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RESOLUTION OF MICROSOMAL MEMBRANES INTO FRACTIONS DIFFERING IN POLYPEPTIDE COMPOSITION

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Summary

Microsomes from rat liver, prepared by gel filtration, were subjected to centrifugation in a continuous sucrose density gradient containing a low concentration of deoxycholate. The membranes were subfractionated into five bands differing in appearance and equilibrium density. Each band, when analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis, displayed a characteristic population of membrane proteins.

Microsomal membranes from rat liver contain over 100 different polypeptides [1]. Although information is accumulating on the transverse disposition of some microsomal proteins [2], the organisation of proteins in the lateral plane of the membrane is at present not well understood. It has been suggested that some of the enzymes of the endoplasmic reticulum which catalyze consecutive reactions, such as the cytochrome *P*-450-linked electron-transfer chain, may be arranged in complexes within the membrane, analogous to those in the inner mitochondrial membrane [3]. So microsomal membranes may be heterogeneous in the membrane plane, with proteins segregated in specific patches of the membrane. In an original approach to this question, Winqvist and Dallner [4] showed that microsomal membranes centrifuged in a sucrose gradient containing sodium deoxycholate can be subfractionated into five bands with specific enrichment of different enzymes in each band, and partial separation of

Abbreviation: SDS, sodium dodecyl sulphate.

electron transfer enzyme activities from glucose-6-phosphatase. Subsequently, Stasiecki et al. [5] have reported on the distribution of further enzymes between microsomal subfractions obtained by this method. These papers, however, do not indicate the extent to which microsomal proteins as a whole are fractionated by this procedure. In this paper, we report characterization by SDS-polyacrylamide gel electrophoresis of the polypeptide composition of subfractions obtained from microsomal membranes by using the method of Winqvist and Dallner [4].

Microsomes were prepared by gel filtration (Sepharose 2B) of a post-mitochondrial supernatant from livers of male Sprague-Dawley rats, as described previously [1,6]. Gel-filtered microsomes were collected by centrifugation (105 000 $\times g_{av}$, 1.5 h) and were then (i) washed in 0.15 M Tris-HCl, pH 8.0, (ii) suspended in water, incubated for 30 min at 30°C and collected by centrifugation as above, (iii) re-washed in 0.15 M Tris-HCl, pH 8.0. The washed microsomal membranes were resuspended in 0.13 M sucrose and gently layered onto a sucrose gradient which was prepared as follows. A continuous linear sucrose density gradient (22 ml, 1.15—1.05 gm/cc was prepared in an MSE mixing chamber and poured onto a 1 ml cushion of 2.0 M sucrose in a 25 ml centrifuge tube. Sodium deoxycholate (0.19%) and Tris-HCl buffer (50 mM, pH 7.5) were present throughout the gradient. Centrifugation was carried out in a swing-out rotor at 80 000 $\times g_{av}$ for 13 h at 4°C; the rotor was allowed to coast to a halt at the end of the run. Gradients were then fractionated from the bottom using a Watson-Marlow flow inducer, to give 80 aliquots each of 0.3 ml. A sample (0.05 ml) of each aliquot was diluted with 1 ml of water and absorbance was

