SHORT COMMUNICATIONS

BBA 73124

The molecular weight of the major protein component from mitochondrial membranes

The molecular weight of structural protein from numerous sources has been estimated to be approx. 22000 (refs. 1–6). Only BLAIR *et al.*[7] have suggested more recently that the molecular weight of beef heart mitochondrial structural protein is 55000–70000, and SCHNAITMAN[8] observed that rat liver membranes contained major proteins with molecular weights in the range of 50000–68000. A procedure for the purification of the major protein component, presumably equivalent to structural protein, from rat liver mitochondrial membranes has been described[9] using chloroform–methanol (2:1, v/v) as a solvent for the membrane proteins. The molecular weight of this major component has been investigated.

The major protein component was prepared as described previously[9] by combining the fractions containing the major component, as judged by gel electrophoresis, after the second cycle of gel filtration. The gel pattern of the final product is compared with that for total membrane protein in Fig. 2 (Gels 1 and 2).

Initial attempts to measure the molecular weight were made by ultracentrifugation using solutions of the protein in 6 M urea or 6 M guanidine·HCl. The protein was transferred from chloroform–methanol to an aqueous medium by precipitating the protein with a slight excess of ethanolamine. The precipitate was dissolved in a minimal volume of methanol containing HCl (2 μmoles H^+ per mg protein) and remaining chloroform removed by a flow of nitrogen. 6 M urea or guanidine·HCl containing 1 % β-mercaptoethanol was then added and the solution dialysed overnight to the required conditions. Protein dissolved in 6 M urea containing 1 M acetic acid gave a single peak that did not disperse in a 2–3-h run in the ultracentrifuge. However, the sedimentation coefficient from sedimentation velocity experiments increased non-linearly with decreasing protein concentration, while the diffusion constant decreased (Fig. 1), so that it was impossible to extrapolate these values to zero protein concentration. Electrostatic repulsion by like charges was presumed to be responsible for the observed behaviour. Under conditions where such electrostatic repulsion would be minimal, *i.e.* at neutral pH and/or high salt concentration, the protein gave a fast-moving peak that dispersed rapidly in the ultracentrifuge. These observations would suggest that the protein is aggregated in 6 M urea or 6 M guanidine·HCl except when there is intra- and intermolecular repulsion by like charges.

However, a value for the molecular weight was obtained by the method of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate[10]. For electrophoresis the protein was transferred from chloroform–methanol to 8 M urea as described previously. The solution was then made 1 % with respect to β-mercaptoethanol and sodium dodecyl sulphate and dialysed overnight against 0.01 M phos-

phate buffer, pH 7.0, containing 0.1 % β-mercaptoethanol and 0.1 % sodium dodecyl sulphate. Approx. 80 μg protein were added to each gel and electrophoresis was performed at constant current of 10 mA per gel. Direct staining of the gels after electrophoresis did not give a major band but several faint bands. However, soaking the gels overnight in methanol–water (1:1, v/v) containing 7 % acetic acid resulted, on subsequent staining and destaining, in a pattern containing one major band and several faint bands (Fig. 2, Gels 3–6). The staining solution was 0.25 % naphthalene

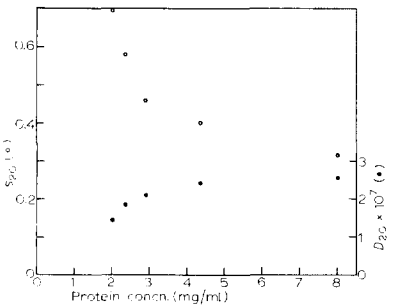

Fig. 1. The effect of protein concentration on s_{20} and D_{20}. The values have not been corrected to water.

