Selective Hepatic Uptake of Synthetic Glycoproteins

Mannosaminated Ribonuclease A Dimer and Serum Albumin

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ABSTRACT The influence of mannose-containing oligosaccharides on the tissue uptake of glycoproteins has been examined with synthetic glycoconjugates. Oligosaccharides obtained from the acetolysis of bakers' yeast mannan have been coupled to the lysine residues of the cross-linked dimer of bovine pancreatic ribonuclease A and of human serum albumin by reductive amination with cyanoborohydride. 14C-labeled derivatives of the two proteins containing two to four mannopyranose residues per 10,000 mol wt were administered intravenously to rats. There was selective (70–80%) uptake of these derivatives by the liver within 10–15 min after injection. A minor site of uptake was the spleen. The extent of hepatic uptake was a function of the number and size of the mannooligosaccharide residues coupled. With the nonglycosaminated derivatives the liver uptake was <5%. Related studies have shown that mannose-containing glycoproteins are taken up by both the endothelial and Kupffer cells of the liver; thus, reductive mannosamination may provide a means of directing to these cells proteins of potential therapeutic interest.

INTRODUCTION

The clearance of glycoproteins from the mammalian plasma depends largely upon the nature of the carbohydrate residues at the termini of the carbohydrate side chains. The pioneering studies of Ashwell and Morell and co-workers (1974) have shown that glycoproteins having terminal galactosyl residues are rapidly cleared from the plasma and taken up by the hepatocytes (liver parenchymal cells). Endocytic uptake of asialoglycoproteins into these cells is mediated by a specific galactose-binding protein (Hudgin et al., 1974). Glycoproteins that contain terminal fucose residues α-1→3-linked to N-acetylglucosamine are also taken up by the hepatocytes, but by a separate recognition system (Prieels et al., 1978). It has also been shown that glycoproteins and certain lysosomal hydrolases, whose carbohydrate side chains ter-
minate in N-acetylglucosamine or mannose, are preferentially removed from
the mammalian plasma and taken up by the nonparenchymal cells of the
liver and tissues characteristic of the reticuloendothelial system (Achord et al.,
1977a; Achord et al., 1978; Brown et al., 1978; Schlesinger et al., 1978).
Although the physiological significance of this clearance process has not been
determined, a mannan-binding protein which is thought to be involved has
been isolated from rabbit liver (Kawasaki et al., 1978).

The presence of at least three distinct carbohydrate recognition systems
responsible for the clearance of glycoproteins from the mammalian plasma
raised the possibility that, by coupling the appropriate sugar moiety to
molecules of potential therapeutic interest, the synthetic glycoconjugates could
be delivered specifically to certain organs or cell types and taken into the cells
by receptor-mediated endocytosis. Rogers and Kornfeld (1971), in model
experiments, first demonstrated that the coupling of an asialoglycopeptide
from fetuin to lysozyme or bovine serum albumin led to hepatic uptake of
these derivatives in vivo, and Krantz et al. (1976) showed that proteins to
which 1-thio-β-D-galactopyranosyl residues had been coupled would bind to
liver membranes in vitro.

We have recently reported that the coupling of lactose residues to proteins
by the reductive amination method of Gray (1974) provided a simple proce-
dure for directing the cross-linked dimer of bovine pancreatic RNase A to the
liver in vivo (Wilson, 1978). Bovine pancreatic RNase B, which contains α-
linked mannosyl residues on its carbohydrate side chain (Plummer and Hirs,
1963), is taken up principally by the nonparenchymal cells of the liver and
also by other tissues characteristic of the reticuloendothelial system (Brown et
al., 1978). We have extended our studies to the coupling of mannosyl residues
to proteins, because this particular chemical modification of enzymes may in
principle be useful in the enzyme therapy of lysosomal storage diseases with
reticuloendothelial cell involvement (Desnick et al., 1976).

