

Receptor-mediated endocytosis of ovalbumin by two carbohydrate-specific receptors in rat liver cells

The intracellular transport of ovalbumin to lysosomes is faster in liver endothelial cells than in parenchymal cells

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1. The uptake of ovalbumin (OVA) in rat liver parenchymal cells (PC) and non-parenchymal cells was studied *in vivo* and *in vitro* in order to compare the cellular expression of glycoprotein receptors and the kinetics of intracellular transport of ligand endocytosed by these receptors. 2. Ovalbumin was labelled with ^{125}I or with ^{125}I -tyramine-cellobiose (^{125}I -TC). By using ^{125}I -TC-OVA the labelled degradation products were trapped in the cells. 3. ^{125}I -TC-OVA was rapidly cleared from blood mainly by receptor-mediated uptake in the liver. At 30 min after injection, 50% of the ligand was recovered in the liver. The endothelial cells (EC) and the PC were the predominant cell types responsible for uptake. 4. The uptake in PC was strongly inhibited by asialo-orosomucoid (AOM), but not by mannan, indicating that the uptake in these cells was mediated by the galactose receptor and not by the mannose receptor. This finding is compatible with the observation that a proportion of the OVA contains terminal galactose residues in the carbohydrate moiety. 5. *In vitro* uptake of OVA in cultured EC was saturable and inhibited by mannan, mannose, fructose, *N*-acetylglucosamine, EDTA or monensin, but not by galactose or AOM. The uptake of OVA in these cells was therefore mediated by the mannose receptor. 6. To label the organelles involved in endocytosis in PC and EC, ^{125}I -TC-OVA was injected intravenously together with an excess of either AOM or mannan. In this way the labelled ligand could be directed selectively to EC or PC respectively. Subcellular fractionation of total liver in sucrose and Nycodenz gradients revealed that in EC the intracellular transport of OVA is so fast that endocytosed ligand accumulates and thus increases the density of the lysosomes. Conversely, in PC transfer of ligand is slower, with the result that accumulation of undegraded ligand in the lysosomes does not occur. These findings are interpreted to mean that in EC the rate-limiting step of handling of endocytosed ligand is intralysosomal degradation, whereas in PC the rate-limiting step is transport of ligand to the lysosomes. 7. Altogether, these findings suggest that endocytosis of OVA by the liver EC and PC is mediated by mannose and galactose receptors respectively, and that the kinetics of intracellular transport of OVA differ in the two cell types.

INTRODUCTION

Glycoproteins with mannose or *N*-acetylglucosamine in terminal non-reducing positions are rapidly cleared from rat and rabbit blood by the mannose receptor. A mannose receptor has been described in rat and rabbit alveolar and bone-marrow-derived macrophages [1–3]. These observations led to the belief that macrophages in general carry the mannose receptor, and it has been suggested that it is characteristic for cells of the reticuloendothelial system [4].

The hepatic mannose receptor has been reported in non-parenchymal liver cells (NPC), which bind and internalize mannose-terminated glycoproteins both *in vivo* [5] and *in vitro* [6]. Parenchymal cells (PC) have been alternately reported to express mannose receptors on their surface. Thus Tolleshaug *et al.* [7] and Blomhoff *et al.* [8] reported that the mannose-terminated glycoproteins invertase and β -galactosidase are taken up in PC, whereas Haltiwanger & Hill [9] found mannose-receptor antigen on Kupffer cells (KC) and endothelial cells (EC), but not on PC.

The purpose of the present study was initially to determine the precise distribution of binding sites for mannose-terminating glycoproteins in PC and NPC, using ovalbumin (OVA) as a model ligand. OVA labelled with fluorescein isothiocyanate

(FITC) has previously been used to identify liver EC by fluorescence microscopy [10], and ^{125}I -OVA has recently been reported to be taken up in rat liver EC in suspension [11].

The results indicate that OVA is endocytosed by both hepatic PC and EC. The uptake of OVA in PC was, however, not mediated by a mannose-specific receptor, but by the asialoglycoprotein receptor. The existence of a receptor specific for glycans terminating in mannose in liver PC is therefore unlikely.

The finding that OVA is taken up both by galactose receptors in liver PC and mannose receptors in liver EC suggested that this particular ligand could be used to identify the organelles involved in endocytosis in the two cell types in subcellular-fractionation studies. To direct the ligand to EC or PC organelles, OVA was coinjected with an excess of unlabelled asialo-orosomucoid (AOM) or mannan respectively. As the ligand is labelled with ^{125}I -tyramine-cellobiose (^{125}I -TC), the labelled degradation products are trapped in the lysosomes after receptor-mediated endocytosis and may therefore serve as lysosomal markers. The TC-residue cannot penetrate biological membranes, and the radioactive degradation product is therefore trapped in the cell where the degradation takes place [12]. With this technique we could show that the transfer of endocytosed material from endosomes to lysosomes was much faster in EC than in PC.

