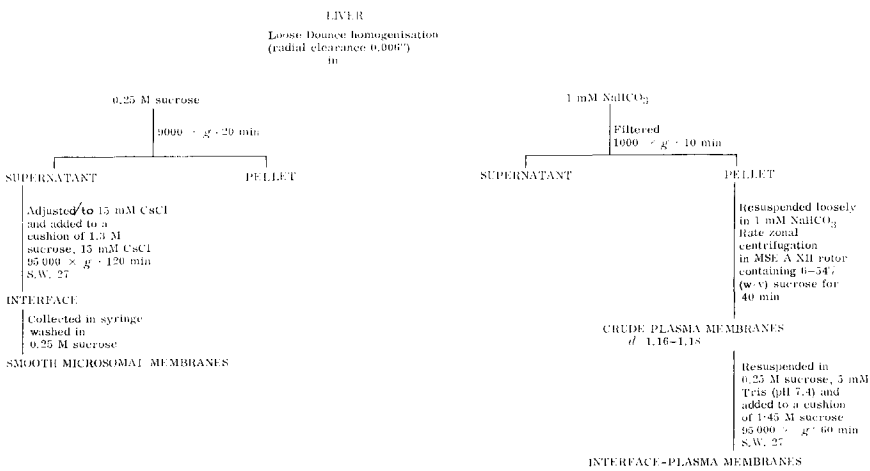
BBA 73126

Glycoproteins of mouse liver smooth microsomal and plasma membrane fractions

Glycoproteins are present in the membranes of most cells where they probably play important structural and functional roles[1]. Yet, current membrane models[2,3] regard the cell membrane as composed almost exclusively of lipid and protein molecules and give little consideration to the presence and role in mammalian membranes of carbohydrate moieties linked covalently to protein. Chemical estimation of cell membranes indicates that up to 10% of the membrane dry weight can consist of carbohydrate[4]. Histochemical studies[5] have shown the presence of carbohydrates, especially in the region of the Golgi apparatus and on the surface membranes of cells. Electrophoretic studies[6] have also pinpointed the presence of sialic acid on the surface of cells. It is therefore pertinent to examine the distribution and approximate molecular size of the glycoproteins present in membranes. In the present report, the glycoproteins of the smooth microsomal and plasma membrane fractions of mouse liver tissue have been studied.

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate represents one of the best analytical systems currently available for examining the complexity and molecular size distribution of the constituent proteins of membranes[7,8]. Membrane proteins resolved by electrophoresis in thin sheets of acrylamide gel containing sodium dodecyl sulphate can therefore be examined for the presence of proteins or carbohydrates. A general stain such as Procian Blue is used to visualise the proteins. The Schiff–periodate procedure used to stain for carbohydrate demonstrates not only carbohydrate constituents containing *vicinal* hydroxyl groups but also any proteins having an N-terminal serine, threonine or an α-hydroxylcarboxylic acid. Membrane proteins so far examined are deficient in N-terminal serine and threonine residues[9], and therefore the most significant reaction of the Schiff–periodate procedure will be with proteins containing covalently bound carbohydrates. This procedure should therefore give an indication of the number and molecular weight of glycoproteins



Scheme 1. Preparation of liver smooth microsomal and plasma membranes.

TABLE I

CHEMICAL AND ENZYMIC COMPOSITION OF MOUSE LIVER SMOOTH MICROSOMAL AND PLASMA MEMBRANE FRACTIONS

The two fractions were prepared as shown in Scheme 1. Neutral sugars and sialic acid were determined on water-washed membranes by the method of DUBOIS *et al.*[11] and AMINOFF[12], respectively.

Fraction	*5'-Nucleotidase (μmoles P_i liberated per mg protein per h)*	*Glucose-6-phosphatase (μmoles P_i liberated per mg protein per h)*	*Neutral sugars (μg hexose/mg protein)*	*Sialic acid (nmoles/mg protein)*
Smooth microsomes	1.8	5.9	46	17.0
Plasma membranes	16.6	0.1	44	34.0

present in relation to the total number of proteins present in the membrane fractions examined by acrylamide gel electrophoresis in sodium dodecyl sulphate.

