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BOVINE HEART LECTIN STIMULATES β-D-GALACTOSIDE α2→6 SIALYLTRANSFERASE OF BOVINE COLOSTRUM

Peter Scudder, Robert A. Childs and Ten Feizi

Applied Immunochemistry Group, Division of Communicable Diseases, Clinical Research Centre, Harrow HA13UJ, U.K.

David H. Joziasse, Wietske E.C.M. Schiphorst and Dirk H. Van den Eijnden

Department of Medical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands.

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Summary. Preparations of the β-galactoside-binding lectin of bovine heart have been shown to stimulate in vitro the sialylation of the oligosaccharide Galβ1→4GlcNAc and asialo-α_1-acid glycoprotein by bovine colostrum β-D-galactoside α2→6 sialyltransferase. Kinetic data revealed that in the presence of lectin the K_m values for Galβ1→4GlcNAc and CMP-NeuAc were reduced from 25.0 to 11.6 mM and from 0.42 to 0.19 mM respectively, but the K_m for asialo-α_1-acid glycoprotein and the V_{max} values for all three substrates were little affected. Stimulation by the lectin was partially inhibited by Fucα1→2Galβ1→4GlcNAc. This, together with the effects of certain plant lectins, suggests that the stimulation of sialytransferase may be mediated through the carbohydrate-binding properties of the lectin.

Many animal tissues contain β-galactoside-binding lectins of unknown function which share the following properties: recognition of terminal non-reducing β-galactosyl groups, a monomer molecular weight between 13K and 17K and a requirement for thiol reducing groups (1). Attempts at demonstrating the sub-cellular localisation of this type of lectin have indicated that it might have a different distribution in different cell types; pericellular, intracytoplasmic or intranuclear (2-4). Among these lectins, one of the most extensively studied is that isolated from bovine heart, which recognises terminal Galβ1→4(3)GlcNAc sequences (5,6). The Galβ1→4GlcNAc structure is an acceptor-substrate for at least two sialyltransferases which catalyse the synthesis of α2→3 and α2→6 glycosidic linkages between sialic acid and galactose (7,8). This shared specificity of lectin and enzymes prompted us to investigate a possible involvement of the lectin in sialyltransferase reactions.

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MATERIALS AND METHODS

Bovine heart lectin. This was isolated by a modification of the procedure described by De Waard et al. (5,6). Heart was homogenised in 0.15 M NaCl, 0.02% NaN_3, 10 mM NH_4HCO_3, 1 mM dithiothreitol (solution A) containing 0.2M lactose and centrifuged for 1 h at 2000 g. The supernatant was made 45% saturated with $(NH_4)_2SO_4$ and centrifuged to recover the precipitate which was dialysed against solution A, and the lectin isolated by affinity chromatography on a column of asialofetuin-Sepharose. The lectin migrated as a single band (13K) in SDS-polyacrylamide gels stained with Coomassie blue and agglutinated a 1% suspension of trypsinised rabbit erythrocytes at 1 µg/ml.

Plant lectins. Concanavalin A, Ricinus communis agglutinin-120 and soybean agglutinin were obtained from Sigma, U.K. Fucose-binding protein from Lotus tetragonolobus seeds was from Miles, U.K. Peanut agglutinin, prepared by the method of Irie (9), was donated by Dr. J. Picard of this Institute.