Abbreviations used: AOM, asialo-orosomucoid; EC, endothelial cell; FITC, fluorescein isothiocyanate; KC, Kupffer cell; NPC, non-parenchymal cell; OVA, ovalbumin; PC, parenchymal cell; TC, tyramine-cellobiose.

MATERIALS AND METHODS

Chemicals and animals

Na^{125}I was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Collagenase, albumin (fraction V), galactose, mannose and monensin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Nycodenz and Maxidens were obtained from Nycomed, Oslo, Norway. Fibronectin (purified from human plasma) was a gift from Kabi Vitrum A.B., Stockholm, Sweden.

Male Wistar rats, weighing 200–250 g, were fed a standard diet. Cell-culture dishes (1.6, 3.5 or 6.0 cm diameter) and RPMI medium were purchased from Costar (Cambridge, MA, U.S.A.) and Flow Laboratories (Irvine, Ayrshire, Scotland, U.K.) respectively. All other chemicals were of analytical grade.

Labelling of OVA

OVA was labelled with ^{125}I by oxidation with sodium hypochlorite [13] or by covalent attachment of ^{125}I -TC as described by Pittman *et al.* [12]. The TC adduct was kindly provided by Dr. H. Tolleshaug (Nycomed). The specific radioactivity of ^{125}I -OVA was 10–15 $\mu\text{Ci}/\mu\text{g}$ and of ^{125}I -TC-OVA, 1–1.5 $\mu\text{Ci}/\mu\text{g}$. Radioactivity was measured in a Kontron MR252 γ -radiation counter.

Determination of radioactivity in blood and various organs

The rats were anaesthetized by intraperitoneal injection of pentobarbital (0.05 mg/g body wt.). Clearance and cellular distribution studies were performed by injecting about 30×10^6 c.p.m. of ^{125}I -TC-OVA through the tail vein or right femoral vein. Radioactivity in blood was monitored by taking 50 μl blood samples from the tail vein or left femoral vein at different times. Various organs were removed 30 min after injection and washed in phosphate-buffered saline (0.02 M-sodium phosphate/0.15 M-NaCl, pH 7.5) before being assayed for radioactivity.

Preparation and cultivation of purified liver cells

Isolated rat liver cells were prepared essentially as described by Seglen [14] and Berg & Blomhoff [15]. PC and NPC were isolated from the total liver cell suspension by differential centrifugation. Pure suspensions of PC and NPC were prepared by centrifugal elutriation [16] or density centrifugation in Percoll [10].

Monolayer cultures of PC, established and maintained in fibronectin-coated dishes (5 μg of fibronectin/ cm^2), contained less than 1% NPC. The suspensions of NPC (containing KC, EC and stellate cells) were seeded on glutaraldehyde-fixed albumin-coated dishes [17] and incubated for 20 min at 37 °C, resulting in attachment and spreading only of KC [18]. Unattached cells were transferred to fibronectin-coated dishes to enable attachment and spreading of EC. The purity of KC and EC in the cultures was at least 99%, monitored by counting the percentage of peroxidase-positive cells (KC [19]) and cells with accumulated fluorescein isothiocyanate (FITC)-labelled OVA (EC [10]). The average numbers of cells grown per cm^2 were 5×10^4 KC, 2.5×10^5 EC and 1×10^5 PC. All cultures were established and maintained in RPMI 1640 medium without serum.

Subcellular fractionation

At different time points after injection of the ligand, liver lobules were tied off, cooled immediately and homogenized in 0.25 M-sucrose solution containing 1 mM-Hepes, pH 7.2, and 1 mM-EDTA using a Dounce homogenizer [20]. A 10% (w/v) cytoplasmic extract was prepared and layered on to linear gradients made either of Nycodenz (density ranging from 1.05 to 1.18 g/ml) or sucrose (density ranging from 1.10 to 1.24 g/ml). The volume of the gradient itself was 34 ml. The tubes were

centrifuged at 85000 g in a Beckman SW 28 rotor at 4 °C for 45 min (Nycodenz gradients) or 4 h (sucrose gradients). After centrifugation the gradients were divided into 18×2 ml fractions by upward displacement using Maxidens as displacement fluid.

