INHIBITION OF THE RAT CLEARANCE SYSTEM FOR AGALACTO-OROSOMUCOID BY YEAST MANNANS AND BY MANNOSE

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SUMMARY

Infused asialo-orosomucoid, agalacto-orosomucoid and purified mannans from Saccharomyces cerevisiae are rapidly cleared from rat plasma. Clearance of ^{125}I-agalacto-orosomucoid is inhibited by simultaneously infused mannans and several simple sugars including mannose. Clearance of ^{125}I-mannans is inhibited by simultaneously infused agalacto-orosomucoid. Although mannans and agalacto-orosomucoid show cross-inhibition of clearance, neither inhibits the clearance of ^{125}I-asialo-orosomucoid. Thus, even though agalacto-orosomucoid is an N-acetyl-glucosamine terminal glycoprotein, a substantial part of its clearance from rat plasma is mediated by a mannose recognition system.

INTRODUCTION

Several systems for clearance of mammalian glycoproteins have been described or suggested. These include the well characterized hepatocyte receptor for asialo-glycoproteins which terminate in galactose (1), a system in avian (2) and rat (3) liver for clearance of agalacto-glycoproteins which terminate in N-acetylglucosamine, and a system for clearance of mannosyl terminal aglycosyl-antibody (4) and RNase B (5). Stahl and co-workers (6,7) found that a variety of rat lysosomal enzymes was rapidly cleared from rat plasma following infusion, and that agalacto-orosomucoid blocked their clearance. These findings led them to attribute clearance of these hydrolases to the system described for agalacto-orosomucoid and to suggest that terminal N-acetylglucosamine was part of the recognition site on the en-

Abbreviations: Wild type mannan contains a 1→6 linked backbone with side chains of 1→3 and 1→2 linked mannose units. Mutant mannan mnn2 (called 1→6 linked) has no 1→3 and 1→2 linked side chain on the polymannose backbone of the outer chain. Mannan from mutant mnn1 (called 1→2 linked) has predominantly mannobiose side chains in 1→2 linkage. Mannan from mutant mnn4 (called 1→3 linked) has many side chains with an additional mannose in 1→3 linkage.

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zymes which were cleared by this system. Infused human placental β-glucuronidase is also cleared rapidly from rat plasma by a system which appears to depend on the carbohydrate structure of the enzyme (8). Evidence presented elsewhere (9,10) indicates that infused human placental β-glucuronidase localizes in rat Kupffer cells, and that clearance of this human enzyme by rat Kupffer cells involves recognition of mannosyl residues on the enzyme. Surprisingly, agalacto-orosomucoid, an N-acetyl-glucosamine terminal glycoprotein, also blocked clearance of human placental β-glucuronidase. This result led us to test the cross-inhibition of clearance of agalacto-orosomucoid and yeast mannans and the inhibition of clearance of agalacto-orosomucoid by simple sugars.

MATERIALS AND METHODS

Orosomucoid was a gift of the American National Red Cross Laboratory, Bethesda, MD. Asialo-orosomucoid was prepared by treating a 1% solution of orosomucoid at 80°C for 1 hour in 0.1 N H_2SO_4 followed by dialysis against water. Agalacto-orosomucoid was prepared by treatment of asialo-orosomucoid with sodium metaperiodate followed by reduction with sodium borohydride using the method of Spiro (11). Mannans from mutant S. cerevisiae (12) were kindly supplied by Dr. Clinton Ballou, Dept. of Biochemistry, University of California, Berkeley, CA.

Asialo-orosomucoid, agalacto-orosomucoid, and S. cerevisiae yeast mannans were labeled using the chloramine-T method (13) with 10 μCi of carrier-free Na ^{125}I per 100 μg of protein. The iodinated proteins were separated from Na ^{125}I by gel filtration on a 1x20 cm Sephadex G-25 column. Specific activities ranged from 0.05 to 0.08 μCi per μg.

Male Sprague-Dawley rats were used for infusion experiments. Animals were anesthetized with ether. Nephrectomies were carried out through a retroperitoneal approach to each kidney, allowing isolation and ligation of renal veins and arteries prior to removal. ^{125}I-glycoproteins in 0.15 M NaCl were infused into the tail vein of rats weighing 150-200 gm and blood samples taken from a cannulated femoral artery into heparinized hematocrit tubes. Radioactivity was determined in plasma samples taken at intervals after infusion with a Packard model 5260 auto-gamma scintillation spectrometer.