Sialyltransferase assay and determination of kinetic parameters in the absence and presence of lectin. CMP-[^{14}C]NeuAc (1.6 Ci/mol) was purchased from New England Nuclear, Boston, Mass., and diluted with unlabelled CMP-NeuAc (10) to a specific activity of 0.8 Ci/mol. The chemically synthesised oligosaccharides Galβ1→4GlcNAc (N-acetyllactosamine) and Fucα1→2Galβ1→4GlcNAc were gifts from Dr. S.David, Université Paris-Sud, Orsay, France and Dr. P. Sinay, Université d'Orléans, France, respectively. α_1-Acid glycoprotein (donated by the American Red Cross National Fractionation Center, Bethesda) was desialylated by mild acid hydrolysis (11). Bovine colostrum α2→6 sialyltransferase was partially purified (2000-fold) by affinity chromatography using CDP-ethanolamine-Sepharose (12). The standard incubation mixture for the assay of sialyltransferase contained the following in a final volume of 80 µl: asialo-α_1-acid glycoprotein, 1 mg (0.42 µmol terminal Gal acceptor sites) or Galβ1→4GlcNAc, 0.52 µmol; CMP-[^{14}C]NeuAc, 0.125 µmol; Tris-maleate buffer pH 6.7, 8 µmol; dithiothreitol, 0.04 µmol; NaN_3, 0.13 µmol; NaCl, 6.0 µmol; NH_4HCO_3, 0.8 µmol; and β-D-galactoside α2→6 sialyltransferase, 0.07-0.15 mU (specific activity 5-10 mU/mg protein; 1 unit of enzyme activity will catalyse the incorporation of 1 µmol NeuAc into asialo-α_1-acid glycoprotein per min at 37°C at pH 6.7). Various amounts of lectin were included in this mixture as described in the results section. Incubation was for 1 h at 37°C when the reaction was stopped by the addition of 20 µl 5% (w/v) aq.$Na_2B_4O_7$ and an aliquot subjected to high voltage electrophoresis on Whatmann 3 MM paper in 1% (w/v) aq.$Na_2B_4O_7$ at 66 V/cm for 1 h at 15°C. After electrophoresis the paper was cut into strips and the radioactivity measured by scintillation counting.

With the concentration of assay components as above, the effect of lectin at 0.5 mg/ml on K_m and V_{max} values for each substrate of sialyltransferase was determined by varying the level of the substrate around its expected K_m (13) as follows: CMP-[^{14}C]NeuAc, 0.018-0.2 mM (corresponding to 1.44-16.0 nmol per assay); Galβ1→4GlcNAc, 4.15-20.7 mM (0.33-1.66 µmol); and asialo-α_1-acid glycoprotein, 3.5-27.7 mM (0.28-2.2 µmol terminal Gal acceptor sites). Values of K_m and V_{max} were determined from Lineweaver-Burk plots (14). The slope and intercept of each line was calculated by linear regression by the method of least squares.

Product characterisation. The linkage formed between NeuAc and α_1-acid glycoprotein was identified by TLC analysis of the permethylated, acid hydrolysed products of sialylated [^{3}H]-labelled α_1-acid glycoprotein as described previously (11).

RESULTS

Stimulation of the incorporation of [^{14}C]NeuAc into N-acetyllactosamine and asialo-α_1-acid glycoprotein. In the presence of lectin there was a concentration dependent stimulation of sialyltransferase activity using either Galβ1→4GlcNAc or asialo-α_1-acid glycoprotein as acceptor (Fig. 1A). At the highest concentrations of lectin tested (1.6 and 1.2 mg/ml), incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc and asialo-α_1-acid