The densities of the fractions were calculated from the refractive indices. Degradation of the labelled ligand was monitored

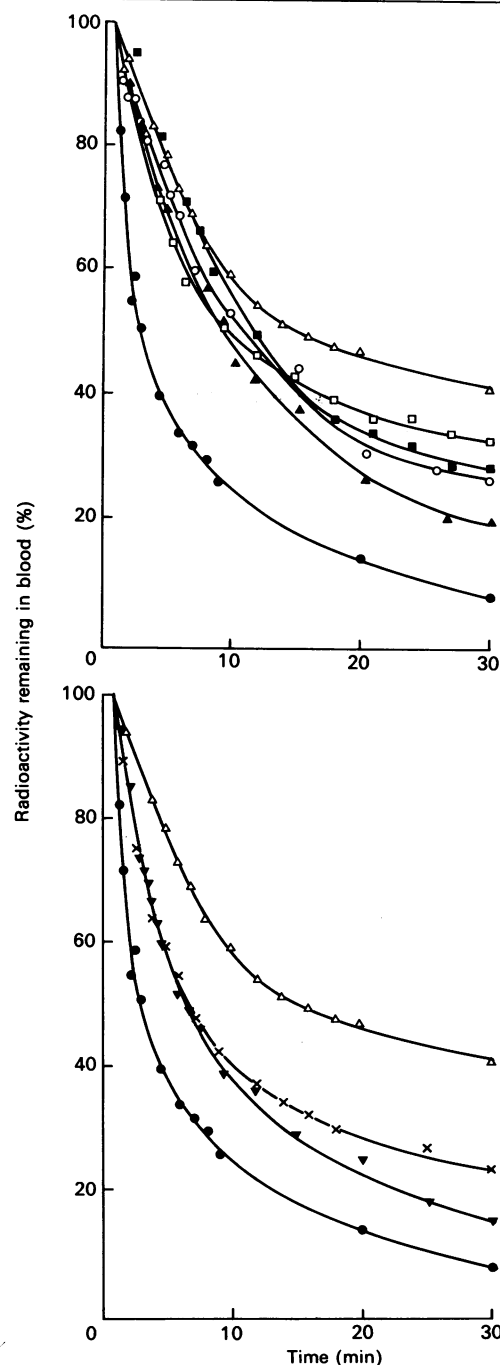


Fig. 1. Clearance of OVA from rat blood

^{125}I -TC-OVA was injected intravenously into rats alone (●; labelled OVA half-life, $t_{1/2}$, 2 min) and simultaneously with 200 nmol of unlabelled OVA (△; $t_{1/2}$ 15 min), 1 mg yeast mannan (M , not known) (□; 9 min), 25 nmol AOM (×; 6 min) or both 1 mg of yeast mannan and 25 nmol of AOM (■; 11 min), 50 mM-mannose (▲; 9 min), 50 mM-galactose (▼; 6 min) or both 50 mM-mannose and 50 mM-galactose together (○; 10 min). Blood samples were removed at different times and expressed as percent of the amount found one minute after the injection. $t_{1/2}$ values for labelled OVA in the blood are shown above for each clearance curve.

by measuring radioactivity soluble in 10% (w/v) trichloroacetic acid. Albumin was added as a carrier to a final concentration of 0.5% (w/v) before acid precipitation.

Number of experiments

All experiments have been performed at least four times with similar results. In all Figures, results from one typical experiment are shown.

RESULTS

Uptake of OVA *in vivo*

After intravenous injection, ^{125}I -TC-OVA disappeared very rapidly from the circulation, with a half-life of approx. 2 min (Fig. 1). The rate of clearance was reduced when a large excess of unlabelled OVA was added, indicating that the uptake process was saturable. The rate of clearance was also decreased by simultaneous injection of mannose or yeast mannan, which inhibited the clearance markedly, and galactose or AOM, which caused a lesser increase in the half-life. By adding mannan and AOM, or mannose and galactose, together with the labelled ligand, the half-life increased additively.

In order to study the uptake of OVA by various organs, ^{125}I -TC-OVA was injected into rats. Fig. 2 shows that the liver is the organ containing most radioactivity 30 min after injection of the ligand (50%). The uptake of OVA in the liver was saturable and could be reduced by simultaneous injection of yeast mannan or AOM. The amount of recovered radioactivity in the kidneys increased with simultaneous injection of excess unlabelled OVA or other compounds that reduced association with the liver; the increased uptake in the kidneys was therefore probably due to an increased amount of ligand filtered in the kidneys. Spleen and lungs contained negligible amounts of the radioactivity.

