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A Self-Adjusting Carbohydrate Ligand for GalNAc Specific Lectins

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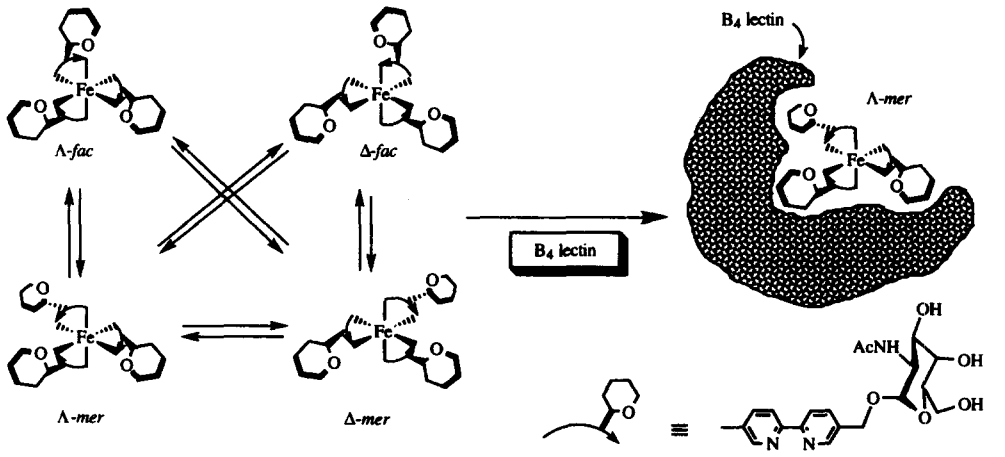
Abstract: Dynamic molecular recognition of synthetic carbohydrate ligands by lectins is described. When bipyridine-modified GalNAc is reacted with Fe(II), four diastereomers of the trivalent GalNAc ligand are formed in dynamic equilibrium at room temperature. The equilibrium allows the three GalNAc residues to adjust their spatial orientation on the metal template to fit into the binding pocket of various GalNAc-specific lectins.

Synthetic carbohydrate ligands are valuable tools to probe carbohydrate-protein interactions at the molecular level.[1-5] We have reported[6] the synthesis of a bipyridine-modified 2–acetamido-2-deoxy-α-D-galactopyranose(bipy-GalNAc). Bipy-GalNAc undergoes Fe(II)-induced self-association to form a trimeric GalNAc ligand (Fe^{II}(bipy-(GalNAc)$_3$) that binds strongly to *Vicia villosa* B_4 lectin, a lectin which recognizes repeating units of GalNAc-modified Ser or Thr residues (Tn antigen). The trimeric GalNAc ligand is a mixture of four diastereomeric isomers that are in dynamic equilibrium at room temperature. This equilibrium allows the



Scheme 1

spatial arrangement of the three GalNAc residues to change in order to fit into a multivalent carbohydrate binding site (Scheme 1). We wish to report here the self-adjusting binding of the trimeric GalNAc ligand to various GalNAc-specific lectins.

Since the bipyridine moiety of bipy-GalNAc is unsymmetrical, four diastereomeric isomers of Fe^{II}(bipy-GalNAc)$_3$, Δ-*fac*, Λ-*fac*, Δ-*mer* and Λ-*mer*, could be formed in an expected statistical ratio of 12.5 : 12.5 : 37.5 : 37.5, respectively.[6, 7] On each isomeric metal template, the three GalNAc residues are expected to have a different spatial organization[6] which should lead to different interactions with carbohydrate-binding proteins. Reverse-phase HPLC analysis of Fe^{II}(bipy-GalNAc)$_3$ showed four peaks in a ratio of 29 : 46 : 10: 15 at retention times of 10.8, 11.1, 12.8, and 13.5 min, respectively (Fig.1a). This ratio deviates slightly from the statistical ratio,[9] and is consistent with the circular dichroism spectra[6] of Fe^{II}(bipy-GalNAc)$_3$, which shows less than a 10% enantiomeric excess at equilibrium. On the basis of their circular dichroism spectra,[8] the four HPLC peaks were assigned to the Λ-*mer*, Δ-*mer*, Δ-*fac*, and Λ-*fac* isomers, in order of their elution from the reverse-phase column.