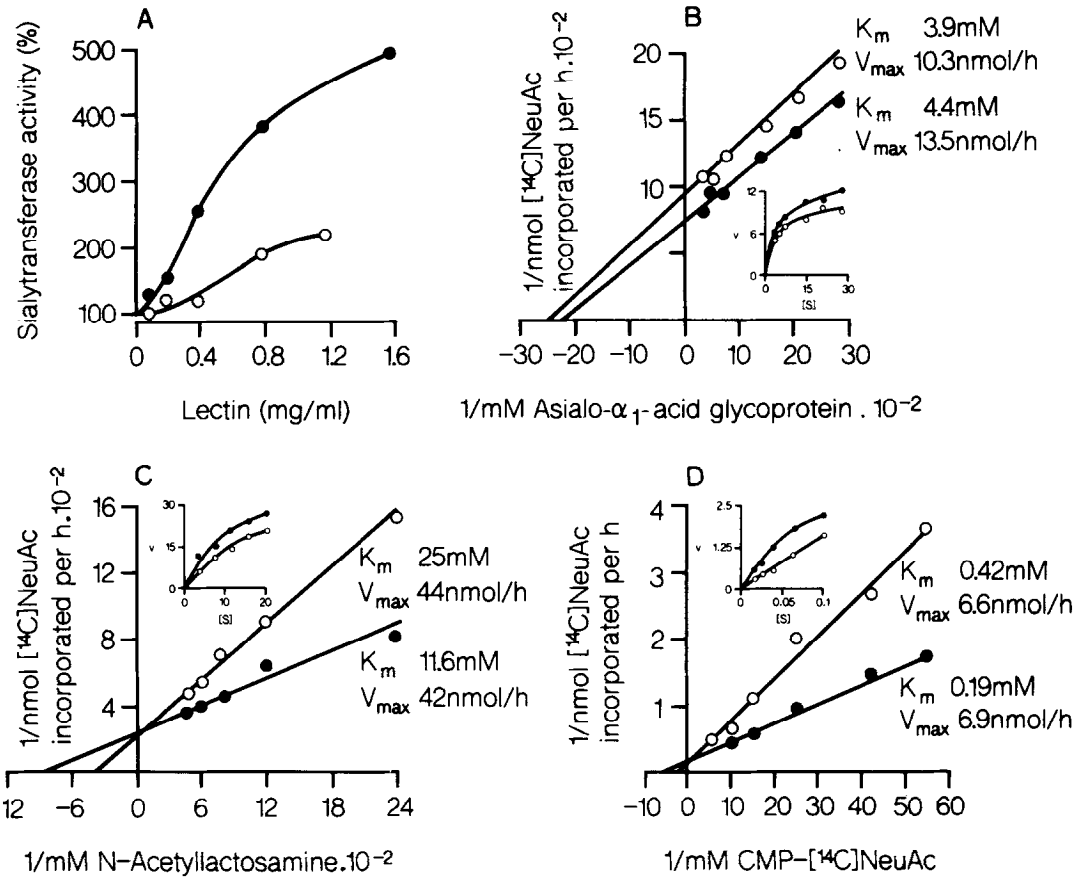

Fig. 1A. The effect of increasing concentrations of bovine heart lectin on the incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc (●—●) and asialo-α_1-acid glycoprotein (○—○). Results have been expressed as a percentage of the control reaction rate without added lectin. Each point represents the mean of duplicate assays. Control values for [^{14}C]NeuAc incorporation into Galβ1→4GlcNAc and asialo-α_1-acid glycoprotein were 2.3 and 8.4 nmol/h respectively.
Figs.1B-1D Lineweaver-Burk plots of sialyltransferase activity in the presence (●—●) and absence (○—○) of 0.5 mg/ml bovine heart lectin. Each plot shows the effect of varying the concentration of one substrate (see Materials and Methods) of the sialyltransferase reaction as follows: Fig. 1B, asialo-α_1-acid glycoprotein was the varied substrate (concentration expressed as terminal galactose residues); Fig. 1C, Galβ1→4GlcNAc was the varied substrate; and Fig. 1D, the substrate varied was CMP-[^{14}C]NeuAc (using 0.52 μmol Galβ1→4GlcNAc as acceptor). The insets are plots of v against [S] in the same experiments.

glycoprotein was 500% and 225% of control values, respectively. Some variation in the degree of stimulation was noted between experiments but stimulation was always greater when the disaccharide rather than the glycoprotein was used as acceptor. Typically, in the presence of 0.5 mg/ml lectin, incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc and asialo-α_1-acid glycoprotein varied from 160%-300% and 120%-200% of control values, respectively.

When lectin was used in the absence of sialyltransferase there was no detectable incorporation of [^{14}C]NeuAc into either of these acceptors.