In order to study the role of the different liver cell types in uptake of OVA, PC, EC and KC were isolated after intravenous injection of the ligand. In control rats, the EC contained nearly 40% of the injected dose, the PC about 10% and the KC less than 5% of the radioactivity (Fig. 3). The uptake in the EC was saturable and inhibited by yeast mannan, but not by AOM. In PC, the uptake was only slightly reduced by the injected excess of unlabelled protein. It was somewhat increased by simultaneous

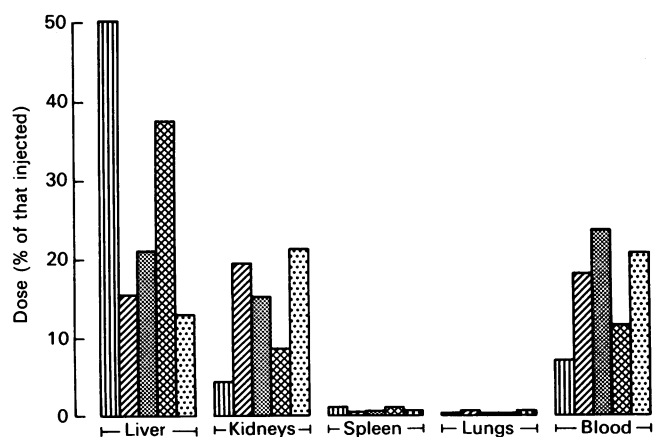


Fig. 2. Distribution of OVA among rat organs

^{125}I -TC-OVA was injected intravenously into rats alone (□) or simultaneously with 200 nmol of unlabelled OVA (▨), 1 mg of yeast mannan (▩), 25 nmol of AOM (⊠) or both 1 mg of yeast mannan and 25 nmol of AOM (⊞). After 30 min the amount of radioactivity recovered in liver, kidneys, spleen, lungs and blood was calculated and expressed as a percentage of the injected dose.

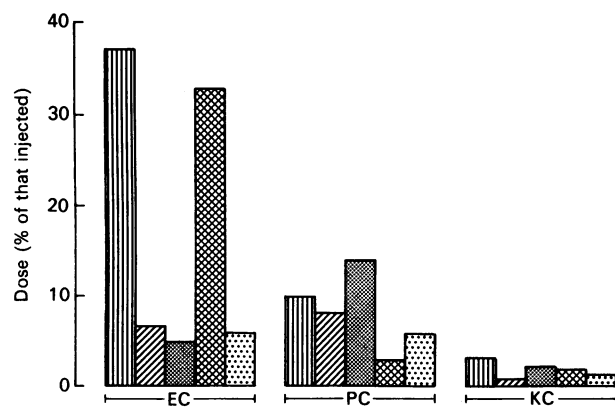


Fig. 3. Cellular distribution of OVA in isolated rat liver cells after intravenous injection

^{125}I -TC-OVA was injected intravenously into rats alone (□) or simultaneously with 200 nmol of unlabelled OVA (▨), 1 mg of yeast mannan (▩), 25 nmol of AOM (⊠) or both 1 mg of yeast mannan and 25 nmol of AOM (⊞). After 30 min the liver was perfused and EC, PC and KC were isolated. The amount of cell-associated radioactivity was determined and expressed as a percentage of the injected dose.

injection of yeast mannan, but inhibited by AOM. Association with the KC was saturable and somewhat inhibited by both yeast mannan and AOM.

Uptake of OVA in cultured liver cells

Cultures of liver EC, KC and PC were incubated with 1 nM ^{125}I -OVA and analysed for cell-associated radioactivity and acid-soluble radioactivity in the media after 60 and 120 min. After 2 h of incubation the total uptake (cell-associated + acid-soluble radioactivity) was 19% in EC and about 2% in PC and KC (expressed as a percentage of the total radioactivity). Acid-soluble radioactivity in the medium was 15% in cultures of EC and about 1% in cultures of KC and PC (Fig. 4).

Directly labelled and TC-labelled OVA were endocytosed to about the same extent in cultures of liver EC (Fig. 5). The specific uptake was approximately linear for at least 60 min. Endocytosis of ^{125}I -OVA and ^{125}I -TC-OVA in cultured EC could be inhibited by the presence of excess amounts of unlabelled OVA (Fig. 6),

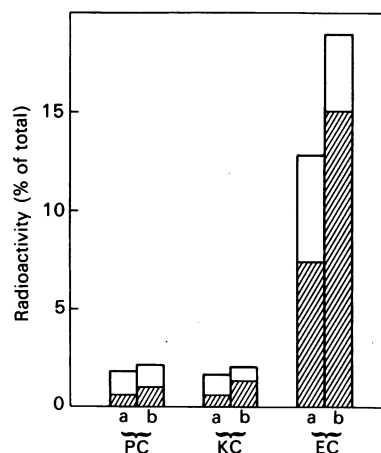


Fig. 4. Endocytosis of OVA in cultured liver PC, KC and EC

Cultures of PC, KC and EC were incubated at 37 °C in the presence of 1 nM ^{125}I -OVA. Cell-associated (□) and acid-soluble (▨) radioactivity were measured after 60 (a) and 120 (b) min and expressed as a percentage of the total amount of radioactivity added.

