein Triton micelle with a high Triton content. Hydrodynamic measurements have to take into account the high content of Triton. Interference by Triton has been overcome according to the techniques described previously for the ADP, ATP carrier [5,6].

2. Materials and methods

Ultrogel AcA 34 was obtained from LKB. All other materials and the protein preparation used in this work were the same as those in [1]. For gel filtration 80 × 1 cm columns were equilibrated and eluted with buffer, containing 20 mM Na_2SO_4, 20 mM MOPS, pH 6.7, 1 mM EDTA, 1 g/l Triton X-100 at 5°C.

Analytical ultracentrifugation was performed in a Beckman model E ultracentrifuge. The An-H Ti rotor was used together with Kel-F standard double sector. Both sedimentation velocity and sedimentation equilibrium were monitored by the photoelectric scanner.

3. Results and discussion

The last step in the purification of the uncoupling protein is sucrose density gradient centrifugation [1]. By this step not only some extraneous proteins are removed but also the bulk of the free Triton and phospholipids are separated from the protein-Triton micelles. Only after this treatment, the amount of free Triton micelles is small enough to permit determination of the bound Triton by gel chromatography (fig.1). Protein-Triton micelles elute a peak before the bulk of the free Triton. The symmetry of the protein peak reflects its near homogeneity. After correcting for absorbance of the protein, the amount of Triton bound is estimated to be 1.9 g Triton/g protein.

Sedimentation velocity runs in the ultracentrifuge

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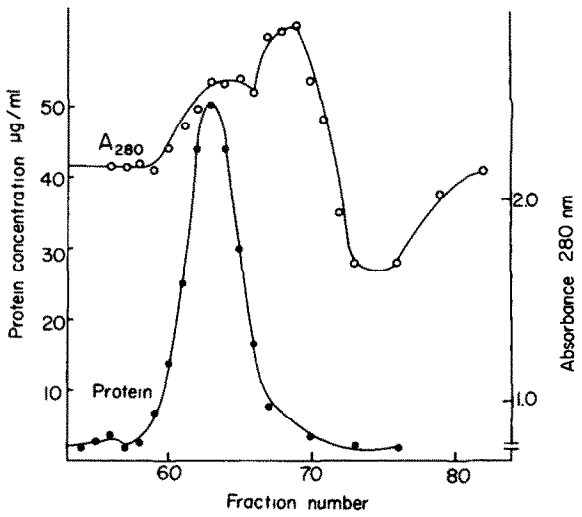

Fig.1. Gel filtration of the uncoupling protein. 750 µl sucrose gradient pretreated coupling protein (0.25 mg protein and 1.67 mg Triton X-100) was applied to the column and eluted with an elution velocity of 2.5 ml/h. The absorbance at 280 nm, after subtraction of the contribution from protein, was used as a measurement for Triton X-100. Each fraction containing 0.5 ml.

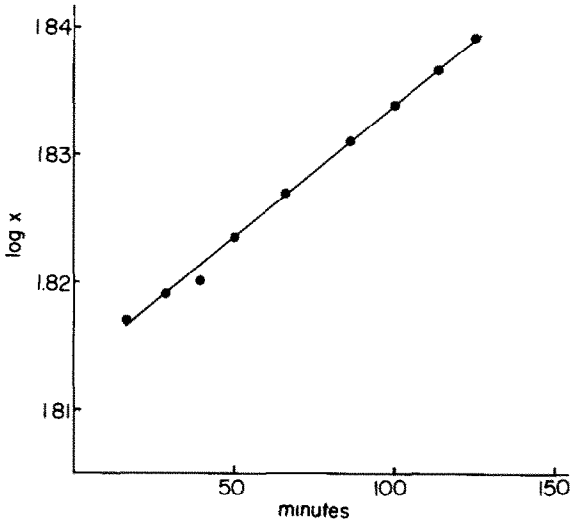

Fig.2. Sedimentation velocity measurements using UV-absorption. A preparation after sucrose gradient centrifugation was used. The sedimentation velocity of the two moving boundaries at 52 000 rev./min at 5°C monitored by its absorbance at 288 nm. X, distance from the center of rotation.

could be performed despite the presence of Triton, making use of the fact that Triton-protein micelles may have a significantly different sedimentation coefficient than the free Triton micelles. This procedure was worked out for the determination of the S-value in ADP, ATP-carrier mixed micelles and becomes possible when the preparation is depleted of the bulk amount of free Triton micelles [6]. Since the protein-Triton micelles are followed by the absorption at 280 nm which is due primarily to Triton the preparation has to be diluted to a protein concentration of 40–60 µg/ml with a Triton concentration of only 0.025%. Under these conditions relatively high s-values between 15 to 25 X 10^{-13} s were routinely observed. This high s-value was attributed to an aggregation of the protein due to a too low Triton concentration which came close to the critical micelle concentration. By shifting the wavelength from 280 to 287–289 nm, the preparation could be scanned at 150 to 230 µg protein/ml and 0.1% Triton. During the run the absorbance scans develop clearly three portions corresponding to the monomeric Triton, protein-free Triton micelles and the more rapidly sedimenting protein-Triton micelles. The evaluation of this portion as a function of time gives a linear relation of the logarithm of the distance from the radius to time (fig.2). From this plot $s^{\circ}_{20,w}$ = 3.9 X 10^{-13} s and in several other scans an average of $s^{\circ}_{20,w}$ = 4.0 is determined.