We have coupled oligosaccharides obtained from bakers’ yeast mannan,
since it has been demonstrated that this polysaccharide will compete with
glycoproteins containing terminal mannosyl or N-acetylglucosamine residues
for the specific receptor sites in vivo (Achord et al., 1977; Brown et al., 1978).
In addition linear oligosaccharides of well-defined structure can be prepared
from yeast mannan using the acetolysis procedure of Ballou and co-workers
(Ballou and Raschke, 1974). Mannobiose and larger oligosaccharides have
been coupled in order to preserve the integrity of the pyranose ring of the
mannose residue at the nonreducing terminus, since in the reductive amination
reaction, the pyranose ring of the reducing sugar coupled to a lysine residue
is destroyed (Fig. 1).

Our previous work (Wilson, 1978) suggested that the cross-linked dimer of
RNase A would be a suitable model enzyme for study since we could measure
the hepatic uptake of the lactosaminated derivative even though the unmod-
ified dimer ($M_r = 27,000$) is subject to rapid renal clearance (Tarnowski et
al., 1976), as are other low molecular weight proteins in the same size range
(Pitts, 1968). However, since in this instance there is significant competition
between tissue uptake and renal clearance, we have also prepared mannosaminated derivatives of human serum albumin for studies on the tissue uptake of a protein that normally has a slow plasma clearance.

MATERIALS AND METHODS

Materials

Ribonuclease dimer with optimal activity toward poly(A)-poly(U) was prepared by cross-linking RNase A (Sigma Chemical Co., St. Louis, Mo., type XII-A) with dimethyl suberimidate (Wang et al., 1976). The dimer contained 16 free lysine residues per molecule as determined by amino acid analysis. Human serum albumin (HSA grade) was obtained from Worthington Biochemical Corp. (Freehold, N.J.). [14C] Iodoacetic acid (57 mCi/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). Sodium cyanoborohydride (recrystallized; lot 03307) was from Alfa-Ventron (Danvers, Mass.). Bakers' yeast mannan (lot 77C-0238) was obtained from Sigma Chemical Co. Elemental analysis of the mannan gave 0.16% N
and 0.38% P. Manno-oligosaccharides (mannobiose, -triose, -tetraose) were prepared by acetolysis of yeast mannan for 13 h, at 37°C (Stewart et al., 1968). After deacetylation, the resulting oligosaccharides were obtained in homogeneous form from a column of Bio-Gel P-2, minus 400 mesh (column dimensions 150 × 2.5 cm) (Bio-Rad Laboratories, Richmond, Calif.), eluted with distilled water at 25°C. The purified oligosaccharides were lyophilized.

**Reductive Amination Reaction**

In a typical experiment 10 mg of protein, 20 mg of oligosaccharide, and 50 mg of cyanoborohydride were dissolved in a total volume of 1 ml of 0.2 M potassium phosphate buffer, pH 8. Phosphate buffer was used to protect Lys-41, at the active site of RNase, from derivatization (Cooke et al., 1963). After incubation at 37°C, the solution was dialyzed (0-4°C) three times for 12 h against 200 vol of 0.15 M NaCl and twice for 12 h against 200 vol of distilled water. The protein concentration of the resulting solution was determined by the method of Lowry et al. (1951). N\(^{-1}\)-(1-deoxymannitolyl)-lysine was prepared by incubation of α-BOC-L-lysine (41 μmol), mannose (283 μmol), and cyanoborohydride (387 μmol), at 37°C for 96 h. After removal of the α-BOC group (by addition of 2 ml 6 N HCl to the lyophilized reaction mixture at 25°C, followed by evaporation to dryness), the product was separated from excess reagents and lysine by ion-exchange chromatography on a 60 × 1.2 cm column of Dowex 50-X8 (200-400 mesh) (Bio-Rad Laboratories, Richmond, Calif.) in the Na\(^+\) form, at 54°C. The sample was dissolved in 0.2 M sodium citrate, pH 4.25; elution was with 0.35 M sodium citrate, pH 5.25.

