THE PROTEINS OF THE INNER MEMBRANE OF RAT LIVER MITOCHONDRIA

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1. Introduction

Purification of membrane proteins and the knowledge of their amino acid sequence are very useful for studies of the structure and the biosynthesis of membranes. The first step for purification is to dissociate the membrane into its constituent polypeptide chains. Schnaitman [1] has compared the band patterns in polyacrylamide gel electrophoresis of the unfractionated proteins of several membranes isolated from rat liver cells. We wish to report here that the proteins of the inner membrane of rat liver mitochondria can be fractionated and purified. One of these proteins, with a molecular weight of about 56,000, has been obtained in a sufficiently pure state to permit amino acid sequence analysis. A fingerprint of the tryptic peptides of this protein is presented.

2. Methods

Rat liver mitochondria were prepared according to Schneider [2]. The inner membrane fraction was isolated by the procedure of Schnaitman and Greenawalt [3]. It was purified further by centrifugation in a continuous exponential sucrose gradient from 18.5% to 38.5% (w/w). This treatment removed some proteins visible as minor bands in polyacrylamide gels.

The purified inner membrane was solubilized and the protein was precipitated by ammonium sulfate as described by Criddle et al. [4]. The precipitate was washed in the centrifuge with a total of 30 volumes of 0.01 M tris-HCl buffer (pH 7.4) containing 0.005 M NaEDTA followed by two washes with distilled water. The precipitate was extracted twice with 10 volumes of acetic acid-acetone (1:9) followed by three extractions with ethanol-ether (2:1) and one with ether. The protein was air dried and could be stored in this form.

3. Results

The proteins were fractionated by chromatography on DEAE-cellulose (fig. 1). The protein of the major peak was reaggregated by dialysis against distilled water. The precipitate was dissolved in 8 M urea. About 20 to 40 mg of protein in 2 to 4 ml were then chromatographed on a 3.5 X 150 cm column of Sephadex G-75 in 8 M urea containing 0.1 M NaCl.

The progress of the fractionation was monitored

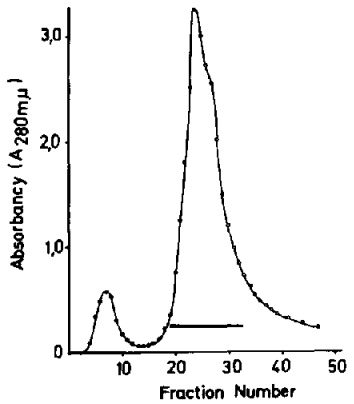

Fig. 1. DEAE-cellulose chromatography of membrane proteins. About 50 mg of proteins was chromatographed on a 2 X 15 cm column in 0.01 M tris-HCl pH 8.0 with 8 M urea, a linear gradient between 0 and 0.5 M NaCl was used. The solid bar indicates the fractions that were pooled.

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by electrophoresis on polyacrylamide gels in the presence of SDS (Na-dodecylsulfate) (fig. 2). Apparently three classes of proteins of different molecular size are present together accounting for about 80% of the total protein mass of the inner mitochondrial membrane (fig. 2a): class I, comprising 25% of the total protein mass contains only one protein species, with a M.W. of about 56,000; class II, with at least 6 species with M.W. ranging from 25,000 to 35,000 accounting for 40% of the total, and class III, with at least two protein species both with M.W. of less than 15,000, constituting about 15% of the total mass.

The major peak eluting right after the void volume of the Sephadex column consists of a single protein species forming one band in the SDS gels (fig. 2d). This protein was also examined by electrophoresis in polyacrylamide gels in the presence of 5 M urea at pH 4.0 [6] and at pH 8.9 [7]. In all instances a single band was obtained indicating that the material is homogeneous in molecular size and has a similar net charge at the two pH values.

A two-dimensional map of the tryptic peptides of this protein (fig. 3) gave about 25 major peptides with some minor ones present.

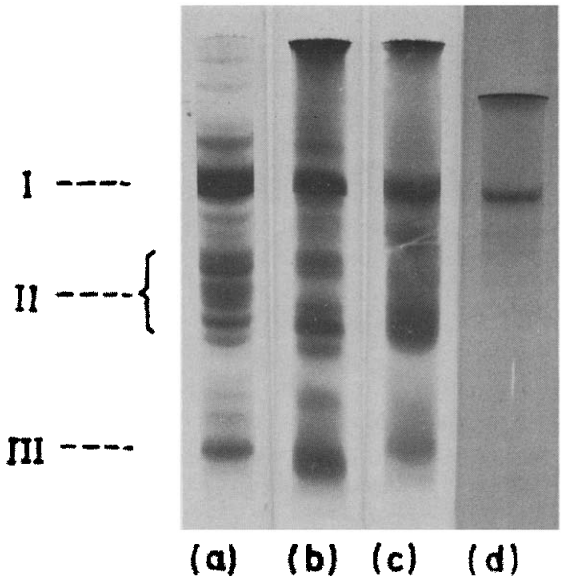

Fig. 2. SDS-polyacrylamide gel electrophoresis of membrane proteins. a) Band pattern of sucrose density gradient-purified intact inner membrane; b) ammonium sulfate precipitate; c) major DEAE peak; d) purified class I protein after Sephadex.