For the determination of the MW of the protein-Triton complex, sedimentation equilibrium runs were performed. At appropriate speed the micelles of free Triton did not develop a boundary so that the protein-Triton micelle could be clearly discerned in the absorbance scan. After a subsequent sedimentation velocity run, the displaced base line was determined according to the procedure previously used for the ADP, ATP carrier [6]. The equilibrium distribution profile is evaluated in fig.3, giving a straight line indicating a monodisperse protein preparation. The MW calculated from the slope gives 180 000. Cor-

Table 1
Some data on the purified uncoupling protein-Triton X-100 micelle

Triton X-100 binding	1.9 mg/mg protein
MW (sed. equil.)	171 ± 9 kD
Sedimentation coefficient:	
sedimentation velocity	4.0 ± 0.1 X 10^{-13} sec
sucrose gradient centrifugation[a]	4.85 ± 0.1 X 10^{-13} sec

[a] $s^{\circ}_{20,w}$ calculated from sucrose gradient centrifugation using the table of McEwen [7].

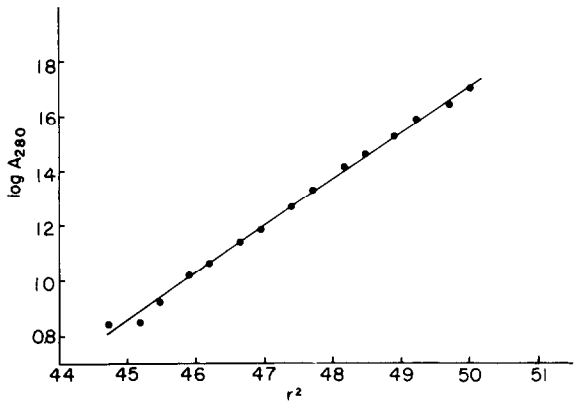

Fig.3. Sedimentation equilibrium measurement. Sedimentation equilibrium pattern at 6000 rev./min and 10°C was recorded with the photoelectric scanner after 72 h. The logarithm of the absorbance at 289 nm of the protein-Triton complex is plotted against the square of the distance from the axis of rotation after subtracting the base line, which was obtained by high speed centrifugation. r, radial distance in centimeters.

recting for the Triton content, the MW for the protein is calculated to 62 000. These values will still have to be corrected for phospholipid that may still be present.

At any rate, this result substantiates a dimeric structure of the isolated uncoupling protein (table 1). Thus we find that the hydrodynamic properties of the uncoupling protein, both in sedimentation velocity and in sedimentation equilibrium runs, are very similar to that of the isolated ADP, ATP carrier [5,6]. Both proteins carry a similar, large Triton micelle which probably reflects the deep imbedment of the proteins in the membrane. The fact that both the uncoupling protein and the ADP, ATP carrier form a dimer with a similar MW and exhibit quantitatively similar interactions with detergents, indicate that the molecular structure is similar. It may finally turn out that the mechanistic properties of these two major proteins in the mitochondrial membrane are also similar.

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References

[1] Lin. C. S. and Klingenberg, M. (1980) FEBS Lett. 113, 299–303.
[2] Riccio, P., Aquila, H. and Klingenberg, M. (1975) FEBS Lett. 56, 133–138.
[3] Klingenberg, M., Riccio, P. and Aquila, H. (1978) Biochim. Biophys. Acta 503, 193–210.
[4] Klingenberg, M. (1978) in: Atractyloside: Chemistry, Biochemistry and Toxicology (R. Santi and S. Luciani, eds) pp. 69–107, Piccin Medical Books, Padova.
[5] Klingenberg, M., Hackenberg, H., Eisenreich, G. and Mayer, I. (1979) in: Functional and Molecular Aspects of Biomembrane Transport (Klingenberg, E. M., Palmieri, F. and Quagliariello, E. eds) pp. 291–303, Elsevier, Amsterdam.
[6] Hackenberg, H. and Klingenberg, M. (1980) Biochemistry, in print.
[7] McEwen, C. R. (1967) Anal. Biochem. 20, 114–149.