The parameters that affect the interaction of ligands with a fucose-binding lectin from rat liver have been examined. 125I-Fucosyl-bovine serum albumin (Fuc-BSA) containing 50 residues of fucose/molecule was used as the standard ligand. At low initial concentrations of ligand (10 ng/ml) and the best ligand (140 ng/ml), the reaction reaches equilibrium at pH 7.8, 23 °C, within 40 min. The binding of ligands is Ca2+ dependent with half-maximal binding occurring at 54 μM Ca2+; of several metal ions tested, only Sr2+ partially replaced Ca2+. Binding was maximal between pH 7.6 and 8.6, fell slightly up to pH 10, but fell markedly below pH 7. The lectin-ligand complexes dissociated at low pH, on removal of Ca2+, or in the presence of a large excess of competing ligand. The apparent association constant (Kₐ) for Fuc-BSA was 7.5 X 10⁴ L/mol⁻¹. The fucose content of the Fuc-BSA also influenced binding, with little apparent binding below 24 fucose residues/molecule and maximal binding from 40 to 50 fucose residues/molecule. With knowledge of the parameters influencing binding, sensitive reproducible assays for the lectin were developed.

The binding specificity of the lectin was examined by measuring the inhibition of 125I-Fuc-BSA binding by neoglycoproteins, monosaccharides, and glycosides or by direct binding of neoglycoproteins. Galactosides and β-linked fucosides were the best ligands among the neoglycoproteins, with much weaker binding by mannosyl- or N-acetylglucosaminyl-BSA. On the basis of the pattern of inhibition of Fuc-BSA binding by various monosaccharides and glycosides, it is possible to propose the conformations of saccharides that best fit the lectin-binding site. The C1 conformation of N-acetyl-d-galactosamine fits best, although other not obviously related monosaccharides such as L-fucose, L-arabinose, and D-mannose can also assume conformations that permit them to be effective inhibitors.

The pattern of binding of neoglycoproteins to the lectin differs from that of other pure hepatic lectins. Thus, the fucose lectin has a high affinity for Fuc-BSA and galactosyl-BSA but a low affinity for N-acetylglucosaminyl-BSA. The galactose lectin binds only galactosyl-BSA and shows little binding with either N-acetylglucosaminyl-BSA or Fuc-BSA. In contrast, the mannose/N-acetylglucosamine lectin binds N-acetylgalactosaminyl-BSA and Fuc-BSA but not galactosyl-BSA.

The preceding paper (1) described the purification to homogeneity of a fucose-binding lectin from rat liver. The apparent binding activity of the lectin was followed throughout the purification by assay with 125I-Fuc-BSA as ligand. By correlating the binding activity with the proteins observed on gel electrophoresis of various fractions, it was possible to identify the desired lectin. Purification was complicated, however, by the fact that the mannos/N-acetylglucosamine-binding lectin (2) also bound 125I-Fuc-BSA and was present in liver extracts in greater amounts than the fucose-binding lectin. Thus, until the contaminating lectin was removed, it was impossible to determine exactly the optimal conditions for assay of the lectin, and an assay with 125I-Fuc-BSA as ligand was performed under conditions similar to those used for other hepatic lectins (3, 4). With the availability of the pure lectin, however, an assay procedure based on knowledge of the parameters that affect the binding of ligand to lectin was designed. We wish to report here the effects of various parameters on the binding reaction and a description of an assay that was subsequently developed.

With a reproducible sensitive assay it become possible to determine the binding specificity of the lectin. Accordingly, the structural basis for ligand binding was examined by determining the inhibitory action of neoglycoproteins, glycosides or monosaccharides on the binding of Fuc-BSA to the lectin or by measuring the binding of neoglycoproteins directly to the lectin. We also wish to report here the results of these studies.