The smooth microsomal and plasma membrane fractions were prepared from liver homogenates as shown in Scheme 1 and the enzymic activities determined as already described[8,10]. The membrane fractions were first examined for enzyme markers and the total neutral sugar and sialic acid contents were determined (Table I). Although both fractions had a similar neutral sugar content, the plasma membrane fraction contained a characteristically higher amount of sialic acid.

Electrophoresis in sodium dodecyl sulphate-containing polyacrylamide gels resolved both the smooth microsomal and plasma membranes into a large number of components (Figs. 1A, 1C). Each fraction is seen to be of a different protein composition, but both membrane fractions contain protein constituents which extend across a wide molecular weight range. Microsomal membranes consistently showed a preponderance of low molecular weight components. Since the smooth microsomal fraction presumably contains some lysosomes, the presence of these low molecular weight components could be the result of the action of proteolytic enzymes.

Gels stained by the Schiff–periodate procedure showed that many of the protein bands also contained carbohydrate. In the plasma membrane fraction (Fig. 1B) a major glycoprotein or group of glycoproteins (Band 4) of approximate molecular weight 140000 was present. Other bands of lesser intensity and covering a wide range of molecular weight were also identified. The intensely staining bands at the electrophoretic front probably represent glycolipid material, since in the sodium dodecyl sulphate procedure, all lipids, being of low molecular weight, would be expected to have migrated to this point. In the smooth microsomal fraction (Fig. 1D) approx. 8 bands covering a wide molecular weight range were stained. The two bands near the electrophoretic front (Bands 6 and 7) may represent nascent glycoprotein chains, for the smooth microsomal fraction would contain membranes derived from elements of the endoplasmic reticulum and the Golgi apparatus, and the latter component is now accepted as being a major subcellular site of glycoprotein synthesis[13].

The molecular weights of membrane proteins and glycoproteins determined by the above procedure are obtained by comparison with the position of migration of standard protein markers of known molecular weight[14]. However, the molecular weights determined for membrane proteins by this method must be regarded as ap-