RESULTS AND DISCUSSION

Figure 1a shows that infused tracer doses of ^{125}I-agalacto-orosomucoid are rapidly cleared from rat plasma, and that this clearance is blocked by large doses of unlabeled agalacto-orosomucoid but not by asialo-orosomucoid. These findings confirm previous reports (3). However, clearance of ^{125}I-agalacto-orosomucoid is also blocked by purified yeast mannan. Figure 1b shows the effect of infused sugars on clearance of ^{125}I-agalacto-orosomucoid in the nephrectomized rat. Note

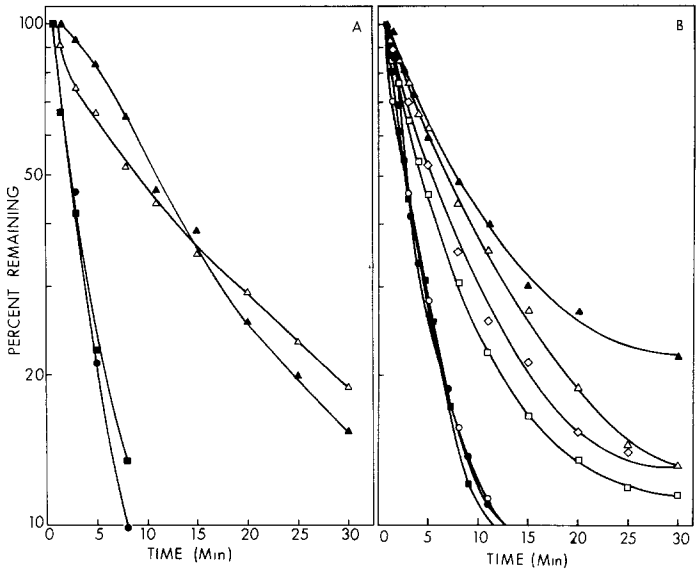

Figure 1. Inhibition of clearance of ^{125}I-agalacto-orosomucoid by other unlabeled glycoproteins or sugars. Rats received 0.5 ml of 0.15 M NaCl containing 10 μg ^{125}I-agalacto-orosomucoid with or without the indicated glycoproteins (Fig. 1a) or 500 μ moles of the indicated sugars (Fig. 1b). Tail vein infusions required 30 seconds. Arterial blood samples were taken at the times indicated following the start of the infusion. The data in Fig. 1b is from rats nephrectomized prior to infusions. Results are expressed as a percentage of the first post-infusion sample taken. Fig. 1a: ●, ^{125}I-agalacto-orosomucoid only; ▲, +2 mg agalacto-orosomucoid; ■, +2 mg asialo-orosomucoid; △, +1 mg mannan. Fig. 1b: ●, ^{125}I-agalacto-orosomucoid only; ■, +galactose; □, +N-acetyl glucosamine; △, +mannose; ▲, +α-methyl-D-mannoside; ○, +glucose; ◇, +L-fucose.

that α-methyl mannoside, mannose, L-fucose, and N-acetyl glucosamine inhibit clearance. α-Methyl mannoside was the most potent inhibitor. Glucose and galactose were without detectable effect.

Mannan from S. cerevisiae is a glycoprotein composed almost entirely of highly branched D-mannose chains. Figure 2 shows the effect of mutant yeast mannans on clearance of ^{125}I-agalacto-orosomucoid. All mutant mannans inhibited clearance of ^{125}I-agalacto-orosomucoid. Inhibition by (1→6) linked mannan is > (1→3) linked is > (1→2) linked. Figures 3 (a-c) show the effect of mutant mannans and agalacto-orosomucoid on the clearance of three different ^{125}I-mannans from S. cerevisiae. Agalacto-orosomucoid inhibits the clearance of mannan from all three mutants, and

