Formation of Highly Ordered Cross-linked Lattices between Asparagine-linked Oligosaccharides and Lectins Observed by Electron Microscopy*

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The interaction of asparagine-linked carbohydrates (N-linked) with carbohydrate binding proteins called lectins has been demonstrated to be involved in a variety of cellular recognition processes. Certain N-linked carbohydrates have been shown to be multivalent and capable of binding, cross-linking, and precipitating lectins (Bhattacharyya, L., Cecconari, C., Lorenzo, P., and Brewer, C. F. (1987) J. Biol. Chem. 262, 1288–1293; Bhattacharyya, L., Haraldsson, M., and Brewer, C. F. (1987) J. Biol. Chem. 262, 1294–1299; Bhattacharyya, L., Haraldsson, M., and Brewer, C. F. (1988) Biochemistry 27, 1034–1041). Recent data have further suggested that certain oligomannose and bisected hybrid-type N-linked glycopeptides form homogeneous cross-linked lattices with concanavalin A (Bhattacharyya, L., Khan, M. I., and Brewer, C. F. (1988) Biochemistry 27, 8762–8767). In the present study, evidence has been obtained from electron microscopy for the formation of highly ordered and distinct lattices for two bivalent complex type oligosaccharides cross-linked with soybean lectin (Glycine max) and isolectin A from Lotus tetragonolobus, respectively. The results indicate a new source of specificity for interactions of N-linked carbohydrates with lectins, namely their ability to form highly ordered homogeneous aggregates.

Oligosaccharides conjugated to proteins and lipids (glycoconjugates) have been implicated as receptors in a variety of biological processes including cellular recognition, adhesion, and signal transduction events (1). In many cases, there is evidence that cross-linking and microaggregation of the receptors is a key step (1–7). We have been investigating the molecular recognition properties of asparagine-linked (N-linked) oligosaccharides and their interactions with lectins which are multivalent carbohydrate binding proteins present in a wide variety of organisms. The results show that many N-linked oligosaccharides are multivalent and can bind, cross-link, and precipitate with lectins (8–11). Recently, we have observed that these cross-linking interactions lead to an important new source of specificity in carbohydrate-protein interactions. For example, for a bisected hybrid-type glycopeptide and a series of oligomannose-type glycopeptides with closely related structures which bind as divalent ligands and precipitate with concanavalin A, a D-glucosyl/D-mannose specific lectin (8), the quantitative precipitation analyses data indicate that each glycopeptide forms a homogeneous cross-linked lattice with the protein, even from solutions containing binary mixtures of the carbohydrates (12, 13). Heterogeneous complexes containing two different glycopeptides bound to the lectin fail to precipitate and exist only as soluble complexes (13). Therefore, the specificity of interactions between the glycopeptides and concanavalin A is much greater in cross-linked complexes than in soluble complexes, which may relate to the biological functions of N-linked carbohydrates and lectins as receptors.

The above findings suggest that the stability of homogeneous glycopeptide-lectin cross-linked lattices is due to long-range order which is not present in heterogeneous complexes. In order to examine this possibility, we have carried out an electron microscopic (EM) study of the precipitates formed by two biantennary complex-type oligosaccharides (Fig. 1) with lectins which possess different binding specificities: the N-acetyl-D-galactosamine/D-galactose-specific lectin from soybean which binds 1 and an L-fucose-specific isolectin (isolectin A) from Lotus tetragonolobus which binds 2 (cf. Ref. 14).

MATERIALS AND METHODS

The lectin from L. tetragonolobus was purchased from Sigma. The major isolectin A (LTL-A) was obtained as described (15). The lectin from soybean was purified from soyfluff (Central Soya, Chicago) as reported previously (11). Oligosaccharides 1 and 2 were obtained as generous gifts from Drs. Martin Haraldsson and Hans Lonn, respectively. Syntheses of the oligosaccharides have been described (16, 17). The structure and purity of the oligosaccharides were checked by 1H NMR at 500 MHz (18). The concentrations of oligosaccharides were measured by the phenol-sulfuric acid method (19) using mixtures of monosaccharides (e.g. L-fucose, D-mannose, and D-galactose) in the appropriate ratio as standards. Quantitative analysis of the precipitation of oligosaccharide 1 with LTL-A was done as described (8, 9). The affinities of oligosaccharide 1 and L-fucose for the lectin were determined by hemagglutination inhibition assays (20).

The electron microscopy of the precipitates collected by centrifugation was done by placing the samples on 300-mesh carbon-coated Parlodion grids which had been freshly glow discharged for 2 min, touched to filter paper and floated on a drop of 1% phosphotungstic acid, pH 7.0, and blotted immediately. Samples were observed at 80 kV in a JEOL 1200EX electron microscope.