Electrophoresis and molecular weight determinations were performed as described by Shapiro et al. [5] using a 10% acrylamide gel. The proteins were dissolved in 3% SDS. The buffer used was 0.05 M tris-acetate pH 7.6 with 0.5% SDS. The gels were stained with coomassie blue.

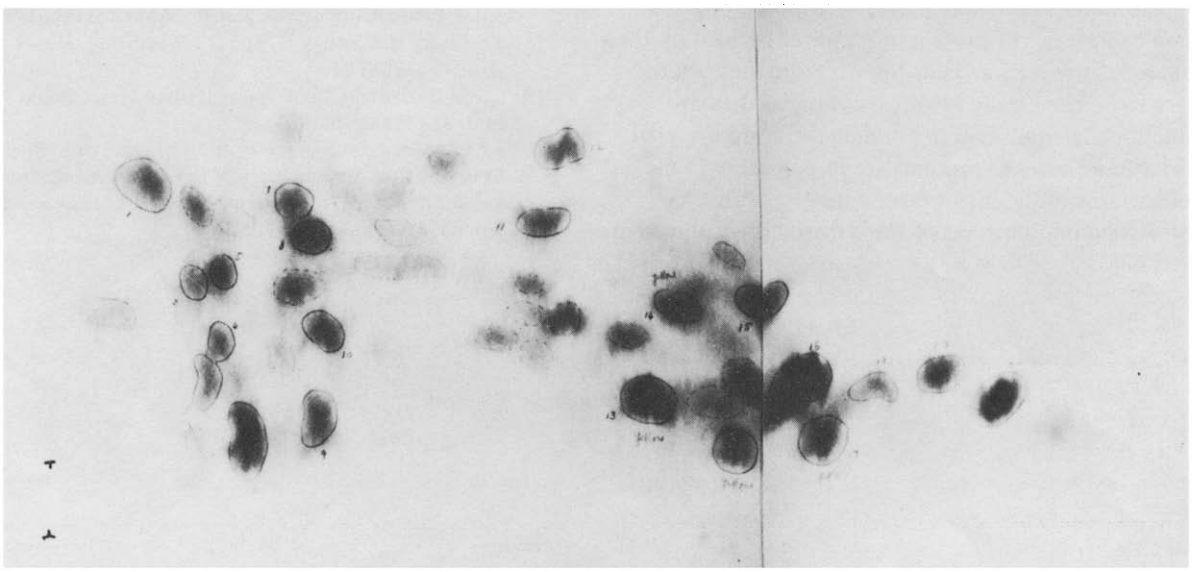

Fig. 3. Map of the tryptic peptides of the class I protein (fig. 2d). The fingerprint was prepared as described by Clegg et al. [8]. The origin and the anode are to the left.

4. Discussion

We have shown that one of the mitochondrial inner membrane proteins can be purified to a degree that an interpretable fingerprint can be obtained. This is a measure of the purity of this protein and shows that it will be possible to determine its amino acid sequence. Amino acid sequence determination will be necessary to test the notion that membrane proteins consist of polypeptide stretches with constant and variable amino acid sequences analogous to the situation in immunoglobulins. In this connection is it noteworthy that on the fingerprint (fig. 3) major and minor spots are visible. Minor peptide spots could arise for such trivial reasons as incomplete digestion, oxidation of methionine residues, etc., or by the presence of a minor contaminating protein. They could, however, also arise if the class I protein consisted of a collection of polypeptide chains of identical chain length and similar net charge with regions of constant and variable amino acid sequence. The strong peptides could then come from the constant region common to all chains whereas the minor peptides could come from the individual region of each chain [9].

The ordered structure of a biological membrane is thought to originate from the noncovalent assembly of structure units in a plane. The units which build the structure in this way may be composed of more than one species of protein molecule as defined by the amino acid sequence. Thus the structure units do not have to be chemically identical as long as they are functionally equivalent in building the structure [10]. Three size classes of proteins are recognizable in the gel patterns of the intact membrane (fig. 2a). It is interesting that proteins of these three classes stayed together during the fractionation procedures involving also a reaggregation step (fig. 2c). This behaviour may be a reflection of the composition of the structure units of this membrane each of which may contain chains of the different size classes.

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