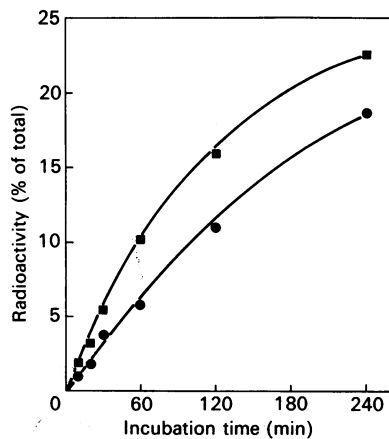


Fig. 5. Endocytosis of labelled OVA in cultured liver EC

Cultures of EC were incubated at 37 °C in the presence of 1 nM-¹²⁵I-OVA (●) and 1 nM-¹²⁵I-TC-OVA (■). After various time intervals, total uptake was determined as a function of time. Total uptake of ¹²⁵I-OVA was obtained by adding acid-soluble and cell-associated radioactivities.

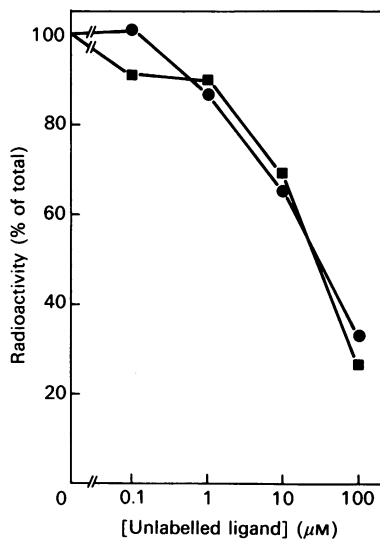


Fig. 6. Effects of increasing concentration of unlabelled OVA on the uptake of ¹²⁵I-OVA and ¹²⁵I-TC-OVA by cultured EC

Cultures of EC were incubated for 120 min at 37 °C in the presence of 1 nM-¹²⁵I-OVA (●) or 1 nM-¹²⁵I-TC-OVA (■) and increasing concentrations of unlabelled OVA. Total uptake is presented as a percentage of uptake in the absence of unlabelled ligand.

showing that the uptake is specific. After 2 h of incubation at 37 °C, the uptake of both ligands was inhibited by 50% in the presence of 50 µM unlabelled OVA.

When increasing concentrations of mannan were included in the incubation medium, endocytosis of ¹²⁵I-OVA and ¹²⁵I-TC-OVA in cultured EC was strongly inhibited. Uptake of ¹²⁵I-TC-OVA and ¹²⁵I-OVA was reduced to nearly 10 and 20% respectively in the presence of 100 µg of mannan/ml (Fig. 7).

Several monosaccharides (at 50 mM concentration) inhibit endocytosis of OVA (Fig. 8). Uptake of ¹²⁵I-OVA by EC was decreased to 20% of the control value by mannose, 30% by fructose, 45% by *N*-acetylglucosamine, 55% by glucose and 80% by galactose. Addition of 2 µM-AOM did not influence the uptake of OVA. The uptake was nearly blocked by the Ca²⁺ chelator EDTA (4 mM), indicating that the binding of OVA to EC is Ca²⁺-dependent. Monensin (1 µM), an ionophore which

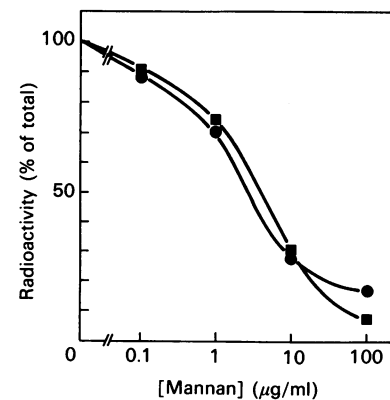


Fig. 7. Effect of mannan on endocytosis of ¹²⁵I-OVA and ¹²⁵I-TC-OVA in cultured EC

Cultures of EC were incubated for 120 min at 37 °C in the presence of 1 nM-¹²⁵I-OVA (●) or 1 nM-¹²⁵I-TC-OVA (■) and increasing concentrations of mannan. Uptake was measured as a percentage of uptake in the absence of added mannan.

