THE LOCATION OF THE MAJOR POLYPEPTIDE OF THE OX HEART MITOCHONDRIAL INNER MEMBRANE

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1. Introduction

A knowledge of the arrangement of the proteins of the mitochondrial inner membrane within the membrane is a necessary requirement for an understanding of the mechanism of respiratory chain function and oxidative phosphorylation. During a study of the location of the inner membrane proteins at the cytoplasmic and matrix faces of the mitochondrial inner membrane, the exposed proteins of ox heart mitochondria were investigated using lactoperoxidase catalysed radioiodination [1]. I found that the outer surface of the inner membrane was accessible to lactoperoxidase (mol. wt 78 000) in mitochondrial preparations. Despite the large number of proteins associated with the inner membrane [2–4] very few polypeptides were detected at its cytoplasmic face. Of these polypeptides one, of apparent mol. wt 29 500 accounts for over 12% of the total inner membrane protein.

2. Materials and methods

Ox heart mitochondria (heavy fraction) were isolated by the procedure of Smith [5] and submitochondrial particles were prepared as described by Low and Vallin [6]. All manipulations were carried out on freshly prepared material.

Iodination was performed in media containing 5 mg/ml mitochondrial protein, 10 mM Tris–HCl pH 7.8, 10 μM $Na^{125}I$ (approx. 12.5 mCi/μmol) and 45 μg of lactoperoxidase per ml. The reaction was initiated by the addition of H_2O_2 (added in five equal aliquots each sufficient to bring the H_2O_2 concentration to 25 μM). The iodinated material was washed five times in 10 mM Tris–HCl pH 7.8 containing 2 mM KI. All operations were done at 0–4°C.

Polyacrylamide gel electrophoresis in the presence of SDS was performed essentially as described by Weber and Osborn [7] but using a horizontal flat plate apparatus which facilitated comparison between electrophoresed samples. Iodinated bands were located either by autoradiography of desiccated polyacrylamide slabs or by liquid scintillation counting of gel slices. Sucrose and Metrizamide [8] density gradient centrifugation were performed essentially as described in [9].

3. Results

3.1. *Iodination of isolated mitochondria*

Mitochondria were subjected to lactoperoxidase catalysed radioiodination and were subsequently fractionated into membranous (submitochondrial particles) and soluble (matrix) fractions. Little radioactivity was incorporated into the matrix protein which is consistent with surface iodination (table 1). SDS gel electrophoresis of the submitochondrial particles revealed that approximately 75% of the incorporated iodine was associated with one component of apparent mol. wt 29 500. Three other components of mol. wts 75 000, 21 000 and 20 000 are also labelled (fig.1) Autoradiography of similar gels after visualization of the protein showed that the 29.5 K iodinated component exactly corresponded

Abbreviations: SDS, Sodium dodecyl sulphate.

Table 1
Fractionation of iodinated mitochondria

Material	cpm/mg protein
Mitochondria	197 000
Matrix fraction	40 900
Submitochondrial particles	278 000

Mitochondria were iodinated as described in Materials and methods and the fractions were separated following ultrasonic disruption of the mitochondria. Incorporated radioactivity was insoluble in 10% trichloroacetic acid. The results quoted are typical for six separate experiments.

with a protein band which constituted 12.5% of the submitochondrial particle protein (fig.2 (a) and (b)). Analysis of iodinated mitochondria prior to fractionation established that no radioactive species had been lost during the preparation of submitochondrial particles (fig.2 (c)).

Monoamine oxidase assays of the submitochondrial particles indicated that the outer membrane could account for only 4% of the total protein based on the data of Hayashi and Capaldi [10]. The large amount of the 29.5 K protein in the submitochondrial particles and the reported absence of polypeptides of this molecular weight from the outer membrane [10]

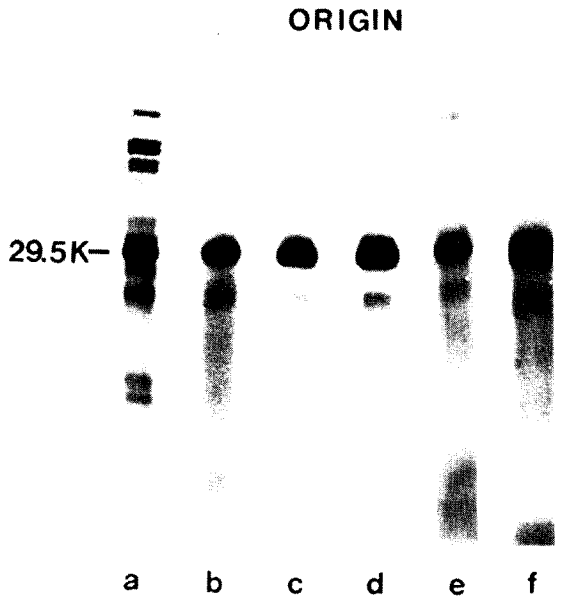

Fig.2. SDS gel analysis of iodinated preparations. (a) Coomassie blue stained gel of submitochondrial particles derived from iodinated mitochondria. (b) Autoradiograph of (a). (c) Autoradiograph of a gel of iodinated mitochondria. (d). As (c) but of digitonin treated mitochondria. (e) As (c) but of mitochondria exposed to a hypotonic medium (buffered 25 mM sucrose). (f) Autoradiograph of mitochondria iodinated in the presence of 200 μM $^{125}I^-$.