Effect of lectin on kinetics of sialyltransferase reaction. Figures 1B - 1D show the effect of 0.5 mg/ml lectin on sialyltransferase activity over a range of concentrations of asialo-α_1-acid glycoprotein (Fig. 1B), Galβ1→4GlcNAc (Fig. 1C) and CMP-[^{14}C]NeuAc (Fig. 1D). The v against [S] curves demonstrate the stimulation of sialyltransferase by lectin at various concentrations of each substrate. Double reciprocal plots show a reduction in the apparent K_m for CMP-NeuAc, from 0.42 to 0.19 mM and for Galβ1→4GlcNAc, from 25.0 to 11.6 mM with little change in V_{max} values. In contrast K_m and V_{max} values for asialo-α_1-acid glycoprotein were slightly increased by the presence of lectin.

Product identification. Only one radioactive galactose derivative, corresponding to the [^{3}H]-2,3,4-trimethyl isomer, was obtained after permethylation and acid hydrolysis of [^{3}H]asialo-α_1-acid glycoprotein which had been sialylated in the presence and absence of lectin. This indicated that in each case NeuAc had been incorporated exclusively at the C-6 position of terminal galactose.

Time course experiments (results not shown) showed that the degree of sialylation of asialo-α_1-acid glycoprotein reached approximately 50% of the theoretical maximum both in the absence and presence of 0.5 mg/ml lectin. This is consistent with previous findings which indicated that certain branches of the oligosaccharide side chains of α_1-acid glycoprotein are sialylated by the α2→6 sialyltransferase at a very low rate only (7,15).

Investigation of the relationship between carbohydrate-binding activity of heart lectin and stimulation of sialyltransferase. Advantage was taken of the recent observation that the chemically synthesised oligosaccharide Fucα1→2Galβ1→4GlcNAc, like Galβ1→4GlcNAc, inhibits haemagglutination by bovine lectin but, unlike the latter, is not an acceptor, nor is it an inhibitor of α2→6 sialyltransferase (S.R. Carding and present authors, unpublished observations). When 0.2 μmol of this trisaccharide was added to 13 μg of bovine lectin (total volume 80 μl) the haemagglutinating activity of the lectin was abolished and when both were included at these concentrations in the sialyltransferase assay mixture with 0.52 μmol Galβ1→4GlcNAc as

Table 1. The effect of plant lectins and bovine heart lectin on the incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc by α2→6 sialyltransferase.

Lectin added	Incorporation of [^{14}C]NeuAc relative to control (%)	
	Experiment 1	Experiment 2
None	100	100
Bovine heart lectin	374	284
Soybean agglutinin	193	162
Peanut agglutinin	140	145
Ricinus communis agglutinin-120	113	138
Concanavalin A	109	128
Lotus tetragonolobus lectin	not tested	97

The amount of sialyltransferase used was 0.08 mU in Experiment 1 and 0.11 mU in Experiment 2 when incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc was 2.1 and 3.5 nmol/h respectively. The concentration of all lectins was 0.5 mg/ml except for bovine lectin in Experiment 1 which was at 0.63 mg/ml. Results are the means of triplicate determinations.

acceptor, the incorporation of [^{14}C]NeuAc was reduced from 342% to 279% above the control value. This corresponds to a 19% reduction in lectin stimulation.

Effect of plant lectins on sialyltransferase activity. Five plant lectins (16,17), were compared with bovine lectin for their effect on the incorporation of [^{14}C]NeuAc into Galβ1→4GlcNAc (Table 1). At the concentration tested, 0.5 mg/ml, the galactose-reactive lectins of soybean, peanut and *Ricinus communis* as well as Concanavalin A, gave some stimulation of sialyltransferase activity though less than that observed with bovine heart lectin. Fucose-binding protein of *Lotus tetragonolobus* had no effect.

DISCUSSION

This study has demonstrated that bovine heart lectin (or a substance co-purifying with it) is a potent stimulator of the α2→6 sialyltransferase of bovine colostrum. Stimulation was apparent using either Galβ1→4GlcNAc or asialo-α_1-acid glycoprotein as an acceptor for the enzyme. Furthermore, the lectin caused no change in linkage specificity of the sialyltransferase or the extent of sialylation of asialo-α_1-acid glycoprotein.