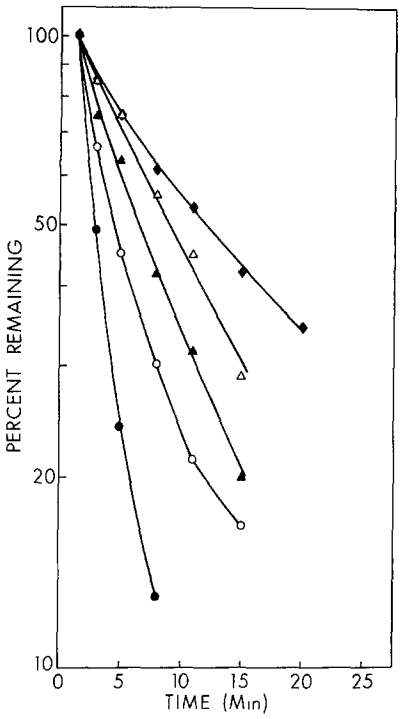

Figure 2. Inhibition of clearance of ^{125}I-agalacto-orosomucoid by unlabeled S. cerevisiae mutant mannans. Rats received infusions containing 10 μg ^{125}I-agalacto-orosomucoid alone, or also containing 200 μg of mannan mnn1 (1→2 linked); mnn2 (1→6 linked); mnn4 (1→3 linked); or 1 mg mnn2. ●, ^{125}I-agalacto-orosomucoid only; ○, +200 μg mnn1; △, +200 μg mnn2; ▲, +200 μg mnn4; ◆, +1 mg mnn2.

each mannan inhibits the clearance of the other two. Note that agalacto-orosomucoid inhibition is greatest for (1→2) linked mannan, less for (1→3) linked, and least for (1→6) linked, the reverse order of inhibition of agalacto-orosomucoid clearance by mannans. Figure 4 (a and b) shows that the only sugar which affects the rapid clearance of asialo-orosomucoid is galactose, and that neither agalacto-orosomucoid nor yeast mannan inhibits clearance of asialo-orosomucoid.

The studies presented show that agalacto-orosomucoid and yeast mannans each inhibit the clearance of tracer doses of the other. Both glycoproteins have been found to inhibit the rapid clearance of human placental β-glucuronidase, which is cleared by a system in rat Kupffer cells which appears to recognize mannosyl determinants on this lysosomal enzyme (9,10). The physiologic significance of this

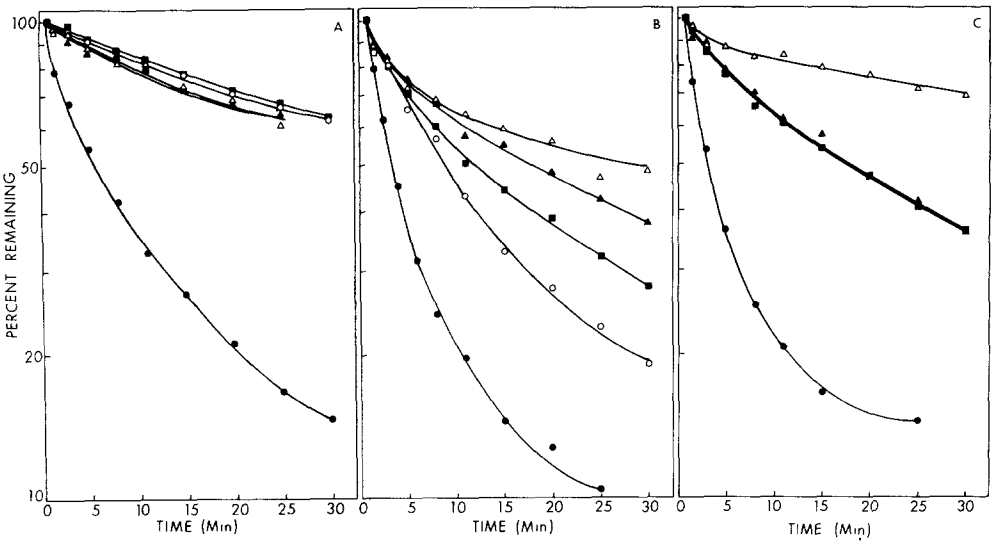

Figure 3. Inhibition of clearance of ^{125}I-mannans by unlabeled mannans or agalacto-orosomucoid in the nephrectomized rat. Rats received 10 μg of different ^{125}I-mannans alone or with 1 mg of agalacto-orosomucoid or the indicated mutant mannan. Clearance of ^{125}I-mnn1 (a), ^{125}I-mnn2 (b), and ^{125}I-mnn4 (c). Fig. 3a: ●, ^{125}I-mnn1 only; ○, +1 mg mnn1; △, +1 mg mnn2; ▲, +1 mg mnn4; ■, +2 mg agalacto-orosomucoid. Fig. 3b: ●, ^{125}I-mnn2 only; ○, +1 mg mnn1; △, +1 mg mnn2; ▲, +1 mg mnn4; ■, +2 mg agalacto-orosomucoid. Fig. 3c: ●, ^{125}I-mnn4 only; △, +1 mg mnn2; ▲, +1 mg mnn4; ■, +2 mg agalacto-orosomucoid.

clearance system is still unclear. Neither agalacto-orosomucoid nor mannans inhibit the clearance of asialo-orosomucoid, which is cleared by a different system involving a receptor for galactosyl groups on hepatocytes (1).

