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Fractionation of the Proteins of Liver Plasma Membranes

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Few analytical studies have been made on the plasma (surface)-membrane proteins of mammalian eukaryotic cells, owing mainly to difficulties in isolating sufficient amounts of pure fractions. The method of Neville (1960) for isolating liver plasma membranes was adapted to a low-speed rate-zonal separation through a sucrose density gradient, thus permitting the preparation of plasma membranes in larger quantities free from other subcellular organelles (Evans, 1970a). Polyacrylamide-gel electrophoresis of liver subcellular membrane fractions dissolved in aqueous sodium dodecyl sulphate has shown the presence of a wide range of proteins, and the plasma-membrane fraction has a characteristic profile distinct from that of intracellular membranes such as the smooth endoplasmic reticulum or mitochondrial membranes (Schnaitman, 1969; Evans, 1970b). The different protein pattern observed by gel electrophoresis is reflected in the different enzymic activities present. Liver plasma membranes are also characterized by the presence of large amounts of glycoprotein components, which are stained in polyacrylamide gels by the Schiff-periodate procedure (Evans, 1970b). Other features of the liver plasma-membrane fraction include the relatively high content of cholesterol and sphingomyelin (Patton, 1970), the presence of the specialized intracellular junctions such as tight junctions and desmosomes (Benedetti & Emmelot, 1968) and the presence of transplantation antigens (Evans & Bruning, 1970). In studying the proteins of the liver plasma membranes, we have devised fractionation procedures that exploit the different solubilities of the components in aqueous and organic solvents and in anionic detergents.

The proteins of mouse liver plasma membranes were fractionated by using the following stepwise procedure. Membranes that had been thoroughly

washed with iso-osmotic sodium chloride were extracted at 37°C for 1 h in 50 mM-carbonate-bicarbonate buffer-10 mM-EDTA, pH 9.8, and 20% of the membrane protein was solubilized. Polyacrylamide-gel electrophoresis in 0.1% sodium dodecyl sulphate showed that the soluble fraction consisted of 20 or more proteins of widely differing molecular weights. The soluble fraction was low in carbohydrate content, and staining of the gels with the periodate-Schiff reagent indicated only one major glycoprotein component. Suspension of the washed residue in cold aq. 33% (v/v) pyridine (Blumenfeld, 1968; Blumenfeld, Gallop, Howe & Lee, 1970) and centrifugation of the resulting mixture (170 000 g_{av} for 60 min) gave a clear supernatant containing 30–40% of the remaining protein, which was slightly enriched in sialic acid. Liver plasma membranes thus differ from erythrocyte membranes, in which the membrane sialic acid was found exclusively in the soluble fraction after fractionation by aqueous pyridine (Blumenfeld, 1968). Less protein was found to be soluble in pyridine when the initial alkaline extraction was omitted or when EDTA was not present during the alkaline treatment. Gel electrophoresis of the pyridine-soluble material showed the presence of 12–15 protein bands, a considerable simplification over the 25 or more bands found in the intact membrane. Further fractionation was obtained by dialysis of the pyridine-soluble fraction against 5% (w/v) sodium dodecyl sulphate-0.5 M-urea-30 mM-2-mercaptoethanol and gel filtration on Sepharose 6B in sodium dodecyl sulphate-urea-tris buffer, pH 8. Gel electrophoresis of pooled fractions showed that resolution on the basis of molecular weight occurred, but all fractions contained more than one protein component. The procedure described represents an appreciable advance towards the goal of resolving the liver plasma membrane into its constituent protein components and provides material for chemical and immunological analyses.