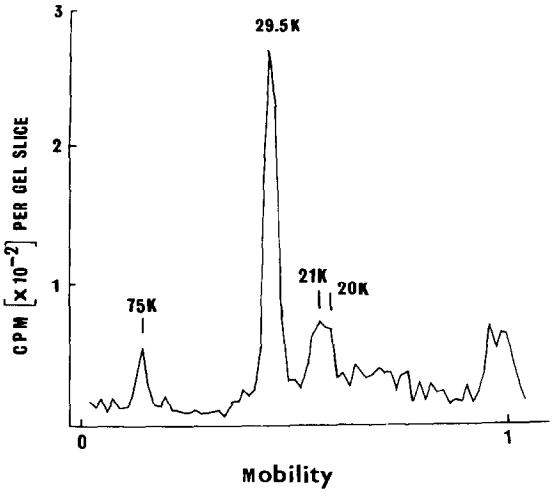

Fig.1. Distribution of radioactivity in a SDS gel of submitochondrial particles prepared from iodinated mitochondria. See Materials and methods for details. Mobility is measured relative to the migration of the marker dye.

suggests that the iodinated component resides in the inner membrane. This implies that the outer membrane did not restrict the access of lactoperoxidase to the inner membrane.

3.2. *Location of the iodinated 29 500 dalton polypeptide*

If the iodinated surface in the above experiment were the outer membrane, then procedures which rupture this membrane should allow the iodination of further components that are located on the outer surface of the inner membrane. Digitonin treatment [11] of the mitochondria and subsequent iodination, failed to alter the spectrum of iodinated polypeptides from that described above (fig.2 (d)). Similar results were found for mitochondria which had been iodinated following exposure to hypotonic (25 mM) sucrose media (fig.2 (e)). Sucrose and Metrizamide density gradient centrifugation of submitochondrial

particles derived from iodinated mitochondria failed to separate the iodinated 29.5 K component from the major staining protein band of that molecular weight. Identical findings were obtained following a similar analysis of ultrasonically disrupted inner membrane/matrix fractions prepared by treating iodinated mitochondria with digitonin [12].

The incorporation of radioactivity during iodination is limited by the low (10 μM) iodide concentration so that in the experiments described only a small proportion of the available sites are modified. However, increasing the iodide concentration (to 200 μM) did not lead to significant iodination of any further polypeptides, indicating that within the specificity limits of lactoperoxidase, the iodinated bands described are representative of the surface polypeptides (fig.2 (f)).

4. Discussion

It appears that the outer membrane did not present a permeability barrier to lactoperoxidase in isolated ox heart mitochondria. Since the membrane is present in the preparations it must be concluded that it is ruptured during isolation. Racker [13] has also reported similar findings. Surface labelling of mitochondria with [^{35}S] diazobenzenesulphonate, a reagent of broad reactivity [14] which would freely permeate the outer membrane, yields a similar almost exclusive radioactive incorporation into the 29.5 K component (P. T. Wingfield, personal communication). It is clear therefore, that the four iodinated species described here must represent the major iodinatable proteins of both the outer membrane and the cytoplasmic face of the inner membrane. From these results it would be predicted that the outer membrane polypeptides are not extensively iodinated, however, attempts at the isolation of this membrane in the author's laboratory have been unsuccessful. Huber and Morrison [15] report that a polypeptide of mol. wt 14 000 is readily iodinated in the outer membrane of rat liver mitochondria, but no similar species was identified in the present work.

It is surprising that relatively few polypeptides can be detected at the outer surface of the inner membrane despite the large number of polypeptides found in this membrane. This distinct asymmetry may have important consequences for the mechanism of respiratory chain action. It should be borne in mind however, that polypeptides of mol. wt less than 10 000 would not be well resolved by the gel system employed.

The 29.5 K component corresponds to the major inner membrane protein whose isolation has previously been reported [14,6]. The function of this component remains to be established. The protein is absent from all the redox complexes [3,4]. However, it appears to be very similar to the carboxy-atractylate binding protein recently isolated by Ricco et al. [17]. Studies on the detailed characterisation of this protein are in progress.

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