**EXPERIMENTAL PROCEDURES**

**Materials**—Pure fucosyl lectin was prepared from rat liver as described in the preceding paper (1). Rat liver mannos/N-acetylglucosamine (3) and galactose (4) lectins were prepared as described earlier. Unless stated otherwise, all Fuc-BSA preparations used here were prepared by the amidination reaction (5) and contained 50 ± 2 fucose residues/molecule of BSA. The following were obtained commercially: D-talose, D-allose, D-altrose, 3-O-methyl-D-glucose, D-mannosamine HC1, N-acetyl-D-mannosamine, 2-deoxy-D-galactose, L-galactose, D-lyxose, L-ribose, and disodium mannos 6-phosphate (Sigma); β-methyl-L-arabinoside (ICN); p-galactose, α-methyl-D-galactoside, β-methyl-D-galactoside, α-methyl-D-mannoside, D-galactosamine HC1, N-acetyl-d-galactosamine, D-glucosamine HC1, N-acetyl-D-glucosamine, L-arabinose, and D-xyllose (Flanstein); D-glucose and sodium D-glucuronate (Calbiochem); L-fucose, D-fucose, and D-mannose/molecule.

The neoglycoproteins are abbreviated with the standard symbols for monosaccharides and bovine serum albumin (BSA). Thus, Fuc-BSA is 1-fucosyl-bovine serum albumin. Unless otherwise designated all monosaccharides used here were of the D-configuration, except for fucose, which was of the L-configuration. The other abbreviation used in: Bicine, N,N-bis(2-hydroxyethyl)glycine.
nose (Aldrich); and α-methyl-D-glucoside and β-methyl-D-glucoside (Pierce Chemical Co.). We wish to thank Dr. Y. C. Lee (Johns Hopkins University, Baltimore, MD) for samples of L-Fuc-α-BSA and L-Fuc-α-BSA prepared by reductive amination and L-Fuc-β-BSA and α-GlcNAc-BSA prepared by amidination (5). All other materials were obtained as described earlier (1).

Binding Assays for the Lectin—The fucose-binding lectin was assayed in a few experiments by the procedure (assay 1) described in the previous paper (1). Another assay (assay 2) was developed when pure lectin became available and the effects of various parameters on the lectin-Fuc-BSA interaction were examined. Assay 2 was used here unless stated otherwise.

Assay 2—Each assay contained 0-1000 ng of lectin, 10 ng of 125 I-fucosyl-BSA, and the same concentration of BSA, sodium chloride, Tris-Cl, pH 7.8, calcium chloride, sodium azide, and Triton X-100 as in assay 1 in a total volume of 1.0 ml. After incubation for 30 min at 23 °C, human fibrinogen (Kabi, 65 μl, 5 ng/ml containing 0.15 M sodium chloride and 0.05 M Tris-Cl, pH 7.8) was then added. After 10 min at 23 °C, the reaction mixture was filtered by vacuum filtration on Whatman GF/C filter discs presoaked as described in assay 1 and rinsed with 10 ml of 0.01 M Na-glycinate, pH 10.0, containing 0.2 M sodium chloride, 0.01 M calcium chloride, and 0.02% sodium azide. 125 I-Fucosyl-BSA that bound was detected in a γ-counter. Nonspecific binding determined in the absence of lectin was less than 0.5% of the counts. One unit of activity was defined as that amount of lectin that bound 0.1 ng of 125 I-fucosyl-BSA in the assay.

Comparison of the Binding Activities of the Rat Liver Lectin—The binding of ligands to the fucose, mannose/N-acetylglucosamine (3), and galactose (4) lectins of rat liver was measured in a reaction mixture described for assay 1 above, except that 8% polyethylene glycol was used to precipitate the ligand-lectin complexes and the ligand used was one of several neoglycoproteins described under "Results." Under these conditions it was found that 13, 77, and 100%, respectively, of the ligand-lectin complexes formed in the assays were precipitated by the polyethylene glycol and collected. These values were obtained as follows. The apparent extent of binding at one ligand concentration by different concentrations of lectin was measured and the ratio of this value by the total amount of ligand in the assay mixture, the fraction of the ligand-lectin complex actually measured was determined. This value by the amount of complex formed in solution from the amount detected in precipitation assays. Over 90% of each of the lectins used in these studies was found to adsorb to a specific affinity adsorbent; thus, inactive lectin that may accumulate on storage was largely absent.