RESULTS AND DISCUSSION

The L-fucose-specific lectin from L. tetragonolobus seeds consists of three isolectins, A, B, and C (14). The major isolectin A (LTL-A) is a tetramer with one carbohydrate binding site/monomer of molecular weight 28,000 (14) and is

* The abbreviations used are: EM, electron microscopy; LTL-A, isolectin A from L. tetragonolobus; SBA, soybean agglutinin.
Interactions of N-Linked Oligosaccharides with Lectins

**Fig. 1.** Structures of synthetic biantennary complex type oligosaccharides. Fuc, Gal, Man, and GlcNAc represent L-fucose, D-galactose, D-mannose, and N-acetyl-D-glucosamine, respectively.

**Fig. 2.** Quantitative precipitation profile of LTL-A in the presence of oligosaccharide 2 at 4 °C in 0.1 M Tris-HCl buffer, pH 7.2, containing 0.9 M NaCl, 1 mM MnCl₂, and 1 mM CaCl₂. The concentration of LTL-A expressed in terms of monomer is 73 μM. The concentration of 2 at the equivalence point is 37 μM.

Therefore tetravalent in its binding activity. Nonbisected biantennary oligosaccharide 2 (Fig. 1) is a synthetic carbohydrate containing α(1-3)-linked L-fucose at the nonreducing termini. Although 2 is not found as a naturally occurring oligosaccharide, it is closely related to the Le⁻ (and type 2 chain of Le⁺) blood group determinant which possesses a β(1-4)-galactose residue attached to the N-acetylglucosamine residue. Interestingly, increased expression of α(1-3)-linked L-fucose has been observed in most common human cancers, particularly in adenocarcinoma (21) and neuroblastoma (22). Such oligosaccharides have also been implicated in recognition involving fertilization of eggs by sperm (23).

Hemagglutination inhibition assays show that LTL-A possesses essentially equal affinity for oligosaccharide 2 as for L-fucose. However, under the appropriate stoichiometric conditions, oligosaccharide 2 precipitates with LTL-A. Fig. 2 shows the quantitative precipitation profile for LTL-A in the presence of oligosaccharide 2 at 4 °C (data are similar at 22 °C). The ratio of the concentration of oligosaccharide at the equivalence point (point of maximum precipitation) to the concentration of protein monomer is 1:2 which gives the stoichiometry of the precipitation reaction (2:4) (24). Since the isolectin possesses one binding site/monomer (14), oligosaccharide 2 is therefore divalent and can bind and cross-link two separate lectin molecules via its two terminal L-fucose residues. The cross-linked complex is a result of specific carbohydrate-lectin interactions, since the precipitates do not form in the presence of 0.1 M L-fucose and nonbinding saccharides have no effect.

EM of the negatively stained precipitates formed at the equivalence point in the precipitation profile of LTL-A and oligosaccharide 2 shows bands of “zipper-like” filamentous structures (Fig. 3B). A similar pattern is obtained by freeze-fracture EM of the precipitates (not shown). When samples at different points across the precipitation profile were examined, similar images were obtained. Thus, only one type of cross-linked complex is formed in a 2:4 stoichiometry between the oligosaccharide and protein. Neither the protein nor carbohydrate alone show any pattern. The results therefore demonstrate long-range order in the cross-linked complex between LTL-A and 2.

SBA is a tetramer with one carbohydrate binding site/monomer of molecular weight 30,000 (14) and is therefore tetravalent in its binding activity. SBA consists of multiple isolectins (cf. Ref. 14), and our preliminary evidence suggests that they have similar binding and precipitation activities. Bisected biantennary oligosaccharide 1 (Fig. 1) is a synthetic analog of a naturally occurring carbohydrate which occurs frequently at the surface of cells and in serum as part of glycoproteins (25, 26). The naturally occurring oligosaccharide has the reducing mannose residue of 1 linked β(1-4) to a...
"core" GlcNAcβ(1-4)GlcNAc disaccharide which, in turn, is linked to an asparagine residue of a glycoprotein (25, 26). Oligosaccharide 1 binds with the same affinity as N-acetyl-
lactosamine to SBA. However, under appropriate stoichiometric conditions, the lectin binds to the two terminal N-
acetylactosamine residues of oligosaccharide 1 and forms a cross-linked complex with a 2:4 oligosaccharide/protein (monom-
ero) stoichiometry (11). Thus, the oligosaccharide is diva-
lent. The naturally occurring glycopeptide of 1 is also diva-
In summary, the results of the present study provide direct
evidence for the existence of long-range order in cross-linked
complexes between N-linked oligosaccharides and lectins. In
fact, the degree of organization in the precipitates formed
between oligosaccharide 1 and SBA resembles a crystalline
lattice. In addition, distinct patterns are observed for the two
different complexes, even when the two are mixed. The pre-
sent results together with our recent findings (12, 13) indicate
a potential new source of specificity not previously recognized
with a variety of cellular events (1–7). Last, the presence of
highly organized lattices for N-linked oligosaccharide-lectin
complexes will allow an analysis of the lattice geometry for
individual complexes and thereby provide structural infor-
mation on the molecules in their bound state.

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