GALACTOSYL TRANSFERASE - THE LIVER PLASMA MEMBRANE BINDING-SITE FOR ASIALO-GLYCOPROTEINS

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SUMMARY

Agalacto-fetuin inhibits the binding of ^{125}I-asialo-fetuin by liver plasma membrane fragments. The chemically prepared agalacto-glycoprotein derivative is not a substrate for plasma membrane sialyl transferase and therefore this indicates that agalacto-fetuin is a true inhibitor of the membrane binding of ^{125}I-asialo-fetuin. The plasma membrane fraction also contains galactosyl transferase activity and the binding of ^{125}I-asialo-fetuin by plasma membranes is prevented by α-lactalbumin, a known inhibitor of glycoprotein-galactosyl transferase. These data indicate that galactosyl transferase is the liver plasma membrane component which binds asialo-glycoproteins.

INTRODUCTION

Morell *et al.*[1] have found that the removal of terminal sialic acid residues from many glycoproteins results in the rapid and specific uptake of these proteins by rat liver tissue within ten minutes after their injection. In the case of ceruloplasmin it has been shown that the asialo-molecule is degraded within the lysosomes upon entering the liver.[2] The signal residue for the uptake is the galactose moiety exposed on sialic acid-free ceruloplasmin.[3] Recent results by Pricer and Ashwell[4] and by Van Lenten and Ashwell[5] characterize properties of the *in vitro* binding of asialo-glycoproteins by isolated rat liver plasma membranes, and in addition, Morell and Scheinberg[6] have reported on the solubilization of the membrane binding site from rabbit liver. We wish to report evidence that indicates the plasma membrane component which binds asialo-glycoproteins is the enzyme, glycoprotein-galactosyl transferase.

MATERIALS AND METHODS

Rat liver plasma membrane fragments were isolated as previously described.[7] In all experiments that fraction of membranes (P_2) obtained by gradient flotation of the microsomal pellet was used. Binding assays were done in a total volume of 0.5 ml containing 0.05 M 2-(N-morpholino)-ethane sulfonate buffer, pH 6.8. After incubation at 37°, the binding mixture was cooled in an ice bath and layered above 4.5 ml of ice-cold 15% (W/W) sucrose in a centrifuge tube for a 50.1 rotor (Beckman Spinco). The samples were centrifuged at 50,000 rpm for 30 min at 2°, all of the supernatant liquid was removed from the resulting membrane pellet with a transfer pipette, and the membranes were resuspended in 0.5 ml H_2O. The membrane suspension was precipitated by the addition of 1.0 ml of cold 4% phosphotungstic acid in 2 N HCl. The precipitate was centrifuged, and the resulting pellet was dissolved in 0.5 ml of Protosol (New England Nuclear Corp.) and added to 10 ml of a standard toluene-Triton X-100 counting solution. The counting solution was neutralized by the addition of 0.1 ml of glacial acetic acid and the radioactivity of each sample was determined in a Beckman Model LS-150 liquid scintillation counter.

For the determinations of galactosyl and sialyl transferase activities, the radioactive glycoproteins were precipitated from the reaction mixtures by the addition of 1.0 ml of cold 4% phosphotungstic acid in 2 N HCl. The precipitates were centrifuged, and the resulting pellets were washed twice with 1 ml of the acid solution. The dissolving of the pellets and the determination of their radioactivity were done as described above for the membrane binding assays. CMP-N-acetyl-^{14}C-neuraminic acid was prepared enzymatically with rat liver CMP-N-acetyl neuraminic acid synthetase.[8] UDP-^{14}C-galactose was purchased from Amersham/Searle Corp.

Fetuin prepared by the Spiro method was obtained from Grand Island Biologicals. Asialo-fetuin was prepared by hydrolysis of fetuin at 80°

TABLE I

SPECIFICITY OF MEMBRANE-BINDING FOR ASIALO-FETUIN

Binding Mixture	Bound (cpm)
Complete	15,554+
Complete	15,451+
+ Asialo-fetuin	808
+ Fetuin	12,793
+ Agalacto-fetuin	3,433

Assays were in 0.5 ml. Complete mixtures contained the buffer system described by Pricer and Ashwell,[4] 0.55 mg of P_2 membranes, and 800 ng of ^{125}I-asialo-fetuin (30,000 cpm). For each glycoprotein inhibitor, 50 µg were added. Incubation time was 1 hr.

+Duplicate experiments.

for 1 hr in 0.06 N H_2SO_4, followed by extensive dialysis against distilled H_2O. Agalacto-fetuin was prepared by Smith degradation of asialo-fetuin.[9] ^{125}I-asialo-fetuin was prepared by iodination of asialo-fetuin with carrier free ^{125}I-iodide.[10] α-Lactalbumin was purchased from Schwarz-Mann.

RESULTS

SPECIFICITY OF BINDING.

P_2 plasma membranes exhibit the same specificity for binding asialo-fetuin as that previously shown for liver membranes isolated by the procedure of Ray[4] (Table I). Asialo-fetuin effectively inhibits the binding of the radioactively labeled glycoprotein, while native fetuin does not, and agalacto-fetuin is intermediate in its ability to inhibit binding. Calcium is required for binding (Table III).

INHIBITION OF BINDING BY AGALACTO-FETUIN.