**Determination of Amount of Oligosaccharide Coupled**

The amount of oligosaccharide coupled to protein was determined by the phenol-sulfuric acid method (DuBois et al., 1956). In a more sensitive assay the number of modified lysine residues was determined by amino acid analysis after hydrolysis in 6 N HCl, at 110°C for 20 h. On a Durrum D-500 analyzer (Durrum Instrument Co., Sunnyvale, Calif.) using pH 6 as the third buffer, unhydrolyzed N\(^{-1}\)-(1-deoxymannitolyl)-lysine was eluted as a single peak located between phenylalanine and histidine. During acid hydrolysis three additional (two major and one minor) ninhydrin-positive peaks were produced (Fig. 2). Similar acid degradation products, identified as anhydrides of mannitolyl-lysine were reported by Robins and Bailey (1972). Since no free lysine was produced, the number of glycosaminated lysine residues could be determined by calculating the difference in the lysine values of the glycosaminated derivatives and the unmodified proteins. In analyses of RNase dimer, the lysine values were corrected for the additional modification that accomplished the cross-linking reaction (Wang et al., 1976). There was good agreement between the results of carbohydrate analyses (number of sugars coupled) and amino acid analyses (number of lysine residues modified), confirming our previous observations (Wilson, 1978) that the secondary amine was the major product in the reductive amination of the lysine residues of RNase dimer, under the conditions described. For routine purposes we have used amino acid analysis as a sensitive method for determining the extent of glycosamination.

**Radioactive Labeling of Proteins**

Proteins were labeled with \(^{14}C\)iodoacetic acid as described previously (Wilson, 1978). The resulting carboxymethylhistidine derivatives of RNase dimer, prepared at pH 5.5, are enzymically inactive. Carboxymethylation of serum albumin, which was
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conducted at pH 8.4, can be expected to derivatize the -SH group, and to a much lesser extent, the lysine residues. The specific radioactivities of mannosaminated derivatives of RNase dimer and serum albumin were in the range $1-3 \times 10^6$ cpm/mg, and $0.6-2 \times 10^6$ cpm/mg, respectively.

**Tissue Uptake Studies**

The $^{14}$C-labeled protein derivatives (5-25 μg in 300 μl of 0.15 M NaCl) were injected into the tail veins of male albino rats (Sprague-Dawley; 200-400 g; age- and weight-matched for each series of experiments). At given times the animals were killed by cervical dislocation, a sample of blood was collected from the inferior vena cava, and the tissues were removed and homogenized in 0.15 M NaCl. Blood (0.5 ml) and portions (0.5 ml) of each homogenate were dried and oxidized in a Packard Tri-Carb sample oxidizer (Packard Instrument Co., Downers Grove, Ill.) ($^{14}$CO$_2$ was trapped in Packard Carbo-Sorb; Permafluor V was the scintillation fluid), and the radioactivity was determined. The recovery of counts of a $^{14}$C standard, after oxidation, was greater than 98%. The blood volume of each rat was calculated as 64.1 ml/kg of body weight (Wang, 1959). The livers were perfused before being removed; 0.15 M NaCl was forced into the portal vein until the color of the liver became pale brown. Samples (200 mg) of muscle and skin, were oxidized after drying (48 h at 37°C). Bone (200-mg) sections cut from femur and pelvis were solubilized in 1 ml Soluene-350 (Packard)

![Figure 2](https://jgp.rupress.org)
for 48 h at 25°C and the tissue was counted in 10 ml Dimilume-30 (Packard) scintillation fluid. Total bone marrow uptake was estimated by multiplying the net counts per minute from one femur by 11.8 (Keene and Jandl, 1965).

**Determination of RNase Activity**

The activity of RNase dimer derivatives toward cyclic 2',3'-cytidylic acid, yeast RNA, and poly(A)·poly(U) was determined as previously described (Wang et al., 1976).