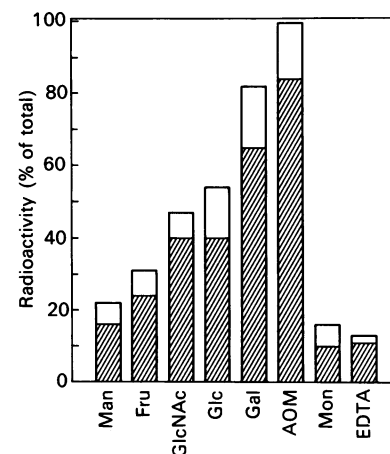


Fig. 8. Effect of inhibitors on uptake and degradation of ¹²⁵I-OVA

Cultures of EC were incubated for 120 min at 37 °C in the presence of 1 nM-¹²⁵I-OVA together with 50 mM-mannose (Man), 50 mM-fructose (Fru), 50 mM-*N*-acetylglucosamine (GlcNAC), 50 mM-glucose (Glu), 50 mM-galactose (Gal), 2 µM-AOM, 1 µM-monensin (Mon) or 4 mM-EDTA. Cell-associated (□) and acid-soluble (▨) radioactivity were measured in the presence of each compound and expressed as a percentage of the control (value) (no inhibitors added).

dissipates H⁺ gradients across membranes and thereby inhibits receptor recycling and endosome-lysosome fusion, strongly inhibited the uptake and degradation of OVA.

Subcellular distribution in liver of intravenously injected ¹²⁵I-TC-OVA

As we wanted to compare the density distribution of compartments involved in endocytosis in EC and PC, we injected ¹²⁵I-TC-OVA together with a large excess of AOM to exclude uptake in PC, thereby obtaining a good marker for the endosomes and lysosomes in EC. Conversely, by injecting ¹²⁵I-TC-OVA together with an excess of mannan, the uptake in EC was nearly blocked and the labelled acid-precipitable and acid-soluble radioactivity formed from OVA could therefore serve as markers for the organelles involved in PC (Fig. 3).

In order to study the intracellular transport and degradation of endocytosed ¹²⁵I-TC-OVA *in vivo*, cytoplasmic extracts were

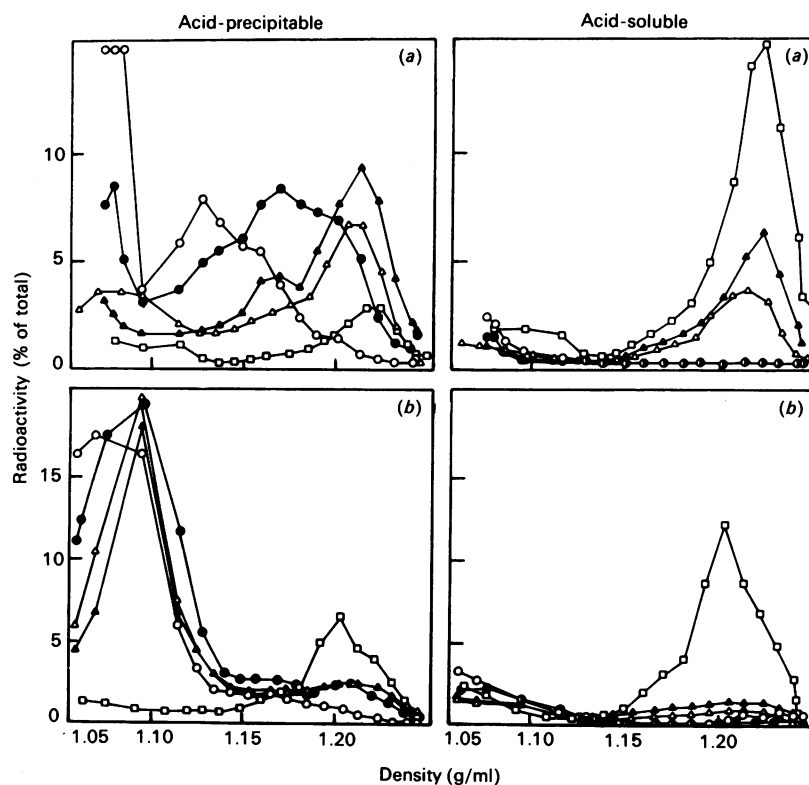


Fig. 9. Fractionation of liver homogenate in sucrose gradients

Postnuclear fractions were prepared from liver 1 (○), 6 (●), 12 (△), 24 (▲) and 60 (□) min after intravenous injection of 0.8 nmol of ^{125}I -TC-OVA simultaneously with 25 nmol of AOM (a) or 1 mg of yeast mannan (b). The sucrose gradients were centrifuged at 85000 *g* for 4 h at 4 °C. After fractionation, acid-precipitable and acid-soluble radioactivity in each fraction were determined and presented as a percentage of total activity in the gradient. The radioactivities are plotted against the densities of the fractions.