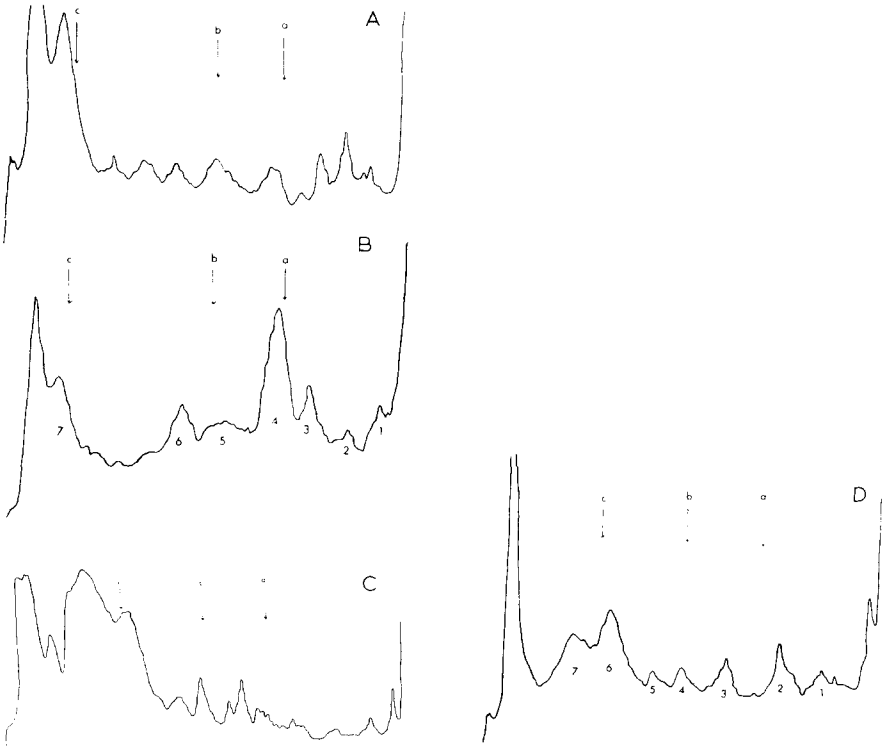


Fig. 1. Polyacrylamide gel electrophoresis densitometer tracings of mouse liver membrane fractions. Direction of electrophoretic migration is from right to left. Plasma membranes, stained for protein (A) or glycoprotein (B); smooth microsomes, stained for protein (C) or glycoprotein (D). Electrophoresis was performed in 7% acrylamide gels containing 0.1% (w/v) sodium dodecyl sulphate in a flat plate electrophoretic apparatus as previously described[8]. Membrane fractions (500 μg) dissolved in 5% (w/v) sodium dodecyl sulphate containing a few drops of glycerol were used per run. Following electrophoresis for 16 h at a constant current of 9 mA, protein bands were visualised in 1% (w/v) Procian Blue in methanol–acetic acid–water (8:1:11, by vol.) and destained in the same solvent. For glycoprotein staining, gels were left for 30 min in 10% (w/v) trichloroacetic acid, and following a water wash, they were reacted with 2.5% (v/v) periodic acid dissolved in 3% (v/v) acetic acid for 60 min. Excess periodic acid was removed by extensive overnight washing with water and the bands visualised by immersion in Schiff reagent (British Drug Houses) for 45 min in the dark. The following marker proteins (50 μg) were used (a) rabbit immunoglobulin G, mol. wt. 150000; (b) adenosine deaminase (Boehringer), mol. wt. 35000; (c) trypsin (Worthington), mol. wt. 24000. Gels stained to visualise protein or carbohydrate were photographed and the prints used to obtain the tracings shown by reflectance using a Joyce-Loebl Chromoscan Mk II densitometer. Glycoprotein tracings were made at about 3 times higher sensitivity than protein tracings.

proximate. It has been shown that most characterised proteins bind 90–100% of their weight of sodium dodecyl sulphate, and the use of secreted glycoprotein markers has shown that this is not greatly affected by the presence of large amounts of carbohydrate on the protein[15]. However, membrane proteins, unlike the hydrophilic marker proteins used, are known to possess hydrophobic properties and may therefore bind additional amounts of sodium dodecyl sulphate, as already shown with viral envelope proteins[16], resulting in slightly erroneous molecular weights being determined by the present procedure.

The results presented clearly indicate that liver smooth microsomal and plasma membrane fractions contain a number of glycoprotein moieties now identified by the Schiff–periodate staining procedure. In addition, the liver plasma membrane fraction possesses a major glycoprotein or group of glycoproteins of similar molecular size. Studies on erythrocyte membranes[17] have shown that two major glycoproteins of molecular weight 30000 can be extracted. Recently[18], it has been shown by acrylamide gel electrophoresis and differential staining that human erythrocyte membranes contain a major glycoprotein of molecular weight 100000. Soluble glycoprotein fragments of molecular weight 70000 carrying the H.2 alloantigenic sites have been isolated from mouse spleen after digestion of cell membranes by papain[19].

The range of glycoproteins now shown to be present in liver membranes may have a number of important functions. Plasma membrane glycoproteins have been implicated in cell contact, metastatic, antigenic and transport phenomena[20]. Further studies on the composition, biosynthesis and turnover of liver membrane proteins and glycoproteins are in progress.

I thank Dr. H. R. Perkins for encouragement and helpful discussions and Mr. R. Fordham for technical assistance. Rabbit immunoglobulin G was kindly donated by Dr. D. G. Smyth of this Institute.

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Received May 11th, 1970