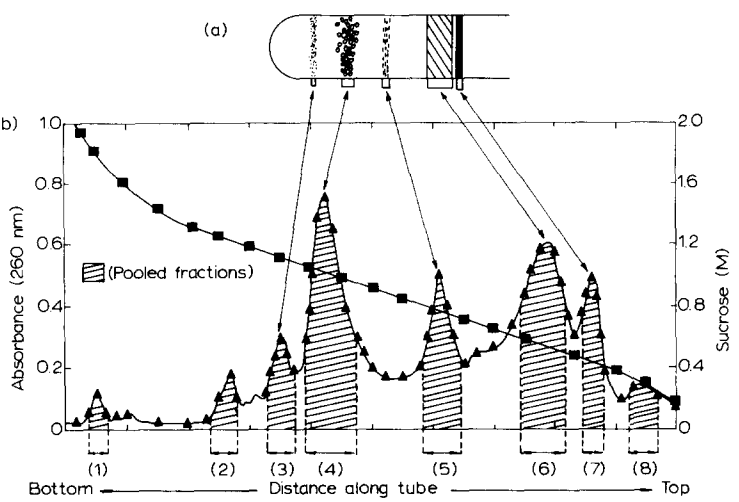

Fig. 1. (a) Diagram illustrating the appearance of the microsomal subfractions after centrifugation in a continuous sucrose gradient containing 0.19% (w/v) deoxycholate. Detailed descriptions of the bands are mentioned in the text. (b) ▲———▲, absorbance profile at 260 nm of the aliquots from the gradient containing subfractionated microsomes. Major absorbance peaks marked by the arrows correspond to the bands visible in the tube after centrifugation. Aliquots forming major absorbance peaks were pooled into fractions 1—8 as shown in the diagram. ■———■, profile of sucrose density gradient along the length of the tube after centrifugation. The gradient was traced by determining sucrose molarity by refractometry in the harvested aliquots.

monitored at 260 and 280 nm. Correction was made for background absorbance by sucrose. Aliquots forming major peaks of absorbance (Fig. 1b) were pooled. Protein content [7], cytochrome *P*-450 [8], cytochrome b_5 [8], NADH and NADPH-cytochrome *c* reductases [9] and glucose-6-phosphatase [10,11] activities were determined in each of the pooled fractions. SDS-polyacrylamide gel electrophoresis was performed in a linear polyacrylamide gradient (16—7%) as described previously [1].

Fig. 1a indicates the appearance of the subfractions obtained after centrifugation in a continuous sucrose gradient containing 0.19% (w/v) deoxycholate. The lowest visible band (density 1.15 gm/cc) was relatively narrow with a fine greyish appearance, the next (density 1.13 gm/cc) was more diffuse with white aggregated particles and the central band (1.10—1.11 gm/cc) was faint, diffuse and flakey in appearance. The two upper bands were in close proximity; the lower of these two appeared as a vivid wide red band (1.07—1.08 gm/cc) and the upper as a narrow creamy band (1.05 gm/cc). Two additional bands in the lowest section of the gradient, and one at the top, were only detectable by their ultraviolet absorbance. Fig. 1b indicates the A_{260} profile, the sucrose molarity gradient, the aliquots which were pooled and the numbering of the pooled fractions.

The distributions of a number of enzyme activities between the isolated subfractions are shown in Fig. 2. As observed by previous workers [4,5], glucose-6-phosphatase activity was found in two regions of the gradient; most was in fraction 3, but significant activity was also present in fraction 7. NADH- and NADPH-cytochrome *c* reductase activities were also partially separated, with the former enriched in fractions 6 and 7, and the latter in fraction 5. The distributions of cytochromes b_5 and *P*-450 matched those of their respective reductases; so that the components of the NADPH-linked electron-transfer chain appeared at a slightly greater median density than those of the NADH-linked

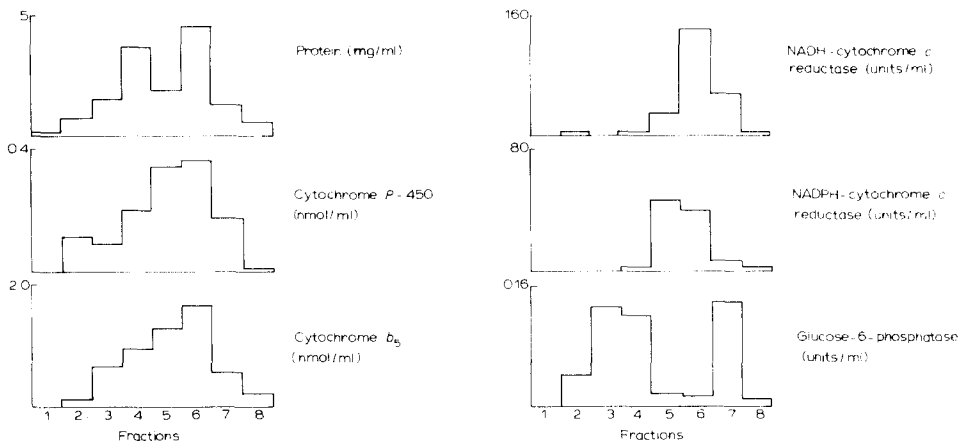

Fig. 2. Distributions of proteins and enzyme activities in the isolated subfractions. Assay methods are referred to in the text. Glucose-6-phosphatase activity was determined at 30°C; NADH- and NADPH-cytochrome *c* reductase activities at 20°C. Control experiments indicate that the effects of the deoxycholate present on the measured enzyme activities are small, and would not significantly alter the observed distributions.

