ISOLATION OF A MAJOR HYDROPHOBIC PROTEIN OF THE MITOCHONDRIAL INNER MEMBRANE

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SUMMARY: A protein of molecular weight 29,000 has been isolated from the mitochondrial inner membrane. It is a major component of Racker's hydrophobic protein mixture and is also rather selectively released from the inner membrane by lysolecithin treatment. Data indicate that the 29,000 component may be as much as 10% of the total protein of the inner membrane.

INTRODUCTION

The mitochondrial inner membrane contains a large number of protein components. These are for the most part organized into five complexes: the four electron complexes isolated by Green and his collaborators (1,2) and the oligomycin sensitive ATPase complex (3,4). The polypeptide compositions of Complexes I (5), II (5,6), III (5,7) and IV (8-10) of the electron transfer chain and oligomycin sensitive ATPase (11,12) have been identified. These are referrable to almost all the major bands seen in sodium dodecyl sulfate polyacylamide gels of inner membrane preparation (ETP) (5). One major component of molecular weight 29,000 is not however found in significant amounts in any of these complexes. We have succeeded in isolating and purifying this component. Its method of preparation is detailed in this paper.

MATERIALS AND METHODS

ETP were prepared according to the method of Linnane and Ziegler (13). They were treated with lysolecithin as described by Komai *et al.* (14). The

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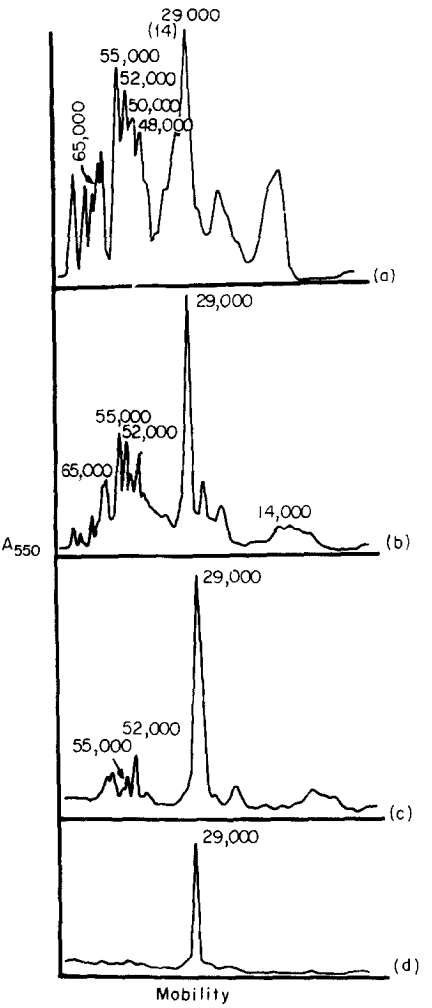

Figure 1. Densitometric traces of various fractions derived from the mitochondrial inner membrane, run on 10% polyacylamide gels in the presence of 1% SDS and 5 mM β-mercaptothanol. Trace a, ETP; Trace b, Racker's hydrophobic mixture; Trace c, the supernatant S_2 from lysolecithin treated ETP; Trace d, pure 29,000 component after chromatography through Biogel A-5 M in 1% SDS and 0.5 mM dithiothreitol. A number of bands are identified by size as follows: 65000, NADH dehydrogenase; 55000 and 52000, the major subunits of F_1; 50000, core protein of Complex III; 48000, one of the b cytochromes of Complex III.

supernatant S_2 was rich in the 29,000 component (15). The hydrophobic protein fraction was prepared as described by Kagawa and Racker (16).

The 29,000 molecular weight protein was further purified from S_2 of the lysolecithin treatment or from the hydrophobic fraction as follows: 5 ml of the above fractions (5-10 mg/ml) were made 5 mM with respect to dithiothreitol

and then were brought to 8 M with respect to urea by addition of the solid reagent. This solution was incubated on ice for 30 min and then centrifuged at 105,000 x g for 120 min. The pellet was resuspended in 3% sodium dodecyl sulphate (SDS) and 5 mM β-mercaptoethanol and dissolved by heating to 100°C for 1 min. This solution was chromatographed through Biogel-A-5 M (Bio-rad Laboratories, Inc.) in 1% SDS and 0.5 mM dithiothreitol. Fractions were monitored for absorbance at 280 nm. SDS was removed from the purified protein by dialysis against a large volume of 90% ethanol in the cold room (4°C) for 48 hours. Ethanol was removed by rotary evaporation and the protein was concentrated by freeze-drying. Protein concentration was estimated by the method of Lowry et al. (17). Polyacrylamide gel electrophoresis was performed and the gels were fixed and stained as described by Fairbanks et al. (18).

RESULTS AND DISCUSSION

A typical densitometric trace of ETP solubilized in 1% sodium dodecyl sulphate and run on 10% polyacrylamide gels is shown in Figure 1 (trace a). Band number 14 is the predominating polypeptide in the preparation. This component has a molecular weight of 29,000 as estimated by its migration on gels in comparison with the standard proteins bovine serum albumin, ovalbumin, lactic dehydrogenase, chymotrypsinogen, ribonuclease and cytochrome c.

The 29,000 dalton protein is the major component in supernatant S_2 of lysolecithin treated inner membrane preparations, as shown in Figure 1 (trace c). It is also the major component of Racker's hydrophobic protein mixture, Figure 1 (trace b).

Treatment of the lysolecithin supernatant or hydrophobic protein fraction with 8 M urea, precipitated the 29,000 molecular weight protein fairly free of other contaminating proteins. Purification to homogeneity was achieved by column chromatography through Biogel A-5 m in 1% SDS and 0.5 mM dithiothreitol, Figure 1 (trace d). Using the lysolecithin method of preparation, the yield of highly purified protein was 5-8% of the starting inner membrane

preparation, even after the manipulations described. This protein therefore is a major component of the mitochondrial inner membrane.

The fact that it is rather selectively "scooped out" and solubilized as a lipoprotein by lysolecithin, suggests that it is distributed in an area of the membrane with a high lipid concentration. This contention is supported by the fact that the protein is solubilized along with a large amount of lipid (about 1.5 mg/mg protein) in the preparation of the hydrophobic protein fraction, while the precipitate left after removal of this fraction is depleted in lipid (0.3 mg/mg protein, cf. 0.5-0.6 mg/mg protein in ETP).

In summary then, we have isolated a major intrinsic protein of the mitochondrial inner membrane with a molecular weight of 29,000. Its function remains obscure at present. It may be significant, however, that the protein is a major portion of Racker's hydrophobic protein fraction, a mixture of proteins which are required for reconstitution of oxidative phosphorylation from the component electron transport and ATP synthesizing complexes (19).

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