Vicia villosa B_4 lectin is a plant lectin whose carbohydrate-binding site recognizes multiple GalNAc residues.[10, 11] When Fe^{II}(bipy-GalNAc)$_3$ was mixed with the B_4 lectin, the isomer ratio gradually changed; after 32 h at room temperature, the Λ-mer isomer made up 85% of the total isomers, as shown in Figure 1b. Ultrafiltration experiments indicated that more than 97% of the Fe^{II}(bipy-GalNAc)$_3$ was bound to the lectin. In the presence of 0.1 M GalNAc, Fe^{II}(bipy-GalNAc)$_3$ does not bind to the B_4 lectin[6] and the original isomer ratio remains unchanged. The binding of the GalNAc moieties of Fe^{II}(bipy-GalNAc)$_3$ to the lectin is therefore responsible for the shift of the isomerization equilibrium. The three GalNAc residues assembled on the Λ-mer isomer of Fe^{II}(bipy-GalNAc)$_3$ appear to provide a better complementarity to the carbohydrate-binding site of the B4 lectin than the other isomers. The relative binding constants of the four isomers to the B4 lectin were calculated to be 5, 1, 1, and 18 for Δ-*fac*, Λ-*fac*, Δ-*mer*, and Λ-*mer*, respectively.[12]

Fe^{II}(bipy-GalNAc)$_3$ binds to other GalNAc-specific lectins with different affinities.[13] *Glycine max* lectin is specific for a terminal α-D-GalNAc residue attached to the 3'-OH of galactose (A antigen),[11, 14, 15] and showed strong binding[13] to Fe^{II}(bipy-GalNAc)$_3$. HPLC analysis of the isomer ratio of Fe^{II}(bipy-GalNAc)$_3$, at 32h after equilibrium was established, revealed the enrichment of both Δ-*fac* and Δ-*mer* isomers (Figure 1c). The relative binding constants of the four isomers to the lectin were calculated to be 48, 2, 21, and 1 for Δ-*fac*, Λ-*fac*, Δ-*mer*, and Λ-*mer*, respectively. Circular dichroism spectra of Fe^{II}(bipy-GalNAc)$_3$ also showed the shift of the isomerization equilibrium to enrich the Δ-isomer in the presence of *Glycine max* lectin. This is completely opposite to the chiral preference of the B_4 lectin, as shown in Figure 2. The striking difference in the isomer selectivities between the B_4 and *Glycine max* lectins must be due to the difference in the shape and functionalities of their carbohydrate binding pockets. The strong binding preference for the Δ-*fac* and Δ-*mer* isomers shown by *Glycine max* lectin suggests that the lectin may have a carbohydrate binding site that can interact with more than two GalNAc residues.[16]

In conclusion, we have demonstrated a dynamic self-adjusting ligand system[17] with tridentate GalNAc ligands assembled on a metal template. The isomerization equilibrium allows the carbohydrate ligand to change its shape to maximize its fit to the binding sites of different lectins. Similar self-adjusting molecules could be devised for other receptor-ligand systems, and the analysis of enriched isomer(s) would provide structural information about their ligand binding sites.

