Human serum contains a lectin which inhibits hepatic uptake of glycoproteins

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Human serum will inhibit the specific uptake of *N*-acetylglucosamine-terminated glycoproteins by isolated rat hepatic sinusoidal cells. The serum inhibitors are not glycoproteins that bind to the hepatic mannose/*N*-acetylglucosamine receptor but have the properties of lectins which bind to mannose/*N*-acetylglucosamine-terminated glycoproteins. They can be isolated from serum by affinity chromatography. The predominant lectin, with a molecular mass of less than 35 kDa, will inhibit the sinusoidal cell uptake of glycoproteins in vitro.

Lectin *Glycoprotein* *Mannose* N-*Acetylglucosamine* *Sinusoidal cell* *Serum*

1. INTRODUCTION

The survival of glycoproteins in the circulation is determined by the terminal non-reducing sugar residues of their oligosaccharide moiety [1]. Galactose-terminated glycoproteins are rapidly cleared from the blood by a galactose-specific receptor on hepatocytes and mannose- and *N*-acetylglucosamine-terminated glycoproteins are cleared from the blood by a Man/GlcNAc specific receptor on hepatic sinusoidal cells (review [2]). Human serum will inhibit the binding of galactose-terminated glycoproteins to the galactose-binding protein in an in vitro assay [3]. The inhibitors are galactose-terminated glycoproteins [4]. We show here that human serum also contains a lectin which will inhibit glycoprotein uptake by the hepatic Man/GlcNAc receptor.

2. MATERIALS AND METHODS

Asialo-orosomucoid and agalacto-orosomucoid

Abbreviations: Man, mannose; GlcNAc, *N*-acetylglucosamine; ASOR, asialo-orosomucoid; AGOR, agalacto-orosomucoid; HBSS, Hank's balanced salt solution.

were prepared by the modification of human orosomucoid (Sigma, Poole), and were radioiodinated with carrier-free $Na^{125}I$, to a specific activity of 5–12 μCi/μg by a solid-phase enzymobead system (Bio-Rad, Richmond, CA) which uses immobilized lactoperoxidase and glucose oxidase [5]. Mannan was also iodinated by this method to a specific activity of 1.5 μCi/μg. Male Sprague Dawley rats bred in the Comparative Biology Unit of the Royal Free Hospital School of Medicine were used. Hepatic sinusoidal and parenchymal cells were prepared by collagenase perfusion followed by differential centrifugation and centrifugal elutriation as in [5]. One fraction containing endothelial and Kupffer cells was collected from centrifugal elutriation by pooling the cells eluted between 13 and 46 ml/min.

Sinusoidal cells ($5–10 \times 10^6$ cells/ml) were suspended in glucose-free HBSS (500 μl) containing ^{125}I-AGOR with or without serum or other inhibitors. After varying lengths of time duplicate samples (200 μl) were placed in 400-μl microfuge tubes and centrifuged in a Beckman microfuge B for 30 s. The supernate was removed by aspiration and the cell pellet was washed once with glucose-free HBSS. Radioactivity in the cell pellet was deter-

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mined. Non-specific binding of AGOR, in the presence and absence of serum, was estimated by measuring binding in the presence of the inhibitor mannan (250 μg/ml) in parallel tubes.

Uptake of ^{125}I-ASOR by parenchymal cells was determined as described above except that the cells (10^6/ml) were suspended in complete HBSS. Non-specific binding of ASOR was estimated using a 100-fold excess of unlabelled ASOR.

AGOR or mannan was coupled to activated CH-Sepharose-4B (Pharmacia, Uppsala). Mannan (Sigma) was purified by Sephadex G-200 chromatography before use. The binding of ligand to Sepharose was estimated by the addition of ^{125}I-AGOR (3×10^6 cpm) or ^{125}I-mannan (1.8×10^6 cpm) to the coupling reaction mixture. AGOR–Sepharose bound approx. 5 mg AGOR/ml Sepharose, and mannan–Sepharose approx. 2 mg mannan/ml Sepharose.