The sialyltransferase reaction is complex; it proceeds most probably by a random order equilibrium mechanism, although an ordered steady state mechanism (with CMP-NeuAc binding to enzyme before acceptor) has not been excluded (7). Therefore, without more extensive investigations, it is difficult to interpret the mechanism of stimulation by the lectin. Since the lectin does not have sialyltransferase activity, the stimulatory effect may

arise as a result of its interaction with the donor-substrate, the acceptor-substrate or sialyltransferase.

Binding of the donor-substrate, CMP-[^{14}C]NeuAc, to lectin could not be demonstrated in an immune precipitation experiment using a reaction mixture of CMP-[^{14}C]NeuAc, bovine heart lectin, rabbit anti-lectin serum and swine anti-rabbit immunoglobulins (unpublished observations).

Both acceptor-substrates of the enzyme, asialo-α_1-acid glycoprotein and Galβ1→4GlcNAc, are known to bind to bovine lectin and inhibit its haem-agglutinating activity (5,6). Stimulation could arise from this binding due to an improved presentation of the acceptor to the enzyme or an increase in acceptor valence. Multivalence may also account for the observation that, in control assays, asialo-α_1-acid glycoprotein is sialylated at a higher rate than Galβ1→4GlcNAc (Fig. 1). Alternatively, if sialyltransferase were a glycoprotein with terminal galactose residues, binding to lectin might cause a conformational change and an increased affinity of the enzyme for either of its substrates. Our kinetic data are consistent with such a mechanism. At each concentration of CMP-[^{14}C]NeuAc and Galβ1→4GlcNAc tested, the velocity of the sialyltransferase reaction was always greater in the presence of lectin without affecting V_{max} for either substrate. The reduced apparent K_m values for CMP-[^{14}C]NeuAc and Galβ1→4GlcNAc may therefore reflect a lowering of the dissociation constant (K_d) of the enzyme-substrate complex.

The involvement of the carbohydrate-binding site of lectin in the stimulation of sialyltransferase is difficult to investigate since most oligosaccharide inhibitors of the lectin are themselves acceptors for the enzyme. Thiodigalactoside is such an example; it reduces incorporation of NeuAc into Galβ1→4GlcNAc and asialo-α_1-acid glycoprotein (results not shown). However, in the presence of Fucα1→2Galβ1→4GlcNAc (which is an inhibitor of the lectin but not an acceptor for the enzyme) there was a 19% decrease in the stimulatory effect of the lectin; this is compatible with competition between the trisaccharide and Galβ1→4GlcNAc (acceptor) for the carbohydrate binding site on the lectin. A greater effect by the trisaccharide was not observed presumably because of the high concentration of lectin and the 2.5 times molar excess of Galβ1→4GlcNAc in the reaction mixture. The stimulation of sialyltransferase by plant lectins provides further evidence for the involvement of carbohydrate-binding in the mechanism

of lectin stimulation. Thus it will be important to investigate (a) whether the bovine colostrum sialyltransferase is a glycoprotein, (b) whether the bovine and plant lectins interact with it and (c) the role of carbohydrate binding in this interaction and the stimulation of sialyltransferase activity.

Earlier studies have shown that certain plant lectins stimulate the activity of membrane-bound enzymes (18-23); their mode of action was assumed to be an indirect effect resulting from membrane perturbations arising from binding with the lectins. Our observations indicate that lectins may also have a direct effect on the activity of enzymes. Whatever the mechanism, the stimulation of sialyltransferase activity by the bovine lectin is of considerable biological interest since it provides novel evidence for a possible regulatory role for this endogenous lectin in the biosynthesis of sialic acid-containing macromolecules. Thus, it will be of interest to investigate whether there is a relationship between changes in lectin levels (24) and sialyltransferase activity (25-28) that have been reported to occur during development. The generality of this phenomenon, namely the effect of endogenous lectins on diverse enzymes, now deserves investigation.

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