Suspension of washed mouse liver plasma membranes in 4% (w/v) sarcosyl (sodium lauryl sarcosinate)-2.3% (w/v) tris, pH 7.8, at 0°C resulted in the solubilization of 80% of the protein and 50–70% of the 5'-nucleotidase activity. The detergent-insoluble fraction was examined by electron microscopy and consisted mainly of amorphous material. However, unit membrane structure was visible in the region of the tight junctions and desmosomes. The sarcosyl-soluble proteins were fractionated on a sucrose density gradient [10–30% (w/v) sucrose dissolved in 0.5% (w/v) sarcosyl-tris, pH 7.8] by centrifuging at 95 000 g_{av} for 24 h (Beckman SW 27 rotor). Most of the phospholipids remained at the top of the gradient, while the proteins entered it. The protein peak sedimented in

the same position as bovine serum albumin (mol.wt. 64000). However, analysis of the gradient for 5'-nucleotidase activity indicated a sharp peak well separated from the major protein peak sedimenting in the same position as immunoglobulin G (mol.wt. 150000). A small amount of organic phosphate was extracted by chloroform-methanol (2:1, v/v) from the fractions corresponding to the enzyme peak. Experiments with membranes derived from animals injected with [32 P]phosphate confirmed the general distribution of phospholipids in the sucrose gradient, and indicated a separate peak of high 32 P specific radioactivity corresponding to the peak of 5'-nucleotidase. Radioautography of phospholipids separated by t.l.c. indicated that phosphatidylethanolamine, phosphatidylcholine, sphingomyelin and phosphoinositides were associated with the enzyme peak.

The separation procedure was repeated on a preparative scale by using zonal centrifugation. Sarcosyl-soluble liver plasma membranes (43ml containing 2.5g of sucrose and 100mg of protein) were introduced into the BXIV rotor (MSE) containing a 10-30% (w/v) sucrose gradient in 0.5% sarcosyl-tris, pH 7.8. An overlay of 200ml of 0.5% sarcosyl-tris was added. After centrifugation for 24h at 43000 rev./min a discrete peak of 5'-nucleotidase activity, which sedimented away from the bulk of the protein, was collected and concentrated by dialysis against Aquacide II (Calbiochem). The enzymically active fraction (5mg) was added to a Sepharose 6B column equilibrated with 0.5% sarcosyl-tris, pH 7.8, and the enzyme peak, corresponding to a small proportion of the protein, was pooled and concentrated.

The enzymically active protein (1mg) was finally purified by gel filtration on Sephadex G-75 equilibrated with 0.5% sarcosyl-tris, pH 7.8. The purified enzyme was examined by gel electrophoresis and consisted of a single band of molecular weight 150000 (Weber & Osborn, 1969). Extraction of the purified enzyme with chloroform-methanol (2:1, v/v) indicated that the protein was still associated with a small amount of phospholipid (1mg of protein/300 μ g of phospholipid). This purified membrane-bound lipid-associated 5'-nucleotidase may be compared with the enzyme isolated from rat liver membranes by extraction with a mixture of Triton X-100 and sodium deoxycholate (Widnell & Unkeless, 1968).

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COMMUNICATIONS

Properties of Mesosomal Membranes Isolated from *Micrococcus lysodeikticus* and *Bacillus megaterium*

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Following the development of methods for obtaining mesosomal membranes from bacteria in the absence of cell lysis (Ferrandes, Chaix & Ryter, 1966; Reaveley, 1968; Ellar & Freer, 1969), several investigators have attempted to determine the function of these unusual membranes and to compare their characteristics with those of the mesosome-depleted plasma membrane. In a careful study Reaveley & Rogers (1969) reported distinct biochemical and morphological differences between these two fractions isolated from *Bacillus licheni-*

formis 6346. A preliminary examination of mesosome and plasma membranes from *Micrococcus lysodeikticus* and *Bacillus megaterium* KM (Ellar & Freer, 1969; Ellar, 1969) revealed significant differences in composition and structure. These experiments have now been extended to cover a wide range of enzyme activities and the differences evaluated quantitatively.

In marked contrast with the plasma membrane, *M. lysodeikticus* mesosomal membranes are deficient in cytochromes *a*, *b* and *c*, succinate dehydrogenase and adenosine triphosphatase and contain smaller amounts of a number of other oxidative enzymes. The specific activities of acid phosphatase and alkaline phosphatase, however, are greater in the mesosome than in the plasma membrane. Mesosome NADH oxidase is completely inhibited by 50mM-magnesium chloride, whereas the activity of the membrane enzyme is not changed under these