RESULTS

The Development of a Binding Assay for the Lectin

The binding activity of the lectin was measured during its purification (1) by incubating the lectin with 125 I-Fuc-BSA at 23 °C for 60 min and measuring the radioactivity in the lectin-125 I-Fuc-BSA complexes. The complexes were collected by filtration after precipitation from the assay mixture by polyethylene glycol (PEG 6000). Although this assay was effective, after highly purified lectin was obtained (1), several parameters that affect Fuc-BSA binding to the lectin led to the development of a reproducible sensitive assay that permitted determination of its binding specificity.

Effect of Polyethylene Glycol Concentration—Fig. 1 shows the apparent binding activity in the presence and absence of lectin as a function of the polyethylene glycol concentration used to precipitate lectin-ligand complexes. Specific binding was estimated from the difference between the binding in the presence of the lectin and the nonspecific binding observed in the absence of lectin. Specific binding and nonspecific binding were about the same from 0-5% polyethylene glycol, and each increased at higher concentrations.

Since the amounts of lectin-ligand complex obtained by filtration of the assay mixtures were about the same at concentrations of polyethylene glycol between 0 and 5%, studies were performed to determine whether the complexes were actually being filtered from solution by the glass filters or directly adsorbed onto the filters. Thus, when one, two, or three glass filters were used together, the specific binding was unchanged although the nonspecific binding increased in proportion to the number of filters. In addition, by use of filters of different pore size (0.7, 1.2, and 1.6 μm) specific binding increased with decreasing porosity, but nonspecific binding was the same for each. Pretreatment of the filters with inert protein solution (0.1% BSA) decreased the nonspecific binding by 50% but was without effect on specific binding. Addition of fibrinogen decreased the nonspecific binding by 65%. Together, these results suggest that Fuc-BSA adsorbs nonspecifically to the glass filters, thereby accounting for the nonspecific adsorption. The lectin-Fuc-BSA complexes, however, are sufficiently large to be trapped by the glass filters either in the presence or absence of polyethylene glycol.

Studies were also performed with ammonium sulfate as a precipitating agent for lectin-Fuc-BSA complexes. It was found that the apparent specific binding decreased as the ammonium sulfate concentration increased to 20% saturation. For this reason and the fact that apparent nonspecific binding increased markedly above 20% saturation in ammonium sulfate, assays based on ammonium sulfate precipitation were not pursued.

Time and Temperature—Fig. 2 shows the effects of time of incubation and temperature on the apparent binding activity of the lectin. Apparent equilibrium in the binding of lectin to ligand was reached after 20 min at 37 °C, 40 min at 23 °C.
and 70 min at 0 °C. No change in binding was observed over 5 h at 23 °C. Moreover, almost twice as much ligand bound an equivalent weight of lectin at 23 °C as at 0 and 37 °C.

Trition X-100 Concentration—Apparent specific binding did not vary between 0.01 and 0.5% Triton X-100 (data not shown). Since nonspecific binding increased markedly below 0.05% Triton X-100, this concentration of detergent was used in both binding assays.

Metal Ion Requirement—Binding of ligands to the lectin was dependent on Ca²⁺ concentration as shown in Fig. 3. Half-maximal binding was at 54 μM Ca²⁺, and no increase in binding was observed above about 1 mM Ca²⁺. Thus, 10 mM Ca²⁺ was used in both assays. Strontium ion (Sr²⁺; 10 mM) gave about 15% of the binding as 10 mM Ca²⁺, but 10 mM concentrations of Mn²⁺, Mg²⁺, Ni²⁺, Ba²⁺, and Co²⁺ did not permit binding.

Effect of pH—Fig. 4 shows the effect of pH on the apparent binding of Fuc-BSA to the lectin. Binding was maximal between pH 7.6 and 8.6 but diminished considerably below pH 7.6 so that at pH 5 no activity was detectable. Binding was only slightly diminished, however, between pH 8.1 and 10. Nonspecific binding decreased with increasing pH, thus to diminish non-specific binding in the assays, the filters containing the lectin-Fuc-BSA complexes were washed with a buffer at alkaline pH. The apparent specific binding was not diminished by this procedure but decreased non-specific binding about 5-fold.