Human serum (6–12 ml), dialysed against glucose-free HBSS, was mixed with an equal column of 0.02 M Tris–HCl (pH 7.4), 40 mM $CaCl_2$, 0.5 M NaCl, and incubated at 4°C with either AGOR–Sepharose (5 ml) or mannan–Sepharose (7.5 ml) suspended in 0.02 M Tris–HCl (pH 7.4), 20 mM $CaCl_2$, 0.5 M NaCl (column buffer). After 1 h the Sepharose suspension was loaded into a column and washed with column buffer until the absorbance at 280 nm of the eluate was less than 0.01. The column was eluted with 0.2 M α-methyl mannoside (Sigma) in column buffer at room temperature and 6-ml fractions were collected. The fractions were dialyzed exhaustively against glucose-free HBSS to remove α-methyl mannoside before testing for inhibitory activity in the ligand cell uptake assay.

3. RESULTS AND DISCUSSION

The specific uptake of ^{125}I-AGOR by isolated rat hepatic sinusoidal cells was inhibited by human and rat serum but not by albumin (up to 40 mg/ml) (fig.1). All further experiments were carried out on human serum. Serum increased the apparent K_{uptake} of AGOR uptake but did not alter the apparent maximal uptake velocity indicating that inhibition was competitive (fig.2). Competitive inhibition of AGOR uptake could have been due to either a serum ligand, like ^{125}I-AGOR, binding to the cell receptor, or a serum lectin, like the cell receptor,

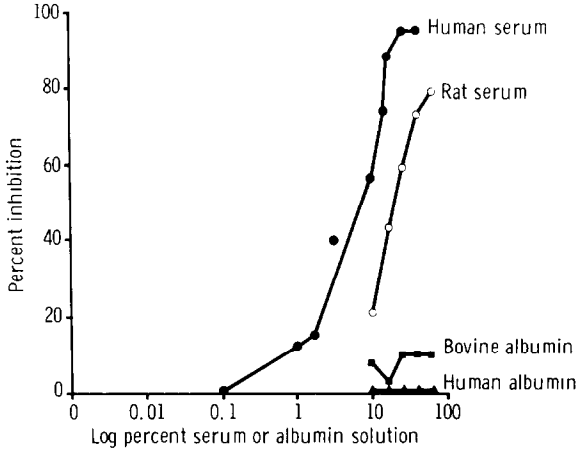

Fig.1. Inhibition of specific AGOR uptake by dialyzed serum. Sinusoidal cells were incubated with ^{125}I-AGOR (0.5 μg/ml) for 30 min at 37°C with increasing amounts of dialyzed serum or albumin (5% w/v in glucose-free HBSS). Representative of 4 experiments.

binding to ^{125}I-AGOR. To distinguish between these mechanisms, we performed assays in which the concentrations of serum and AGOR were kept constant but the number of sinusoidal cells added were varied. We postulated that if inhibition was due to a serum ligand, the amount of inhibition observed would depend on the ratio of serum ligand to ^{125}I-AGOR and would be independent of cell receptor number (i.e., to cell number), whereas if it was due to a serum lectin, the amount of in-

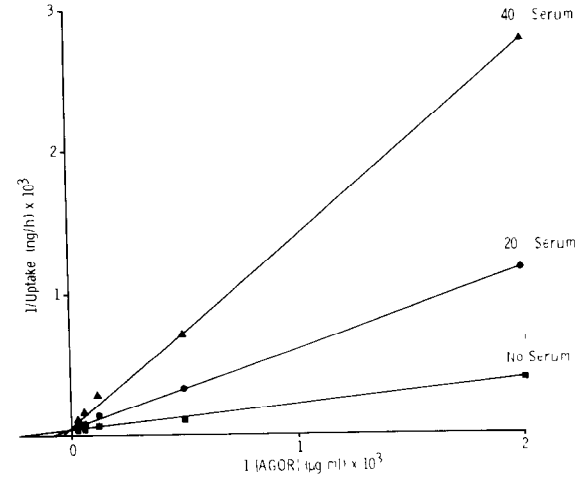

Fig.2. Double-reciprocal plot of AGOR uptake in the presence of serum. Sinusoidal cells were incubated for 10 min at 37°C with ^{125}I-AGOR (0.5–32 μg/ml) and serum. Representative of 3 experiments.