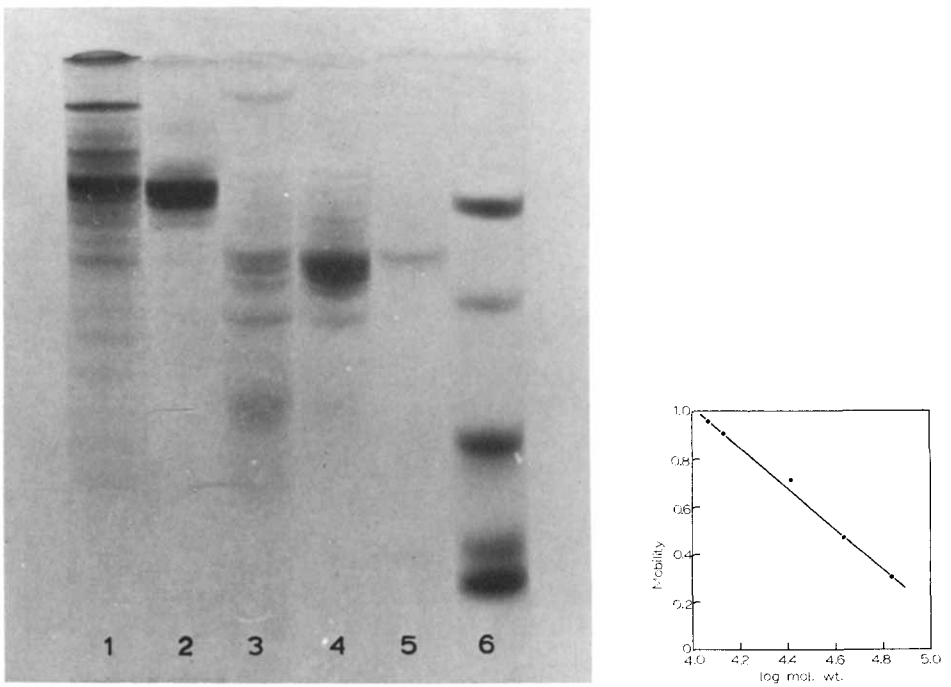

Fig. 2. Polyacrylamide gel electrophoresis. Gel 1, whole membrane protein; 2, major protein component; 3, whole membrane protein; 4 and 5, major protein component; 6, protein standards. Gels 1 and 2 in the Neville buffer system; 3–6, sodium dodecyl sulphate buffer system. Approx. 80–100 μg protein added to each gel except Gel 5 where 30 μg added.

Fig. 3. Typical result of mobility (relative to bromophenol blue) *vs.* log mol. wt.

black in methanol–water (1:1, v/v) containing 7% acetic acid. The gels were destained electrophoretically in methanol–water containing 7% acetic acid and then were allowed to swell in 7% acetic acid in water before measurements were again taken. Bovine serum albumin, ovalbumin, chymotrypsinogen, ribonuclease and cytochrome *c* were run as standards in each experiment (Fig. 3). The conditions were varied from 0.05 M phosphate buffer containing 0.05% sodium dodecyl sulphate to 0.1 M phosphate buffer containing 0.1% sodium dodecyl sulphate, 5–7.5% gel and in the presence and absence of 4 M urea. The value obtained under these various conditions ranged from 52000–60000, but the range obtained using 7.5% gel, 0.1 M phosphate buffer containing 0.1% sodium dodecyl sulphate and no urea was 54000–59000 from twelve determinations, and the average value was 57000.

The major protein component from rat liver mitochondrial membranes isolated by gel filtration in chloroform–methanol did not appear to give rise to multiple components in the sodium dodecyl sulphate electrophoretic system and behaved as a single species, though the fact that the bands, in particular the major bands, do not stain equally and the presence of minor bands in the preparation of the major protein component do not completely preclude other interpretations of the results. There was no major protein component with a molecular weight about 22000. The difficulty of staining the major protein component, also observed by SCHNAITMAN[8], suggests that this band has a higher affinity for sodium dodecyl sulphate as might be expected of a structural element of the mitochondrial membranes.

I would like to thank Dr. J. Paul for his encouragement, Dr. A. Hell for preparing the figures, and the Medical Research Council and Cancer Research Campaign for financial support.

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Received May 19th, 1970