Effect of Lectin and Ligand Concentrations—Fig. 5 shows the apparent binding of Fuc-BSA to the lectin as a function of lectin concentration. At the concentration of Fuc-BSA used, insufficient lectin could be added to the assay to bind all of the Fuc-BSA. Under these conditions 1.4 ng of Fuc-BSA bound 1 μg of lectin. Various lectin preparations differed slightly from one another in binding activity, with activities ranging from 1.0 to 2.5 ng of Fuc-BSA bound per μg of lectin. Fig. 6 shows the apparent binding of Fuc-BSA to the lectin as a function of Fuc-BSA concentration. The extent of binding was linear up to 50 ng/ml Fuc-BSA but nonlinear at higher concentrations. The high nonspecific binding above 1.0 μg/ml Fuc-BSA prevented accurate binding measurements above this concentration. Fig. 6 (inset) shows a Scatchard plot of the binding data. An apparent association constant (Kₐ) of 1.75 x 10⁸ M⁻¹ was calculated from these data.

![Fig. 3. The effect of Ca²⁺ on the binding of lectin to ¹²⁵I-Fuc-BSA. The binding of Fuc-BSA to lectin was measured as in assay 2 except that the Ca²⁺ concentration was varied during the assay and in the rinse of the filters. © binding in the presence of lectin; ○, binding in the absence of lectin.](image)

![Fig. 4. The effect of pH on the binding of Fuc-BSA to the lectin. The lectin (280 ng) was assayed by assay 2 except that different buffers at the pH values indicated were used. The buffer concentration was 0.04 M in the assay and 0.01 M for rinsing the filters. The specific binding (closed symbols) was calculated by subtraction of the nonspecific binding (no lectin in the assay) (open symbols) from the total binding (lectin in the assay). The buffers are acetate-maleate (O, ●); Tris-glycine (□, ■); Bicine (▲, ▼); and Tris-chloride (△, ▲).](image)

![Fig. 5. The binding of ¹²⁵I-Fuc-BSA as a function of lectin concentration. Lectin (0–200 ng) was assayed in duplicate by assay 2.](image)

![Fig. 6. The binding of Fuc-BSA to lectin as a function of concentration of Fuc-BSA. The binding of Fuc-BSA to lectin (115 ng/assay) was measured in assay 2 at the ¹²⁵I-Fuc-BSA concentrations indicated. Nonspecific binding (no lectin) was subtracted from total binding to give specific binding. Inset, the data were recalculated in a Scatchard plot (7) assuming the lectin has a molecular weight of 67,000.](image)
Reversibility of Binding—Fig. 7 shows that the lectin-ligand interaction is reversible at low pH. Thus, after formation of lectin-ligand complexes at pH 8.1, less than 5% of the ligand remained bound after 5 min at pH 5.4, in good agreement with the pH dependence of the reaction (Fig. 4). Addition of a 1000-fold excess of unlabeled Fuc-BSA to the lectin-ligand complexes formed at pH 8.1 gave a slower rate of reversal, but after 60 min in the presence of excess Fuc-BSA, only 20% of the complex remained.

Effect of the Fucose Content in Fuc-BSA on Lectin Binding—The fucose content of Fuc-BSA markedly influenced the apparent binding of ligand to the lectin as shown in Fig. 8. Fuc-BSA preparations ranging in fucose content from 8 to 50 residues of fucose/molecule of BSA were used in the experiments in Fig. 8A to inhibit the interaction of lectin and 125I-Fuc-BSA. In Fig. 8B, these same preparations were iodinated and used as ligands in the binding assay. Both types of experiment clearly show that binding of ligand (Fuc-BSA) increases with increasing amounts of fucose/molecule of BSA. Under the conditions of the inhibition assays, in which the 125I-Fuc-BSA used contained 50 fucose residues/molecule of BSA, little inhibition was observed by Fuc-BSA containing less than about 25 fucose residues/molecule of BSA. Similarly, little binding to lectin was observed with Fuc-BSA preparations containing less than about 25 residues of fucose/molecule of BSA. Fig. 9 shows the concentration of Fuc-BSA preparations with different fucose contents that is required to inhibit 50% of the binding of 125I-Fuc-BSA to the lectin. The linearity of this plot over 4 log units for Fuc-BSA preparations ranging from 13 to 42 fucose residues/BSA molecule indicates a highly cooperative effect in ligand-lectin binding under these conditions.

