DISTRIBUTION OF INSULIN RECEPTOR SITES AMONG LIVER PLASMA MEMBRANE SUBFRACTIONS

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1. Introduction

In the subcellular fractionation of cells, the assignment of the separated membrane elements to their topographical location in the intact cell is usually accomplished by using morphological, biochemical and chemical markers [1]. In the case of the cell surface membrane, isolated as a plasma membrane fraction, the use of biochemical, chemical and immunological markers has sufficed only to determine the extent of contamination of this fraction by intracellular membranes [2]. In fractionation studies of liver, membrane fragments connected by intercellular junctions are identified as being derived from the contiguous faces of hepatocytes, but the surface topographical origin of the large numbers of nondescript vesicular profiles in the fraction is unknown.

Liver plasma membranes were fractionated to give vesicular elements of differing densities [3,4], and to assign these functionally to either the blood sinusoidal, or the bile canalicular faces of the hepatocyte, we have examined the distribution of hormone binding activity. The presence of specific polypeptide hormone binding activity in isolated liver plasma membranes has been shown by a number of studies [5–7]. We have assumed that the insulin-binding receptors of the hepatocyte are located mainly on the sinusoidal region of the hepatocyte surface, and have employed an insulin binding assay [8] to determine the distribution of these receptors among the plasma membrane subfractions. The results show that when plasma membranes are isolated from a low speed 'nuclear' pellet, the subfractions of sucrose density 1.16 and 1.18 show a higher capacity to bind specifically [^{125}I]insulin than the subfraction of density 1.13.

2. Materials and methods

Mouse and rat liver plasma membrane fractions were prepared by a rate zonal centrifugation method and subfractionated as previously described [3,4]. In the present experiments, discontinuous gradients were employed to separate the subfractions. The light (density 1.13), heavy-A (density 1.16), and heavy-B (density 1.18) subfractions were collected at 8–39%, 39–44%, and 44–49% (w/v) sucrose interfaces, respectively. Unfractionated membranes were collected at 8–47% (w/v) sucrose interface [9]. The biochemical, morphological, immunological and biosynthetic properties of the light and heavy subfractions have been described [3,4,9,10]. Plasma membrane intercellular gap-junctions were prepared as previously described [11]. Membranes were stored in isotonic sucrose at −70°C until required. The protein content of membranes was determined by the method of Lowry et al. [16].

Polyacrylamide slab gel electrophoresis was carried out in sodium lauryl sulphate—Tris glycine buffers by using the E.C. apparatus (Philadelphia, Pa., USA) essentially as described by Maizel [12]. Gels were discontinuous, with a 3.6% (w/v) acrylamide spacer gel pH 6.7, and an 8.5% (w/v) acrylamide resolving gel pH 8.3. Plasma membrane samples, 80 μl containing 100 μg protein [13] were dissolved by heating in 2%

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sodium lauryl sulphate, 1% mercaptoethanol, 0.1 M
Tris pH 6.7 at 90°C for 3–5 min and the total sample
was applied to the gel. Electrophoresis was carried
out at 30 mA for 16 hr and the bands were stained
with Coomassie Blue [12]. The following reo-viral
polypeptides were used to calibrate the gels: λ_1, mol.
wt 150 000; μ_2 mol. wt 72 000; Σ_3 mol. wt 36 000
[14].

The insulin binding assay used was as follows: crys-
talline porcine insulin (Novo Industries, Copenhagen;
Batch 66/1 activity 22.6 units/mg) was iodinated with
Na ^{125}I (carrier free, The Radiochemical Centre,
Amersham, U.K.) to a specific activity of 1.25 Ci/
μmol [15]. Hormone binding was determined by in-
cubating membrane fractions at 25°C for 40 min in
0.15 ml Krebs—Ringer bicarbonate buffer containing
1% bovine albumin [15] in the presence of [^{125}I]in-
sulin (1.8 × 10^{-9} M final concn., total radioactivity
300 000 cpm). Two 50 μl aliquots were transferred on
to Whatman glass filter discs (2.4 cm) previously
soaked in the above buffer and the membranes were
rapidly washed with 10 ml of buffer under vacuum fil-
tration. Moist filters were dried and radioactivity de-
termined. Approx. 0.7% of the total radioactivity was
attached to the filters in the absence of cell mem-
branes. Specific binding was calculated by subtracting
radioactivity not displaced when the assay was run in
the presence of 9.2 × 10^{-6} M native insulin. Non-
specific binding varied with the type and amounts of
membrane fractions used; with plasma membranes,
non-specific binding was 5–30% of the total radioac-
tivity bound in different experiments, and with iso-
lated intercellular junctions it amounted to 100%.