**RESULTS**

The amounts of mannobiose coupled to RNase dimer, at pH 8 and 37°C, are shown in Fig. 3. The rate of the reductive amination reaction could be enhanced by increasing the concentration of disaccharide, or by using pH 9, in the reaction medium, consistent with the results of Baues and Gray (1977). Amino acid analysis of the glycosaminated proteins gave the same characteristic peaks obtained when $N^\alpha$-1-(1-deoxymannitoyl)-lysine was hydrolyzed (Fig. 2), confirming that when the disaccharide was coupled the derivatized proteins contained 1-deoxymannobitolyl groups linked to the $\varepsilon$-amino groups of lysine residues. Since RNase dimer contains NH$_2$-terminal lysine residues, it is likely that some modification of the $\alpha$-NH$_2$ groups on those residues may occur. Glycosaminated derivatives of human serum albumin were similarly prepared; up to 14 mannobiose residues were coupled after 96 h at 37°C.

The enzymic properties of mannobiosaminated RNase dimer are similar to those of the lactosaminated derivative (Wilson, 1978). Derivatives of the dimer

![Figure 3. Coupling of mannobiose to RNase dimer at pH 8.0, and 37°C. The reaction mixture contained a nine-fold molar excess of disaccharide and a 118-fold molar excess of cyanoborohydride over the free -NH$_2$ groups of the protein. There was good agreement between the number of moles of lysine modified (amino acid analysis) and the number of moles of mannobiose coupled (carbohydrate analysis) per mole protein.](image-url)
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that contained up to eight 1-deoxymannobiitolyl-lysine residues per molecule retained >70% of their activity toward cyclic 2',3'-cytidylic acid and yeast RNA (Table I); the coupling of additional mannobiase residues resulted in a progressive loss of activity. The unique activity of RNase A dimer toward poly(A)·poly(U), which is thought to be related to the number and precise location of positive charges on the molecule (Libonati et al., 1970) was, however, significantly reduced upon glycosamination; the coupling of as few as four mannobiase residues per molecule of enzyme resulted in an 80% loss of activity toward the double-stranded substrate.

RNase dimer is rapidly removed from the blood of rats, with a half-life of <10 min (Tarnowski et al., 1976). After intravenous injection of 14C-labeled RNase dimer into rats, the bulk of the radioactivity, 10 min after injection, is found in the kidneys; <4% is detected in the liver (Wilson, 1978). After injection of a [14C]RNase dimer derivative containing eight 1-deoxymannobiitolyl-lysine residues per molecule, 70% of the radioactivity was found in

<table>
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<th>Moles mannobiase/mole RNase dimer</th>
<th>Activity* toward</th>
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<tr>
<td></td>
<td>Cyclic 2',3'-cytidylic acid</td>
</tr>
<tr>
<td>4</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
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* Expressed as the percentage of the activity of the nonglycosaminated RNase dimer.
have not detected any radioactivity in this organ after i.v. injection of lactosaminated RNase dimer derivatives. It is unlikely that contamination of the spleen by plasma would contribute significantly to the detected counts, since the total counts found in the blood were <5% of the radioactivity.

![Graph showing tissue uptake of \[^{14}C\]RNase dimer containing eight N'-1-(1-deoxymannobitolyl)-lysine residues per molecule after injection into the tail vein of rats. (O) Liver; (A) kidney; (■) blood. The amount of radioactivity (percent of total counts recovered) in the blood, liver, and kidneys was determined in two to three separate experiments for each time point. The amounts of radioactivity in the lungs, thymus, heart, brain, stomach, intestines, muscle, testes, and skin were either very low (<0.2% of total counts recovered) or undetectable, when these tissues were removed 10 min or 24 hr after injection. Radioactivity could be detected in the bladder contents ~1 h after injection; at 3 h up to 19% of the injected counts could be detected in the unvoided urine.](image)