The Binding Specificity of the Lectin

The Inhibition of the Binding of Fuc-BSA to the Lectin by Different Neoglycoproteins—The binding of lectin to 125I-Fuc-BSA was measured at different concentrations of an unlabeled neoglycoprotein, and the amount of neoglycoprotein necessary to give 50% inhibition of binding was estimated. Since small changes in the number of glycosyl groups/molecule of neoglycoprotein markedly influence the extent of inhibition, as shown for Fuc-BSA in Fig. 9, it was necessary to correct the observed inhibition for differences in saccharide content of the inhibitory neoglycoproteins. Thus, from the protein...
concentration of each neoglycoprotein required for 50% inhibition, Fig. 9 was used to estimate the number of fucose residues/molecule that would give the equivalent extent of inhibition. An index of binding affinity was calculated by dividing the number of fucose residues/molecule estimated from Fig. 9 by the number of glycosyl residues/molecule in the neoglycoprotein being tested. This quotient was 1.0 for any preparation of Fuc-BSA containing from 20-50 residues of fucose/molecule and less than 1.0 for any neoglycoprotein that was a poorer inhibitor than Fuc-BSA. In some cases, it was not possible to add sufficient neoglycoprotein to obtain 50% inhibition; thus it was assumed that 10 times the highest noninhibitory concentration would be required to give 50% inhibition.

Table I lists the binding affinities of the different neoglycoproteins tested as inhibitors of Fuc-BSA binding. The type of structure linking a saccharide group and BSA was without much effect on the binding, as shown for fucose and galactose, which were linked by either amidination or reductive amination. Fucose and galactose in \( \beta \)-linkage were the best inhibitors, whereas \( \alpha \)-linked fucose was much poorer. N-Acetylglucosaminyl and mannoseyl BSA were very poor inhibitors.

**Inhibition of Fuc-BSA Binding to the Lectin by Monosaccharides and Glicosides**—Table II lists the monosaccharides and glicosides tested as inhibitors of \(^{125}\)I-Fuc-BSA binding to the lectin and the concentration of each required to give 50% inhibition of binding. In accord with the results of inhibition of Fuc-BSA binding by neoglycoproteins, galactose and fucose were among the best inhibitors. However, N-acetylgalactosamine was also a potent inhibitor along with methyl-\( \beta \)-D-galactoside, which was a considerably better inhibitor than methyl-\( \alpha \)-D-galactoside. In contrast to the results with the neoglycoproteins, mannose proved to be a potent inhibitor. N-Acetylglucosamine, however, like the N-acetylgulosamine-BSA was a very poor inhibitor.

**Comparison of the Binding Specificities of Three Rat Liver Lectins**—Although the galactose and mannose/N-acetylglucosamine lectins of rat hepatocytes were found to differ structurally from the fucose lectin (1), it was of interest to determine whether they differed as well in their binding specificities. Accordingly, four \(^{125}\)I-labeled neoglycoproteins were used as ligands for each of the three lectins as shown in Fig. 10. Fuc-BSA was bound by both the fucose and the mannose/N-acetylglucosamine lectins but not by the galactose lectin. N-Acetylglucosaminyl-BSA bound only to the mannose/N-acetylglucosamine lectin. In contrast, Gal\( \beta \),4GlcNAc-BSA and Fuc\( \alpha \),3(Gal\( \beta \),4)GlcNAc-BSA bound to the fucose and galactose lectins but not to the mannose/N-acetylglucosamine lectin. Thus, the binding specificity of the fucose lectin as judged by direct binding of ligands clearly differs from that of either of the other lectins and is in general accord with that determined from inhibition studies (Tables I and II).

The binding specificities of the three lectins as judged by measurement of the binding of neoglycoproteins (Fig. 10) was confirmed by inhibition studies (Table III). Thus, the fucose lectin bound Fuc-BSA and Gal-BSA, both of which were good inhibitors of binding, but GlcNAc-BSA was not bound nor an effective inhibitor of binding. In contrast, the mannose/N-acetylglucosamine lectin bound both Fuc-BSA and GlcNAc-BSA, and each was an effective inhibitor, but Gal-BSA was neither bound nor an inhibitor. Finally, the galactose lectin bound Gal-BSA but not Fuc-BSA or GlcNAc-BSA.