3. Results and discussion

The two major plasma membrane subfractions of
rat or mouse liver have been investigated in detail
[3,4,9,10]. The properties of the two subfractions
may be briefly summarized. The light subfraction
possessed the highest specific activities of 5'-nucleo-
tidase, Mg^{2+} adenosinetriphosphatase and leucylnaph-
thylamidase, and contained more cholesterol and sial-
ic acid relative to protein. However, the specific activ-
ity of Na$^+$K$^+$ adenosinetriphosphatase was higher in
the heavy subfraction. Both subfractions were shown
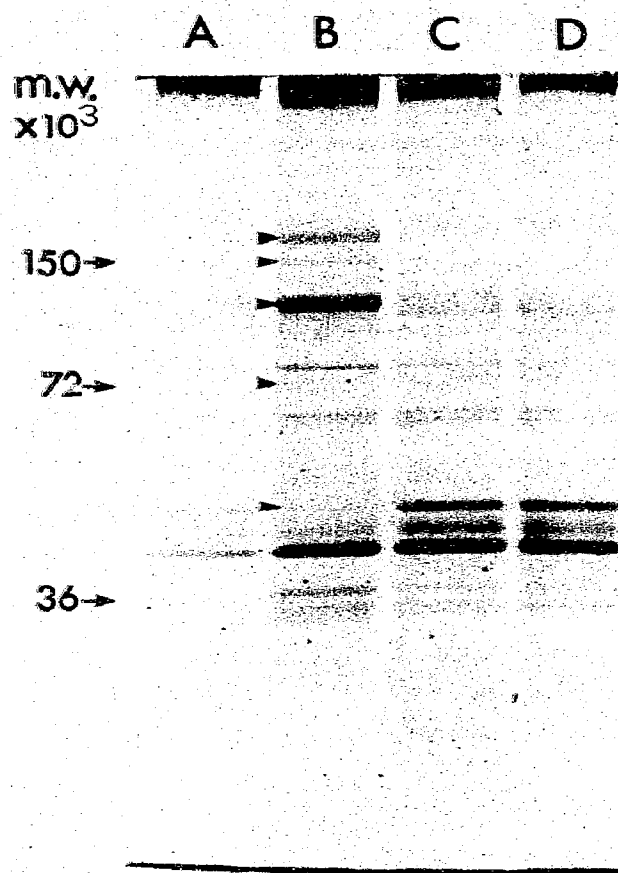by electron microscopy to be composed of vesicles

Fig. 1. Polyacrylamide gel electrophoresis in sodium lauryl
sulphate—Tris glycine pH 8.3 of (A) - unfractionated rat liver
plasma membranes and (B) 'light' (C) 'heavy A' and
(D) 'heavy B' subfractions. Arrows indicate differences be-
tween the light and heavy subfractions. The positions of the
molecular weight markers are indicated.

but the heavy subfraction contained, in addition, the
strips of paired membranes with intercellular junc-
tions which betray an origin from the faces for join-
ing parenchymal cells.

The separation of the heavy subfraction into two
fractions, with approximately equal amounts of pro-
tein, resulted in no significant segregation of the en-
zymic and chemical properties and electron micro-
scopic analysis showed the presence of vesicles and
junctional complexes in both subfractions. The light
subfraction accounted for 15–20% of the total pro-
tein content of the unfractionated plasma membrane
fractions [4,11], and both heavy and light subfrac-
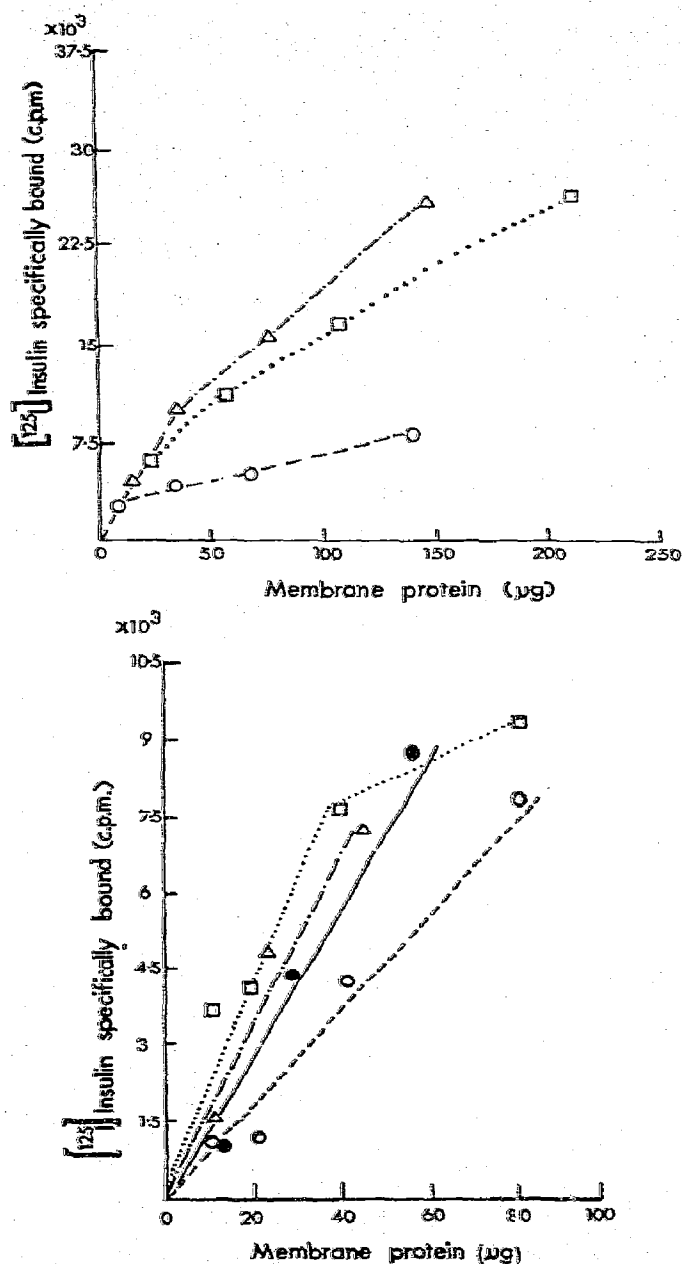tions isolated accounted for 20–25% of the total 5'-