hibition would depend on the ratio of serum lectin to cell receptor and would therefore decrease as the number of cells in the assay was increased. Fig.3 shows that the inhibition of ^{125}I-AGOR uptake by serum decreased as the number of sinusoidal cells in the assay was increased. In the absence of serum the specific uptake of ^{125}I-AGOR expressed as ng/5 × 10^6 cells, remained constant (not shown), showing that the decrease in percent inhibition was due to the presence of serum. Inhibition by glucose [5] remained constant as cell number increased (fig.3). The results indicated that the serum inhibitor(s) was competing with the sinusoidal cell Man/GlcNAc receptor for the binding of ^{125}I-AGOR. In contrast, although serum also inhibited the uptake of ASOR by hepatocytes, the percent inhibition was independent of cell number (fig.4). This is consistent with the serum inhibitions of ASOR uptake being asialoglycoproteins [3].

As the inhibitors appeared to bind to AGOR and prevent it binding to the receptor, they should also bind to AGOR or mannan immobilized on Sepharose and be eluted by an appropriate monosaccharide. Fig.5 shows the result of passing serum through a column of mannan–Sepharose. There was an initial peak of inhibitory activity, representing material passing unbound through the column and indicating that the serum was not completely stripped of inhibitory material. However, some inhibitory material was eluted by 0.2 M α-methyl mannoside. The eluted fractions were combined, concentrated, and radioiodinated. Sephacryl S200 chromatography of the radiolabelled material showed 3 peaks (fig.6) with a molecular mass greater than 68 kDa, and a broad peak of smaller material of molecular mass less than 35 kDa. The first peak eluted in the void volume and therefore had a molecular mass of at least 250 kDa. Sephacryl S200 chromatography of serum proteins eluted from AGOR–Sepharose gave the same profile (not shown).

The effect of each Sephacryl S200 peak on the uptake of ^{125}I-AGOR by sinusoidal cells was tested

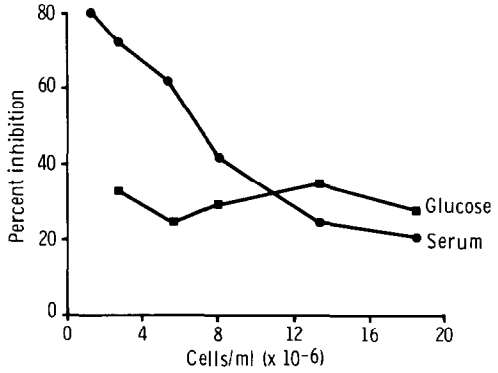

Fig.3. Effect of increasing cell number on percent inhibition of AGOR uptake. Sinusoidal cells (1.3–18.5 × 10^6/ml) were incubated for 30 min at 37°C with ^{125}I-AGOR (0.5 μg/ml) and 10% serum or 200 mg% glucose. Representative of 2 experiments.

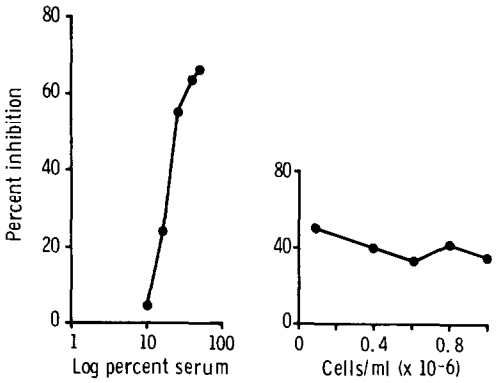

Fig.4. (a) Effect of serum on ASOR uptake by parenchymal cells. Parenchymal cells (10^6/ml) were incubated for 45 min at 37°C with ^{125}I-ASOR (0.5 μg/ml) and human serum (10–70%). (b) Effect of increasing cell number on percent inhibition of ASOR uptake. Parenchymal cells (0.15–1 × 10^6/ml) were incubated at 37°C for 45 min with ^{125}I-ASOR (0.5 μg/ml) and serum (10%). Representative of 4 experiments.