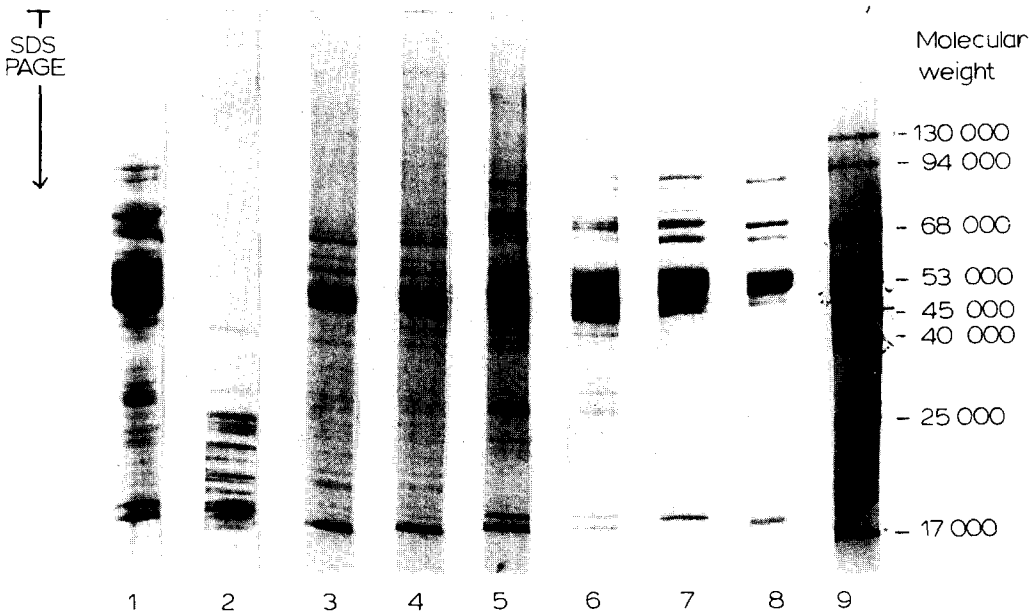

Fig. 3. SDS-polyacrylamide gel electrophoresis (SDS PAGE) (linear gradient 16—7%). 1, total microsomal proteins; 2, fraction 1; 3, fraction 2; 4, fraction 4; 5, fraction 5; 6, fraction 6; 7, fraction 7; 8, fraction 8; 9, marker proteins (molecular weight in parentheses), haemoglobin (17 000), α-chymotrypsinogen (25 000), aldolase (40 000), ovalbumin (45 000), glutamate dehydrogenase (53 000), bovine serum albumin (68 000), phosphorylase a (94 000), β-galactosidase (130 000).

chain. These enzyme distributions are in agreement with those determined by previous investigators [4,5].

These microsomal subfractions have, until now, only been characterised in terms of a few enzyme activities. The exposure to sodium deoxycholate may inactivate or modify some of the enzymes in the subfractions. So it would be useful to have a more comprehensive view of the extent to which this procedure distributes microsomal proteins specifically into subfractions. We have characterised the isolated subfractions by SDS-polyacrylamide gel electrophoresis (Fig. 3). It is clear that while most proteins appear in more than one fraction, there is significant concentration of most proteins into specific regions of the gradient. Thus, fraction 1 contains mostly low molecular weight proteins; this fraction had the highest $A_{260} : A_{280}$ ratio, suggesting that mainly ribosomal proteins are present in this fraction. Of the two proteins in the region of mol. wt. 17 000 and 18 500, only the former is segregated in fractions 2 and 3, and only the latter in fractions 7 and 8. Both of the proteins are present in fractions 5 and 6. The microsomal electron-transport enzymes with molecular weights in the region of 45 000—56 000 appear to be localised in fractions 5 and 6. Low molecular weight components appear to be present in fractions derived from the lower section of the gradient but are absent in upper fractions. Fraction 8 may comprise proteins that are completely solubilised from the membrane by the detergent.

Together with further enzyme studies, this SDS-polyacrylamide gel electrophoretic analysis may throw light on the mechanism by which microsomal membranes are subfractionated in the detergent-containing density gradient, and on the organisation of proteins in the microsomal membrane. It also suggests that this procedure may be a useful preliminary fractionation step in the purification of microsomal proteins.

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