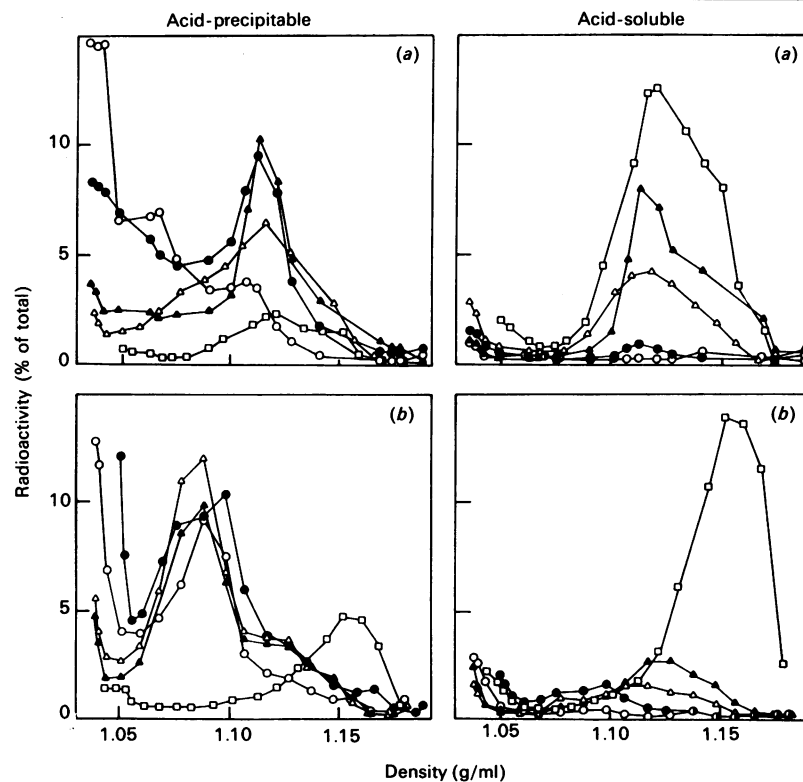


Fig. 10. Fractionation of liver homogenate in Nycodenz gradients

Postnuclear fractions were prepared from liver 1 (○), 6 (●), 12 (△), 24 (▲) and 60 (□) min after intravenous injection of 0.8 nmol of ^{125}I -TC-OVA simultaneously with 25 nmol of AOM (a) or 1 mg of yeast mannan (b). The gradients were centrifuged at 85000 *g* for 45 min at 4 °C. After fractionation, acid-precipitable and acid-soluble radioactivity in each fraction were determined and presented as percentages of total activity in the gradient. The radioactivities are plotted against the densities of the fractions.

prepared from liver 1, 6, 12, 24 and 60 min after injection. The distributions of radioactivity were analysed after centrifugation in Nycodenz and sucrose gradients.

Three steps in the endocytic pathway can be seen in EC after fractionation in sucrose gradients (Fig. 9). Undegraded ligand was rapidly transferred through two kinds of endosomes (densities 1.14 and 1.17 g/ml) before delivery to the lysosomes. Already by 12 min after injection the degradation was initiated were both acid-soluble and acid-precipitable radioactivity distributed at 1.21 g/ml in sucrose gradients. At later time points (24 and 60 min) acid-soluble radioactivity accumulated in this region of the gradients.

In PC, undegraded OVA was localized in endosomes (density 1.09–1.10 g/ml in sucrose gradients) 1, 6, 12 and 24 min after injection. Only small amounts of acid-soluble radioactivity was seen at a density of 1.19–1.20 g/ml at all time points tested before 60 min after injection. At 60 min after injection both acid-precipitable and acid-soluble radioactivity accumulated in this region of the gradients.

The results show that the acid-soluble degradation products formed in EC equilibrate at a higher density than those formed in PC, indicating that sucrose gradients can separate endothelial and parenchymal lysosomes.

The use of Nycodenz gradients showed that, 1 min after uptake in EC, the ligand was associated with two types of organelles banding at 1.07 and 1.11–1.12 g/ml (Fig. 10). At later time points undegraded ligand distributed as a single peak at 1.11–1.12 g/ml. In this region of the gradient, acid-soluble activity appeared 12 min after injection. At 24 and 60 min after injection the degradation products were still seen in this region in addition to a shoulder at higher densities.

In PC, acid-precipitable activity was seen in an endosome banding at 1.08–1.09 g/ml in Nycodenz gradients for all time points tested before 60 min. The degradation was initiated in an organelle banding at 1.12–1.13 g/ml, and 60 min after injection, both undegraded and degraded ligand were distributed at 1.15 g/ml.