**DISCUSSION**

With the availability of a fucose-binding lectin free of other rat liver lectins (1), it was possible to evaluate the parameters that affected its binding with ligands. Thus, with \(^{125}\)I-Fuc-BSA as the standard ligand, it was found that maximal formation of lectin-ligand complexes occurred at low initial concentrations of lectin and ligand within 40 min at 23 °C and that the complexes were stable for several hours (Fig. 2). Complex formation was totally dependent on Ca\(^2+\) concentration (Fig. 3). In addition, maximal lectin-ligand formation occurred between pH 7.6 and 8.5, with binding persisting at more alkaline pH values but dropping sharply at lower pH values (Fig. 4). The amount of ligand bound varied from about 1.0 to 2.5 ng/g of lectin, depending on the preparation. Scatchard plots of the binding revealed, however, that at satura-

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**Table I**

| Neoglycoprotein*  | Glycosyl content | Binding affinity |
|-------------------|-----------------|-----------------|
| L-Fuc (\( \beta \)) | 50              | 1.0             |
| L-Fuc (\( \beta \)) | 41              | 0.92            |
| L-Fuc (\( \alpha \)) | 44              | <0.57*          |
| L-Fuc1,2Gal\( \beta \),4GlcNAc | 26              | >0.46*         |
| D-Gal (\( \beta \)) | 42              | 0.89            |
| D-Gal,4GlcNAc | 35              | 0.96            |
| L-Fuc1,3Gal\( \beta \),4GlcNAc | 35              | 0.74            |
| GlcNAc (\( \beta \)) | 35              | <0.53*          |
| Man (\( \alpha \)) | 40              | <0.38*          |

* All saccharides were coupled to BSA by the amidination reaction (6) except for L-Fuc (\( \beta \))-BSA (41 residues/molecule) and L-Fuc (\( \alpha \))-BSA, which were coupled by reductive amination (6).

**Table II**

| Inhibitor                | Concentration for 50% inhibition (mM) | Inhibitor | Concentration for 50% inhibition (mM) |
|--------------------------|--------------------------------------|-----------|--------------------------------------|
| N-Acetyl-D-galactosamine | 3.0                                  | D-Glucosamine | 28 |
| D-Fucose                 | 3.0                                  | L-Ribose   | 30 |
| \( \beta \)-Methyl-D-galactoside | 3.5                                  | \( \alpha \)-Methyl-D-mannoside | 32 |
| L-Arabinose              | 4.0                                  | \( \beta \)-Methyl-D-glucoside | 37 |
| D-Galactose              | 5.0                                  | D-Xylose   | 38 |
| D-Mannose                | 6.0                                  | D-Altrose  | 38 |
| L-Fucose                 | 12                                   | N-Acetyl-D-mannosamine | 40 |
| \( \alpha \)-Methyl-L-Galactoside | 12                                  | D-Allose   | 43 |
| D-Talose                 | 15                                   | 3-O-Methyl-D-glucose | 47 |
| L-Galactose              | 16                                   | D-Lyxose   | 50 |
| D-Glucosaminolytoside    | 19                                   | D-Glucuronic acid | 50 |
| D-Mannosamine            | 21                                   | D-Mannose 6-phosphate | 54 |
| \( \alpha \)-Methyl-D-glucoside | 24                                  | 2-Deoxy-D-glucose | 60 |
| \( \beta \)-Methyl-L-arabinodiside | 25                                  | Glycerol   | 220 |
| D-Arabinose              | 26                                   | N-Acetyl-D-glucosamine | 300 |
| D-Glucose                | 26                                   |           |           |

* Insufficient neoglycoproteins could be added to obtain an exact binding affinity; thus, the values indicated are maximal possible values.
excess of a competing ligand (Fig. 7).