Several explanations are possible for the observed cross inhibition of clearance of agalacto-orosomucoid and mannans. First, the two ligands might interact with each other and this interaction could reduce the accessibility of the radiolabeled ligand to its receptor. Secondly, the two ligands could compete for the same receptor which recognizes either internal or branch mannose groups of agalacto-orosomucoid or mannose groups of mannan. The impressive inhibition of agalacto-orosomucoid clearance by mannose and α-methyl mannoside suggests that clearance of agalacto-orosomucoid involves a mannose recognition system. A third possibility is that there are distinct receptors on Kupffer cells for N-acetyl glucosamine and

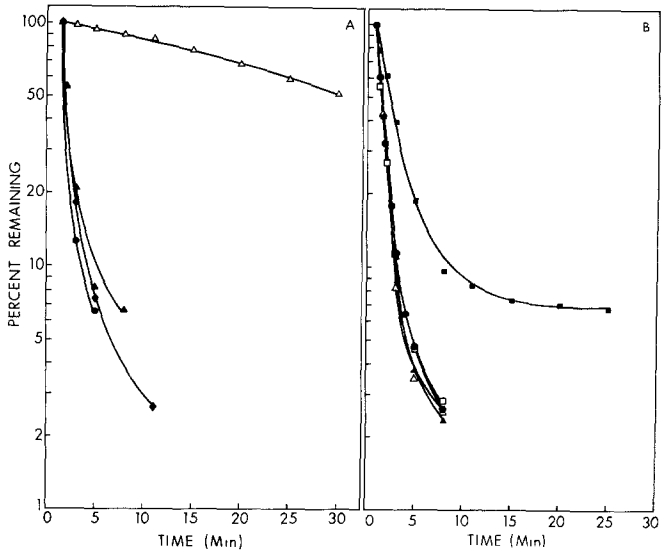

Figure 4. Clearance of ^{125}I-asialo-orosomucoid in the presence of unlabeled sugars or glycoproteins. All rats received 10 μg ^{125}I-asialo-orosomucoid. (a) Effect of 5 mg asialo-orosomucoid, 2 mg agalacto-orosomucoid, or 1 mg wild type mannan on ^{125}I-asialo-orosomucoid clearance. (b) Effect of 500 μ moles of various sugars on ^{125}I-asialo-orosomucoid clearance in the nephrectomized rat. Fig. 4a: ●, ^{125}I-asialo-orosomucoid only; △, +5 mg asialo-orosomucoid; ▲, +2 mg agalacto-orosomucoid ◆, +1 mg wild type mannan. Fig. 4b: ●, ^{125}I-asialo-orosomucoid only; ■, +galactose; □, +N-acetyl glucosamine; △, +mannose; ▲, +α-methyl-D-mannoside.

mannose terminal glycoproteins, with binding to one receptor interfering with function of the other. Saturating doses of ligand for one receptor might delay clearance of tracer doses of the other ligand by a separate receptor-mediated uptake process in the same cell if the cell's capacity to internalize membrane were exceeded.

If the inhibition of ^{125}I-agalacto-orosomucoid clearance by simple sugars represents haptene inhibition of ligand binding to receptor, one might infer that the receptor has greatest affinity for mannosyl determinants since α-methyl mannoside and mannose are the best inhibitors. Admittedly, different rates of clearance or metabolism of the simple sugars could cloud the interpretation of relative potency of the sugar inhibitors. The fact that mannose, L-fucose, and N-acetyl glucosamine all give some inhibition of clearance suggests either that more than one type of glycosyl unit is involved in the determinant on the glycoprotein that interacts with the receptor,

or that the mannosyl glycoprotein clearance system fails to distinguish several glycosyl configurations on the glycoproteins. Stowell et al (14) recently showed that the hepatic asialo-glycoprotein receptor cannot distinguish between the galactosyl and glucosyl configurations.

Studies with isolated binding proteins from liver analogous to those with the receptor for terminal galactose residues should allow definition of the receptor(s) involved in clearance of mannose and N-acetyl glucosamine terminal glycoproteins.

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