DISCUSSION

The present study shows that OVA is rapidly cleared from the rat circulation mainly by receptor-mediated uptake in the liver. The uptake takes place primarily by the mannose receptors in liver EC and, to a lesser extent, in PC. In addition the data show that OVA is not taken up by a mannose-specific mechanism in PC.

The signal for uptake in PC is probably terminal galactose, present in the carbohydrate side chain of OVA. OVA is a glycoprotein with oligosaccharide side chains largely composed of mannose and *N*-acetylglucosamine but, in addition, a proportion of the molecules have terminal galactose residues [21].

An excess amount of unlabelled OVA had no effect on the uptake of labelled OVA in the PC. This could be explained by assuming that the proportion of OVA carrying terminal galactose is so low that, even at high concentrations of OVA, the concentration of galactose-terminated OVA is well below saturation of the galactose receptors in PC.

The hepatic uptake pattern of OVA is similar to that reported for lactoperoxidase [22] and tissue plasminogen activator [23,24], which are cleared by mannose and galactose receptors in the liver. The present findings are in accordance with immunocytological studies suggesting that the PC are devoid of the mannose receptor, whereas sinusoidal cells contain significant amounts [9]. However, the results are in contrast with previous reports of a mannose-specific uptake of β -galactosidase and invertase in PC as well as NPC [7,8]. The uptake of these two

ligands in PC was therefore probably due to some sort of adsorptive endocytosis, independent of the mannose receptor.

The results *in vitro* confirm that OVA is taken up and degraded by EC and, to a lesser extent, by PC and KC. Unlabelled OVA and yeast mannan effectively inhibited the endocytosis of labelled OVA in EC. Mannose and fructose inhibited endocytosis more effectively than *N*-acetylglucosamine and glucose. Galactose and AOM had no significant effect. Furthermore, the finding that EDTA reduced the endocytosis markedly is compatible with uptake via the mannose receptor, which has been reported to require Ca^{2+} [25]. Monensin inhibited the uptake and degradation markedly, indicating that endocytosis of OVA by the mannose receptor follows a classical endocytic pathway in cultured EC.

These results confirm that uptake of OVA in cultured EC is mediated by the mannose receptor. The inhibition effects on cultured cells are in accordance with results obtained in suspended EC [11].

The results showed that acid-soluble degradation products formed from ^{125}I -TC-OVA may serve as markers for the lysosomes in the two cell types. By simultaneous injection of ^{125}I -TC-OVA and mannan or AOM the degradation products serve as markers for the lysosomes in PC and EC respectively. It was found, in accordance with earlier studies [20], that the lysosomes of EC equilibrate at a higher density in a sucrose gradient than those from the PC. On the basis of the difference in density distribution of PC and EC lysosomes in sucrose gradients, subcellular fractionation of a total liver homogenate can be used to differentiate between the cell types responsible for endocytosis of injected OVA upon simultaneous administration of the appropriate inhibitors.

The results obtained by means of subcellular fractionation showed that the kinetics of the intracellular transport of endocytosed ligand were distinctly different in PC and EC. The endocytic pathways in the two cell types could be compared in this study since the ligand taken up via the galactose receptors and the mannose receptors were identical except for the terminal galactose residues in a subgroup of OVA. It has previously been shown that the mannose receptor in the EC internalizes ligands at least 10 times as rapidly as the galactose receptors in PC [11]. The main kinetic difference in the intracellular handling of OVA in PC and EC is seen in the step between endosomes and lysosomes.

In the PC the bulk of the ligand is located in the endosomes and it is transported relatively slowly to the lysosomes, where degradation takes place very efficiently. Only small amounts of undegraded OVA is found in the lysosomes at all time points studied. This is in accordance with previous studies in isolated PC [26,27].

In EC the ligand is transported very rapidly from the endosomes to the lysosomes; after 12 min, most of the OVA is already in the lysosomes, as judged by the subcellular distribution pattern. As a result of the rapid transport, a relatively large proportion of the ligand is undegraded in the lysosomal fractions after 24 min. Eskild *et al.* described that this was the case also for formaldehyde-treated serum albumin taken up by the scavenger receptor in liver EC [28]. Accumulation of undegraded ligand may also be the reason why the lysosomes of EC are relatively dense in sucrose gradients. Owing to the very rapid intracellular transport of ligand in EC, lysosomal degradation is the rate-limiting step in the handling of endocytosed ligand in these cells. In contrast, intracellular transport seems to be rate-limiting in PC.

We are grateful to Ms. Vivi Volden for expert technical assistance. This work was financially supported by the Norwegian Cancer Society, the Nordic Insulin Foundation and King Gustav V's 80-year Fund.

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Received 11 December 1989/5 March 1990; accepted 15 March 1990