Variation of the fucose content of the 125I-Fuc-BSA had a great effect on its binding to lectin. No binding was observed with Fuc-BSA containing less than 24 residues of fucose/molecule of BSA, and maximal binding occurred with preparations containing 40 or more residues/molecule. This does not mean that lectin-ligand complexes were not formed with ligands of lower fucose content, just that they were not detectable in the assay employed. The binding of galactosyl-BSA to the liver galactose lectin (9) varies with galactose content of the BSA in a similar manner. The increase in binding with increasing fucose content or the inhibition of binding of 125I-Fuc-BSA (50 residues of fucose/molecule) by Fuc-BSA with varying fucose contents appears to be exponentially related to the fucose content (Figs. 8 and 9). This is possibly the result of formation of highly cross-linked lectin-ligand complexes analogous to antigen-antibody complexes. Alternatively, if the lectin must bind two or more fucose residues for complex formation, then increasing the fucose content of Fuc-BSA would increase the probability of its binding to lectin. Further studies will be needed to clarify the exact nature of the lectin-ligand complexes.

The binding specificity of the lectin was tested by inhibition of binding of Fuc-BSA by neoglycoproteins, glycosides, and monosaccharides as well as by direct binding of neoglycoproteins. From the neoglycoprotein studies (Table I) the lectin has a good affinity for fucose and galactose in /3-linkage to BSA. Fucose in a-linkage, the linkage found in naturally occurring oligosaccharides, has a much lower affinity for lectin. Mannosyl and N-acetylgalactosaminyl-BSA also have a weak affinity for the lectin. It is evident, however, that binding of neoglycoproteins differs among the three rat liver lectins (Fig. 10 and Table III). Thus, the fucose lectin binds Fuc-BSA and Gal-BSA but not GlcNAc-BSA, whereas the mannosyl/N-acetylgalactosamine lectin binds Fuc-BSA as well as Man-BSA and GlcNAc-BSA but not Gal-BSA. Moreover, galactose lectin binds only Gal-BSA, but not Fuc-BSA, Man-BSA, or GlcNAc-BSA. The pattern of binding of these ligands provides a clear means for distinguishing among the three lectins.

The inhibition of ligand binding by monosaccharides (Table II) offers some interesting insights into the binding specificity of the lectin. The conformation of D-galactose, presumably the C1 conformer, provides ligands with the highest affinity for the lectin. Thus, N-acetyl-D-galactosamine and D-fucose (6-deoxy-D-galactose) are the best inhibitors followed closely by 3-methyl-D-galactoside and D-galactose. This suggests that the orientation of substituents in the galactose C1 conformer are best accommodated into the binding site of the lectin. But if this is the case, then it becomes necessary to explain the fact that L-arabinose, L-fucose, and L-galactose are also effective inhibitors. A possible explanation became evident on examination of the 1C conformer of L-fucose (6-deoxy-L-galactose), as shown in Fig. 11, and comparison of it with the C1 conformer of D-galactose. When carbon atoms 1 and 4 of the C1 conformer of fucose are superimposed on carbon atoms 1 and 4 of galactose and the conformer rotated 180° around an axis through atoms 1 and 4, then the corresponding atoms in each conformer have substituents with the same orientation; C-4 in each has an axial hydroxyl group and all others an equatorial substituent. The ring oxygens in each structure are in the position of a carbon atom in the other ring with an equatorial hydroxyl group. Thus, fucose is a good inhibitor because it binds in a conformation similar to that of D-galactose and key substituents on ring carbon atoms are properly oriented. L-Galactose and L-arabinose are good inhibitors.

**Table III**

The inhibition of the binding of different neoglycoproteins by three rat liver lectins

The Fuc-BSA, GlcNAc-BSA, and Gal-BSA contained 49, 35, and 42 residues, respectively, of monosaccharide/molecule of BSA. The fucose lectin was assayed by assay 2 and the mannosyl/N-acetylgalactosamine (3) and galactose (4) lectins by published procedures.

| 125I-Ligand   | Specific binding | Inhibition by 100-fold excess of |
|--------------|------------------|---------------------------------|
|              | ligand/µg lectin | Fuc-BSA | GlcNAc-BSA | Gal-BSA |
| Fucose lectin|                  |         |           |         |
| Fuc-BSA      | 2.82             | 100     | 11.4      | 70      |
| GlcNAc-BSA   | 0.046            | 77.9    | 3.9       | 100     |
| Gal-BSA      | 2.27             | 91      | 69        | 0       |
| Mannose-N-acetylgalactosamine lectin | | | | |
| Fuc-BSA      | 2.15             | 87      | 94        | 17      |
| GlcNAc-BSA   | 0.81             | 91      | 69        | 0       |
| Gal-BSA      | 0.035            | 0       | 0         | 91      |
| Galactose lectin | | | | |
| Fuc-BSA      | 0.043            | 0       | 0         | 91      |
| GlcNAc-BSA   | 0.023            | 0       | 0         | 91      |