In a quantitative binding assay it has been observed that the agalacto-derivatives of orosumucoid and ceruloplasmin are 970- and 1800-fold less active than their asialo-derivatives at inhibiting the plasma membrane binding of radioactive asialo-proteins.[5] One possibility for the observed inhibition by the agalacto-derivatives is that during their

TABLE II

EFFECT OF GLYCOSYL TRANSFERASES ON AGALACTO-FETUIN

Substrate	^{14}C-Glycoprotein (cpm)
Sialyl Transferase*	
Asialo-fetuin	3936
Control***	241
Fetuin	914
Control***	157
Agalacto-fetuin	202
Control***	166
Galactosyl Transferase**	
Agalacto-fetuin	4598+
Agalacto-fetuin	4562+
Control***	341

* Sialyl transferase assays were in a total volume of 0.1 ml containing 1% Triton X-100; 5 µmoles MES, pH 7.0; 0.09 µmoles CMP-^{14}C-sialic acid (200,000 cpm); 0.2 mg asialo-fetuin or 0.2 mg fetuin, or 0.25 mg agalacto-fetuin; and 13 µg of P_2 membranes. Reactions were for 30 min at 37°.

** Galactosyl transferase assays were in a total volume of 0.12 ml containing 1% Triton X-100; 10 µmoles MES, pH 6.8; 7.5 µmoles $MnCl_2$; 0.2 µmoles UDP-^{14}C-galactose (200,000 cpm); 0.5 mg agalacto-fetuin; and 165 µg of P_2 membranes. Reactions were for 105 min at 37°.

*** All controls were run by adding the glycoprotein substrate to the reaction mixture immediately before precipitation with acid.

+ Duplicate experiments.

preparation a sufficient quantity of the strongly binding parent ligand, asialo-glycoprotein, remains; and this latter contaminant is in fact the only true inhibitor of asialo-glycoprotein binding by liver membranes. To test this hypothesis chemically prepared agalacto-fetuin was examined as a possible substrate for plasma membrane sialyl transferase (Table II). There was no transfer of sialic acid from CMP-sialic acid to agalacto-fetuin by the enzyme. This indicates that the agalacto-fetuin preparation does not contain any residual asialo-fetuin, and therefore the galactose-free protein is a true inhibitor of membrane binding. Fetuin exhibited about 20% of the acceptor ability of asialo-fetuin in the transferase

TABLE III

α-LACTALBUMIN INHIBITION OF MEMBRANE BINDING

Experiment 1

Binding Mixture	Bound (cpm)
Complete	9,083
- Ca^{++}	694
+ 0.25 mg α-lactalbumin	643
+ 62.5 μg α-lactalbumin	867
+ 6.25 μg α-lactalbumin	5,076

Experiment 2

Complete	8,816
- Ca^{++}	637
+ 6.25 μg α-lactalbumin	6,165
+ 6.25 μg α-lactalbumin*	6,547

The assays were done as described in Methods. The complete mixture contained 10 μmoles $CaCl_2$, 1.8 μg of ^{125}I-asialo-fetuin (45,000 cpm) and 0.42 mg of P_2 membranes. Incubations were for 10 min.

* This binding sample contained an additional 10 μmoles of $CaCl_2$.

assay. This could be due to the presence of terminal galactosyl residues in the commercial product, a possibility which would explain the slight inhibitory ability of native fetuin in the binding assay (Table I). Table II also shows that the P_2 plasma membrane fragments contain a galactosyl transferase which utilizes agalacto-fetuin as a substrate.

INHIBITION OF BINDING BY α-LACTALBUMIN.

A likely plasma membrane component binding asialo-glycoproteins would be sialyl transferase, since this enzyme is present in liver plasma membranes[4] and it has the specificity to bind its own substrate, asialo-glycoproteins. Our attempts to show this involvement have failed. Another enzyme which would also have a specific recognition for asialo-proteins would be membrane galactosyl transferase, since the product of its catalytic reaction is asialo-glycoprotein. This could also explain membrane-binding inhibition by agalacto-fetuin, since it is a substrate for the proposed binding enzyme, galactosyl transferase. Table III shows

that α-lactalbumin, a specific inhibitor of glycoprotein-galactosyl transferase activity from both mammary gland and liver,[11] strongly inhibits the binding of ^{125}I-asialo-fetuin by plasma membranes. 62.5 μg of α-lactalbumin eliminates the binding of ^{125}I-asialo-fetuin by the membranes, and 6.25 μg of the protein partially inhibits binding from 33 to 48 percent in two experiments. The fact that increasing the calcium concentration 2-fold does not restore any of the decrease in the binding capacity in the partially inhibited assay eliminates the possibility that α-lactalbumin inhibits by lowering the effective calcium concentration (Experiment 2, Table III).

CONCLUSIONS

Galactosyl transferase is indicated to be the liver plasma membrane binding-site for asialo-glycoproteins based on two significant observations: (1) Agalacto-fetuin is a true inhibitor of the binding process, and (2) α-lactalbumin, a highly specific inhibitor of galactosyl transferase, inhibits the binding of ^{125}I-asialo-fetuin by liver plasma membranes. Three possible physiological functions for the binding of asialo-glycoproteins by liver plasma membranes are: (1) The liver is the tissue most active in the synthesis of serum glycoproteins, and during their secretion, Golgi vesicles may merge with the plasma membrane and thus incorporate Golgi-derived galactosyl transferase into the external surface of liver cells.[12] This function would confirm the theory of Eylar on the role of the carbohydrate residues of glycoproteins in the secretion process;[13] (2) Roseman has proposed that within a tissue, the specific adhesion of cells to one another is due to enzyme-substrate complexes between glycosyl transferases and their appropriate glycoprotein substrates.[14] This would indicate that membrane binding is involved in cell adhesion. The results here extend this proposal in that an enzyme-product complex should also be considered to be involved; (3) The work of

Ashwell has clearly shown that asialo-ceruloplasmin upon entering liver is degraded by the lysosomal system of enzymes.[2] Thus, the binding phenomenon would function to initiate the phagocytic process leading to the degradation of serum glycoproteins. Which, if any, of these functions is biologically significant remains to be shown.

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