Fig. 2. Specific binding of [^{125}I]insulin to (a) mouse and (b) rat liver plasma membrane subfractions. Details of the binding assay are related in the text. (●—●—●) Unfractionated plasma membranes; (○—○—○) light; (△—△—△) heavy A; (□—□—□) heavy B subfractions.

nucleotidase content of the homogenate. A polyacrylamide gel electrophoretic analysis of the three subfractions prepared from rat liver homogenates is shown in fig. 1. Although the two heavy subfractions

showed an identical polypeptide composition, they differed from the light subfraction (arrowheads fig. 1). The polyacrylamide gel patterns of the liver plasma membrane fractions differ radically from those of other intracellular membranes, especially Golgi and smooth microsomal fractions [16].

The specific binding of [^{125}I]insulin to the three subfractions is shown in fig. 2a (mouse) and fig. 2b (rat). In both cases the light subfraction has the least binding activity. The distinction in binding efficiency between the light and heavy A and B subfractions became more marked when larger amounts of membranes were used, and was most evident with mouse liver plasma membranes (fig. 2a). The insulin binding activities of the heavy A and B subfractions are similar to one another. With isolated plasma membrane intercellular junctions [11] binding of [^{125}I]insulin was high, but it was not diminished by unlabelled insulin and was therefore considered to be nonspecific.

The hepatocyte possesses a surface membrane which is morphologically and functionally specialized. Three major areas are recognised — blood sinusoidal, bile canalicular and contiguous surfaces [18, 19]. However, only the intercellular junctions (i.e. tight, gap and intermediate junctions and the desmosomes) present in the plasma membrane fraction could be assigned to a particular surface location, and uncertainty has centred around the exact origin of the vesicles present. The isolation of plasma membranes of different densities [20—22] and the subfractionation of liver plasma membranes [3, 4, 22] into two or more fractions have provided membranes enriched in cell surface markers, but an assignment to the sinusoidal or canalicular faces was not made. Histochemical studies [23] have indicated the preferential location of 5'-nucleotidase at the canalicular surface and this was used tentatively to assign the light subfraction, which possesses the highest 5'-nucleotidase specific activity, to this area of the hepatocyte surface [4].

The higher insulin-binding activity demonstrated in the heavy A and B subfractions of mouse and rat liver plasma membranes leads to the conclusion that these two fractions are enriched in vesicular elements of the hepatocyte surface derived from the sinusoidal face and the intercellular face, since in the intact liver insulin must reach the hepatocyte from the blood sinusoids. Conversely, it would appear that the light subfraction is enriched in membrane elements from

the canalicular face, which are released during the tight Dounce homogenisation of the 'unfractionated' plasma membranes [3,4]. Hence the subfractionation used apparently separates the canalicular elements from the sinusoidal and intercellular membranes.

The conclusion that plasma membranes of sucrose density 1.16–1.18 possess the hormone binding activity is in full agreement with the extensive work on unfractionated plasma membranes of similar density which has laid the groundwork of our current knowledge of the properties of hormone receptors and adenylate cyclase activity of liver plasma membranes [5–8]. It is also consistent with the preferential location of the Na^+K^+ATP-ase in the heavy plasma membrane subfraction [3,4] since it has been shown that polypeptide hormones influence the activity of this enzyme in isolated liver plasma membranes [24] and lymphocytes [25]. Subfractions prepared from rat liver homogenates by House and Weidemann [26,27] were used to show that a 'light' subfraction was enriched in insulin binding and hormone-sensitive adenylate cyclase activities. Although these authors suggested that their light subfraction corresponded to the light subfraction used in the present studies [4], our results do not support this contention. The differences may reflect the radically different method of preparation of the plasma membrane subfractions.

Acknowledgements

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