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The conformations of D-galactose, L-fucose, and D-mannose that are suggested to occupy the binding sites of the lectin. It is proposed that the substituents of the galactose ring interact through noncovalent bonds with complementary groups in the site. When the conformers of fucose (1C) and mannose (C1) are spatially oriented as shown, they also are proposed to be able to occupy the site. Thus, C4 of galactose and C2 of mannose with their axial hydroxyl groups occupy the same complementary site; C5 of galactose and C3 of fucose and mannose occupy the same site and so forth around the ring.

Inhibitors because their conformers are exactly like that of L-fucose except that carbon 6, which is a methyl group in fucose, is a hydroxymethyl group in galactose and is replaced by a hydrogen atom in arabinose. It is possible that the binding of fucose to the galactose lectin (10) could be explained by the same structural similarities between the two monosaccharides.

The structures in Fig. 11 also offer an explanation for the good inhibitory properties of D-mannose (Table II). When the C1 conformer of mannose is rotated 120° in a plane parallel to the ring, then the single axial OH group (C-2) in mannose becomes aligned with the single axial OH group (C-4) in galactose, and all other ring substituents except the ring oxygen atoms have equatorial substituents. This suggests that when bound in this manner, mannose becomes a good inhibitor. This type of structural resemblance between mannose and fucose was used earlier to explain the binding of fucose to the mannose/N-acetylglucosamine lectin (11). D-Talose (Table II) is also a reasonably good inhibitor, perhaps because it has axial substituents on carbon atoms 2 and 4 that would permit it to bind to the lectin like both galactose and mannose. Of course, since mannose-BSA is a poor inhibitor of Fuc-BSA binding (Table I), it is likely that glycosides of mannose are prevented sterically from orienting into the lectin-binding site in the same way as free mannose (Fig. 11). This is in accord with the fact that β-methyl-D-mannoside is a much poorer inhibitor than mannose itself.

The other inhibitors in Table II, though weaker than those considered above, reveal other aspects of the structural specificity of the lectin. Thus, an ammonium group on C-2 decreases the inhibitory properties (N-acetylglucosamine, D-mannosamine, and D-glucosamine), likely because of its positive charge. An N-acetylamino group on C-2 enhances the binding of the galactose derivatives (N-acetyl-d-galactosamine) but decreases the binding of both mannose (N-acetyl-D-mannosamine) and glucose (N-acetyl-D-glucosamine). Indeed, glucose is about 5 times less effective than N-acetyl-D-galactosamine, the best inhibitor, but N-acetylgalactosamine, the worst inhibitor tested (Table II), is about 100 times less effective. β-Galactosides tend to be more effective inhibitors than α-galactosides although β-methyl-L-arabinose and β-methyl-D-mannose are considerably less effective than L-arabinose and D-mannose, respectively. This may well result from the incompatibility of the C-1 O-methyl group to fit into the lectin-binding site when the glycosides fit as mannose or fucose (Fig. 11). Finally, charged substituents on C-6 weaken the binding considerably, as shown for glucuronic acid and mannose 6-phosphate. Further studies in which the ring substituents are varied more systematically will be required to understand in greater detail the binding specificity of the lectin.

Of course, a major question that remains unanswered concerns the ligands that the lectin recognizes in vivo. Likely, nonreducing galactose and N-acetylglactosamine in glycoproteins are the best candidates although perhaps other sugars would also bind since their spatial orientation to one another as in Fuc-BSA (Fig. 9) may be more important than their exact conformations. In a sense it is unfortunate that the name fucose-binding lectin has been given to the lectin studied here, since α-linked L-fucose, the natural linkage, is not a good ligand, but since it does bind fucosides of the type used to purify (1) and assay it, the name is not strictly a misnomer and serves to distinguish it from other hepatic lectins. Moreover, its binding specificity is generally compatible with the structures of the neoglycoproteins that were used in clearance studies from blood (12) to determine whether a heretofore unknown lectin was present in liver.

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