**Figure 4.** Tissue uptake of \[^{14}C\]RNase dimer containing eight N'-1-(1-deoxymannobitolyl)-lysine residues per molecule after injection into the tail vein of rats. (O) Liver; (A) kidney; (■) blood. The amount of radioactivity (percent of total counts recovered) in the blood, liver, and kidneys was determined in two to three separate experiments for each time point. The amounts of radioactivity in the lungs, thymus, heart, brain, stomach, intestines, muscle, testes, and skin were either very low (<0.2% of total counts recovered) or undetectable, when these tissues were removed 10 min or 24 hr after injection. Radioactivity could be detected in the bladder contents ~1 h after injection; at 3 h up to 19% of the injected counts could be detected in the unvoided urine.

<table>
<thead>
<tr>
<th>Time after i.v. injection (h)</th>
<th>[^{14}C] in tissues</th>
<th>% of recovered counts†</th>
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<tr>
<td></td>
<td>Blood</td>
<td>Liver</td>
</tr>
<tr>
<td>3</td>
<td>2.5±1.5</td>
<td>64.5±2.5</td>
</tr>
<tr>
<td>24</td>
<td>2.3±1.7</td>
<td>140±7.0</td>
</tr>
</tbody>
</table>

* Eight 1-deoxymannobitolyl-lysine residues per molecule.
† Expressed as the percentage of the total counts recovered in the blood, liver, kidneys, and spleen. In general, the recovery of radioactivity in these organs was 80-100% of the injected counts. The results (mean ± SD) for each time point were obtained from three separate experiments.
injected. The amounts of radioactivity in other tissues examined were either very low (<0.2% of the total counts recovered) or undetectable, when these were removed either 10 min or 24 h after injection.

In studies with a low molecular weight protein such as RNase dimer (Mr = 27,400), the rate of specific tissue uptake of glycosaminated derivatives must exceed that of renal clearance. The extent to which this competition affects the amount and specificity of tissue uptake of the mannosobiosaminated protein was tested by using mannosobiosaminated derivatives of human serum albumin (Mr = 67,000) in control experiments with a protein that is less subject to renal clearance. After i.v. injection of 14C-labeled serum albumin, >90% of the counts were recovered in blood. In contrast, after injection of a 14C-labeled derivative of serum albumin containing 14 1-deoxymannobiosaminyl-lysine residues per molecule, ~79% of the radioactivity was detected in the liver either 10 min or 1 h after injection (Table III). In addition to a small amount of radioactivity in the spleen, a small proportion of the total recovered counts was also found in the femurs. The amounts of radioactivity in other organs examined (lungs, thymus, brain, heart, stomach, intestines, muscle, skin) were either very low (<0.5% of the total counts recovered) or undetectable.

The extent of hepatic uptake of mannosobiosaminated RNase dimer was related to the number of disaccharide residues coupled (Fig. 5). At least five

<table>
<thead>
<tr>
<th>Protein derivative</th>
<th>10 min</th>
<th>1 h</th>
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<tbody>
<tr>
<td>[14C]Serum albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Mannobiosaminated serum albumin‡</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>% of recovered counts*</th>
<th>14C in tissues</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Liver</td>
</tr>
<tr>
<td>[14C]Serum albumin</td>
<td>93</td>
<td>5</td>
</tr>
<tr>
<td>[14C]Mannobiosaminated serum albumin‡</td>
<td>18</td>
<td>79</td>
</tr>
</tbody>
</table>

* Expressed as the percentage of the total counts recovered in the blood, liver, kidneys, and spleen. Results are the average of duplicate experiments. In these experiments the recovery of radioactivity was 86-100% of the injected counts. At 10 min and 1 h after injection ~0.16% of the total radioactivity was detected in each femur. This represents only a very small proportion of the counts recovered; the total bone marrow radioactivity was estimated as 1.8% of the recovered counts. The amounts of radioactivity in the lungs, thymus, brain, heart, stomach, intestines, muscle, testes, and skin were either very low (<0.5% of total counts recovered) or undetectable when these tissues were removed 10 min or 1 h after injection.