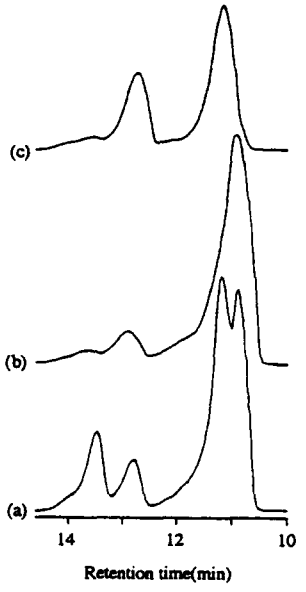


Figure 1

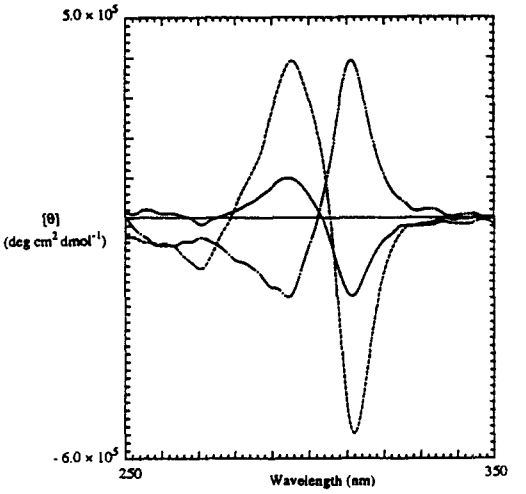


Figure 2

Figure 1: HPLC analysis of four diastereomers of Fe^{II}(bipy-GalNAc)$_3$ in the presence of lectins. [Fe^{II}(bipy-GalNAc)$_3$] = [lectin] = 1.67 x 10^{-5} M^{-1}. Fe(II)(bipy-GalNAc)$_3$ was incubated with lectins in 20mM Tris/HCl, 150mM NaCl, 1 mM $CaCl_2$ pH 7.2 for 32 hrs at 22°C. The complex was analyzed by C4 reverse-phase HPLC, using a linear gradient of 12 - 24% CH_3CN, 0.1% TFA, and a flow rate of 1.0 mL / min over 15 min. (a) Fe^{II}(bipy-GalNAc)$_3$ control, (b) Fe^{II}(bipy-GalNAc)$_3$ + *Vicia villosa* B_4 lectin, (c) Fe^{II}(bipy-GalNAc)$_3$ + *Glycine max*.

Figure 2: Circular dichroism(CD) spectra of Fe^{II}(bipy-GalNAc)$_3$ in the presence of lectins. [Fe^{II}(bipy-GalNAc)$_3$] = [lectin] = 1.67 x 10^{-5} M^{-1}. Fe^{II}(bipy-GalNAc)$_3$ was incubated with lectins in 20mM Tris/HCl, 150mM NaCl, 1 mM $CaCl_2$, at pH 7.2 for 32 hrs at 22°C. Both lectins showed negligible CD absorption at wavelengths longer than 290nm. (—) Fe^{II}(bipy-GalNAc)$_3$ control, (---) Fe^{II}(bipy-GalNAc)$_3$ + *Vicia villosa* B_4 lectin, (·--) Fe^{II}(bipy-GalNAc)$_3$ + *Glycine max*..

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(9) The peak ratio in the original mixture of $Fe^{II}(bipy\text{-}GalNAc)_3$ was unchanged by heat (70°C, 24 h) or guanidine hydrochloride (3.0 M). The order of elution of the four isomers is the same as that of the analogous $Fe^{II}(bipy\text{-}alanine)_3$ system. See ref.7.

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(12) Relative binding constants of $Fe^{II}(bipy\text{-}GalNAc)_3$ isomers to lectins were calculated from the isomer ratio determined by HPLC in the absence and presence of lectins. The original isomeric mixture of $Fe^{II}(bipy\text{-}GalNAc)_3$ has been shown to be 15 times more potent than GalNAc itself in the inhibition of the binding of peroxidase-labeled B_4 lectin to GalNAc immobilized on agarose.[6] The Λ-mer isomer of $Fe^{II}(bipy\text{-}GalNAc)_3$ is therefore calculated to be 45 times more potent than GalNAc. The calculated affinity of the Λ-mer isomer for the B_4 lectin is approximately three times higher than that of asialoglycophorin that contains a Tn-antigen.[2]

(13) GalNAc specific lectins we examined include *Dolichos biflorus, Erythrina corallodendron, Glycine max, Helix pomatia, Phaseolus limensis, Vicia villosa A4 and B4, and Wisteria floribunda*. Except for *Vicia villosa B4* (97% binding) *and Glycine max*(93% binding), all lectins showed less than 65% binding to $Fe^{II}(bipy\text{-}GalNAc)_3$ in 20mM Tris/HCl, 150mM NaCl, 1 mM $CaCl_2$ pH 7.2 at 22°C. $[Fe^{II}(bipy\text{-}GalNAc)_3] = [lectin] = 1.67 \times 10^{-5}\ M^{-1}$.

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(16) Steric interactions of the bipyridine moiety of $Fe^{II}(bipy\text{-}GalNAc)_3$ with amino acid residues near the GalNAc binding site may also be responsible for the diastereo-selective binding.

(17) A similar self-adjusting system has recently been constructed with a ubiquinone and four atropisomers of meso-tetrakis(2-hydroxy-4-nonylphenyl)porphyrin. See Hayashi, T.; Asai, T.; Hokazono, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1993**, *115*, 12210-12211.

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