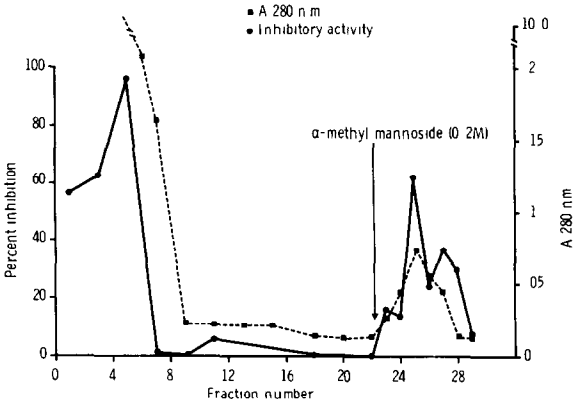

Fig.5. Affinity chromatography of serum on mannan–Sepharose. Each fraction was dialyzed against glucose-free HBSS and tested for inhibitory activity by incubating with sinusoidal cells for 30 min at 37°C with ^{125}I-AGOR (0.5 μg/ml).

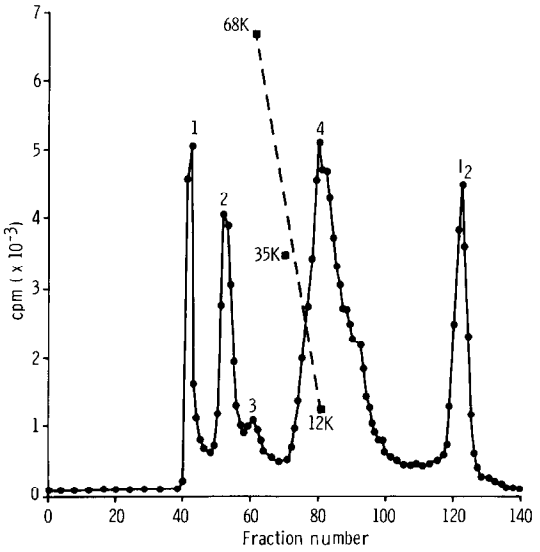

Fig.6. Sephacryl S-200 chromatography of proteins eluted from mannan–Sepharose. Column (2.5 × 90 cm) eluted with 0.02 M Tris–HCl (pH 7.4), 1 mM $CaCl_2$, 0.5 M NaCl.

after unlabelled proteins eluted from mannan–Sepharose were mixed with a trace of ^{125}I-labelled material and separated on Sephacryl S200. The fractions from each peak were combined, concentrated using 30% polyethylene glycol (w/v), dialyzed against 0.9% saline, and adjusted to a final volume of 5 ml. Table 1 shows the amount of protein (estimated by a modified Lowry procedure [6]) in each peak that was obtained from 40 ml serum. Only the smaller molecular mass material (peak 4), which accounted for approximately half of the protein eluted from the mannan column, caused inhibition (table 1). Since smaller amounts of the larger proteins were used in the inhibition assay, it is possible that these would also inhibit AGOR uptake at higher concentrations.

Table 1

Inhibition of sinusoidal cell uptake of ^{125}I-AGOR by Sephacryl S-200 fractions

Peak[a]	Total protein (mg)[b]	Inhibition[c]
1	0.28	9
2	1.42	4
3	0.82	33
4	2.55	73

[a] See fig.6 for peak assignment

[b] Isolated from 40 ml serum by mannan affinity chromatography

[c] Sinusoidal cells incubated for 30 min at 37°C with ^{125}I-AGOR (0.5 μg/ml) and 300 μl concentrated Sephacryl S-200 fractions (see section 2). Mean of 2 experiments

A mannan-binding protein has been isolated from rabbit serum [7] with a molecular mass of about 500 kDa, composed of subunits of 29 kDa. It is immunologically identical to the mannan-binding protein isolated from whole rabbit liver [8]. The protein of molecular mass >250 kDa that we isolated may be similar to the rabbit serum lectin. However, in contrast to the rabbit serum lectin, our data indicate that in human serum the predominant lectin has a molecular mass <35 kDa.

It is intriguing to speculate on the physiological role of these lectins in serum. Conceivably, the lectins interact with potentially toxic mannose-terminated enzymes such as lysosomal hydrolases which escape from cells into the serum. They could act in an analogous way to α_1-antitrypsin and the other components of the serum anti-protease system. The exact nature of these serum lectins with Man/GlcNAc specificity needs to be defined more clearly.

ACKNOWLEDGEMENT

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