‡ Containing 14 1-deoxymannobiosaminyl-lysine residues per molecule. Injection of a 14C-labeled derivative of serum albumin containing 10 1-deoxymannobiosaminyl-lysine residues per molecule resulted in uptake of 59% of the total counts by the liver 10 min after injection; 39% of the radioactivity remained in the blood.
residues of mannobiose per molecule of protein are required to give >50% uptake by the liver. Intravenous injection of derivatives containing up to four residues of oligosaccharide per molecule of protein suggests that the extent of hepatic uptake is also related to the length of the manno-oligosaccharide coupled (Table IV). These results are consistent with the in vitro studies of Kawasaki et al. (1978) who showed that the inhibition of binding of *Saccharomycyes cerevisiae* mannan to a mannan-binding protein from rabbit liver was related to the size of the oligosaccharide competitor. We have observed a similar effect of oligosaccharide size on the hepatic uptake of galactosaminated RNase dimer,¹ the coupling of a trisaccharide² containing a terminal galactosyl residue resulted in a greater hepatic uptake than when equal numbers of the disaccharide, lactose, were linked to the protein.

**DISCUSSION**

Reductive amination using cyanoborohydride provides a simple method for coupling sugars to proteins under a variety of experimental conditions (Baues and Gray, 1977). In the present experiments linear oligosaccharides containing α1→2- and α1→3-linked mannopyranosyl residues have been coupled to

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¹ Wilson, G. Unpublished results.
² Galactosyl α 1→6-galactosyl α 1→6 glucose; prepared by mild acid hydrolysis of the nonreducing tetrasaccharide stachyose.
RNase dimer with good retention of enzymic activity toward cyclic 2',3'-cytidylic acid and yeast RNA. The only precaution that was taken to retain enzymic activity was to protect the essential lysine residue at the active site of the enzyme from derivatization by using phosphate (pH 8) in the reaction mixture (Cooke et al., 1963). The reductive amination of proteins with reducing sugars is, however, a relatively slow reaction presumably because in aqueous solution only a small proportion of the reducing sugar exists in the free aldehyde form capable of reacting with protein amino groups. As a result we have used a large molar excess of oligosaccharide over the free amino groups of the protein, a temperature of 37°C, and pH 8 for the reaction. To provide a method that might be useful with less stable enzymes, Lee and Lee (1978) have synthesized derivatives of 1-thioaldoses containing a free aldehyde moiety, which can be rapidly coupled to proteins by reductive amination.

### TABLE IV

<table>
<thead>
<tr>
<th>Moles manno-oligosaccharide/mole</th>
<th>Oligosaccharide coupled†</th>
<th>Oligosaccharide coupled‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase dimer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td></td>
<td></td>
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</table>

* Expressed as the percentage of the total counts recovered in the blood, liver, kidneys, and spleen. Results expressed as mean ± SD, obtained from three experiments.
† Derivatives of RNase dimer to which up to four residues of either mannotriose or mannotetraose had been coupled retained full activity towards yeast RNA.

The coupling of mannobiose to either RNase dimer or human serum albumin resulted in hepatic uptake of the derivatized proteins. Glycoproteins such as RNase B, which are rapidly cleared from the plasma following i.v. injection, and taken up principally by the liver (Brown et al., 1978), contain several α-linked manno pyranosyl residues in their oligosaccharide moiety. α-Mannosidase treatment of RNase B produced a derivative that was not cleared from the blood stream of nephrectomized rats (Baynes and Wold, 1976). Since this derivative contained a single mannosyl residue at the nonreducing terminus of the remaining trisaccharide moiety (manα → [GlcNAc]2-Asn), it suggested that one or more α-linked manno pyranosyl residues must be present for recognition. Hepatic uptake of mannosaminated derivatives of RNase dimer or human serum albumin occurs when single α-

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When mannobiose is coupled to a protein, the pyranose ring of the reducing sugar moiety is destroyed during the reductive amination reaction; thus a single intact manno pyranosyl moiety is exposed for each disaccharide residue coupled.
mannopyranosyl residues are present on these synthetic glycoproteins. Stahl et al. (1978) have shown that a derivative of bovine serum albumin, to which single mannopyranosyl residues had been coupled, will bind to specific carbohydrate binding receptors on alveolar macrophages. Although the presence of more than one α-linked mannopyranosyl residue per derivatized amino group is not mandatory for hepatic uptake of the mannosaminated proteins, we have observed increased liver uptake of these derivatives (for a given number of sugar residues per mole of protein) when larger oligosaccharides are coupled. For enzymes that are inactivated by extensive modification of lysine residues, it may be preferable to couple a large oligosaccharide rather than mannobiose, since a given hepatic uptake could be achieved by the modification of fewer lysine residues.

The major site of uptake of mannosaminated derivatives of RNase dimer and human serum albumin, following intravenous injection into rats, was the liver; minor sites of uptake were the spleen and bone marrow. The uptake of mannosaminated RNase dimer by the kidneys is presumably related to the normal process of renal clearance of the low molecular weight protein that has not been taken up by the liver, as with the nonglycosaminated protein (Wilson, 1978). There was minimal kidney uptake of mannosaminated serum albumin, a derivative which is not subject to renal clearance. Brown et al. (1978) found that the liver and spleen were the most active organs, on a weight basis, in the uptake of I-1-labeled RNase B from the blood stream of nephrectomized rats. The involvement of other tissues of the reticuloendothelial system in the clearance of mannose-containing glycoproteins from the circulation was demonstrated by Achord et al. (1977 a), who showed that the clearance of human placental β-glucuronidase, from the blood stream of rats, was mediated primarily by the liver and spleen in normal rats, and in the bone, lung, heart, and kidneys of eviscerated rats.

In studies on the clearance of antibodies (Winkelhake and Nicholson, 1976) and ahexosamino-orosomucoid (Stockert et al., 1976) from the blood stream of rats, significant amounts of radioactivity were detected in the kidneys, suggesting that this organ may also function in the specific uptake of glycoproteins whose oligosaccharide side-chains terminate in mannosyl residues. The absence of significant uptake of [14C]mannosaminated human serum albumin into the kidneys implies that this particular recognition system has much more rigid requirements, in terms of the structure of the mannosyl residues on the carbohydrate side chain, than are present in the synthetic derivatives. At 24 h after injection of a 14C-labeled derivative of RNase dimer, containing eight 1-deoxymannobitolyl-lysine residues per molecule, most of the radioactivity could be identified as low molecular weight material (probably 14C-carboxymethyl-histidine) in the urine. Thus, although RNase in the absence of denaturing agents, is highly resistant to proteolysis in vitro (Spackman et al., 1960), it appears to have a relatively short half-life in the liver in vivo, compared to human placental β-glucuronidase (Achord et al., 1977 a) which has a half-life in the liver of rats of >2 d. The precise reasons for the stability of lysosomal hydrolases in vivo have not been established.
Previous studies on RNase B and several lysosomal hydrolases (Achord et al., 1977 a; Achord et al., 1978; Brown et al., 1978; Schlesinger et al., 1978) have shown that mannose-containing proteins are taken up by the nonparenchymal cells of the liver following i.v. injection in rats. Recent EM-autoradiography studies (Hubbard et al., 1979) have shown that 125I-labeled mannobiosaminated RNase dimer is taken up by both the Kupffer and endothelial cells of the rat liver, in contrast to the lactosaminated RNase dimer which localized exclusively in the hepatocytes. Thus, reductive glycosamination of lysine residues with different sugars provides a simple method for directing proteins of potential therapeutic interest to different target cells in the liver. The coupling of mannosyl residues may be an additional approach to the modification of enzymes for replacement therapy of lysosomal storage diseases which involve the reticuloendothelial cells of the liver (Desnick et al., 1976).

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