Glucosylated Tris-bipyridine Ferrous Complexes: Construction of Hexavalent Saccharide Clusteres via Self-Assembly and Their Recognition by Lectin

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Hexavalent glycoconjugates were prepared via self-assembly of divalent glycosylated 2,2'-bipyridine derivatives with ferrous chloride. The Λ- and Δ-stereoisomeric complexes were preferentially formed from α- and β-glucoside derivatives, respectively. The α-glucoside cluster exhibited an enhanced affinity for concanavalin A.

Multivalent or clustered saccharide chains of glycoproteins and glycolipids on cell surfaces are key substances in various cellular recognition events and signal transductions.[1,2] Recently, artificial glycoconjugate polymers, dendorimers, calixarenes, cyclodextrins, and porphyrins have been developed as clustered saccharide models[3-5] to investigate their high affinities to lectins, toxins, viruses, and cells. In the series of our research for functional glycoconjugate materials,[6-9] we are now interested in clustered glycoconjugate assemblies on metal templates. These asemblies can be simply prepared only by mixing of glycosylated bipyridine ligands with metal ions. In addition, transition metal complexes are redox-active and some are fluorescent (e.g., $[Ru(bpy)_3]^{2+}$).[10] In this respect, transition metal complex-based glycoconjugates will be useful for sensing various saccharide recognition phenomena. However, little has been reported on coordinate-bonded glycoconjugates, except of those by Sakai et al.[11,12] They prepared trivalent glycoconjugate clusters based on tris-bipyridine metal complexes, which exhibited unique CD spectral change on binding to lectins. We here report that hexavalent glycoconjugates based on tris-bipyridine ferrous complex (Chart 1) displayed high diastereoselectivity and acquired a strong affinity to lectin.

The tris-bipyridine ferrous complex bearing hexavalent α-glucoside was prepared from *p*-nitrophenyl α-D-glucopyranoside (α-Glc-*p*NP) as follows. The glucopyranoside moiety was acetylated and the nitro function was hydrogenated and amidated with 4-azido-*n*-butyryl chloride. The azido group was hydrogenated and amidated with 2,2'-bipyridyl-4,4'dicarboxyl chloride, followed by deacetylation to afford divalent α-glucosylated 2,2'-bipyridyl-ligand (α-Glc-3-bpy) with a flexible C_3 spacer. When α-Glc-3-bpy was mixed with $FeCl_2$ in water/methanol (1/1 v/v) at room temperature, the solution turned red immediately. The formation of tris-complex ($[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$) was confirmed by the characteristic shift of ^{1}H-NMR signals of bipyridyl moieties, the appearance of metal-to-ligand charge transfer (MLCT) absorption band at 544 nm, and the stoichiometry demonstrated by Job method. The complex bearing hexavalent saccharide terminals was well soluble in water. The corresponding β-*O*-glucosylated 2,2'-bipyridine (β-Glc-3-bpy) and ferrous tris-complex ($[Fe(\beta\text{-Glc-3-bpy})_3]Cl_2$) were also prepared from *p*-nitrophenyl β-D-glucopyranoside (β-Glc-*p*NP).

$[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$ and $[Fe(\beta\text{-Glc-3-bpy})_3]Cl_2$ gave almost symmetrical CD spectra assignable to Λ- and Δ-$[Fe(bpy)_3]^{2+}$, respectively, as shown in Figure 1. The opposite stereoisomers of the bipyridyl array should arise from the opposite chirality of their anomeric positions of the saccharide moieties. Both molar ellipticities due to π-π* transition (ca. 300 nm) and MLCT (ca. 540 nm) were smaller than those of enan-

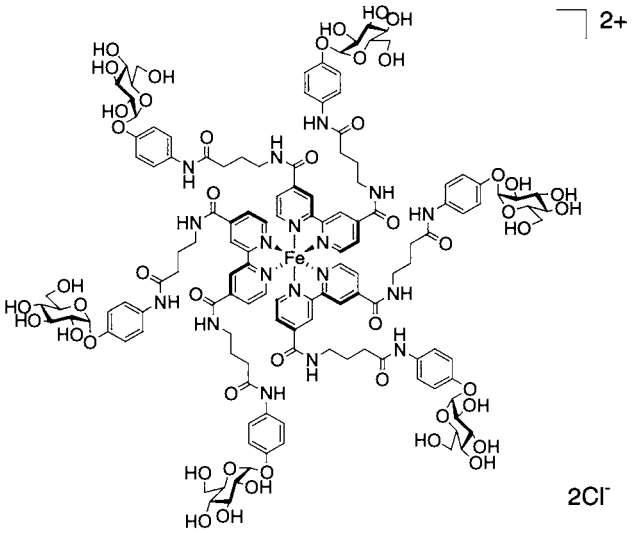

Chart 1. Structure of α-glucosylated tris-bipyridine ferrous complex, $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$.

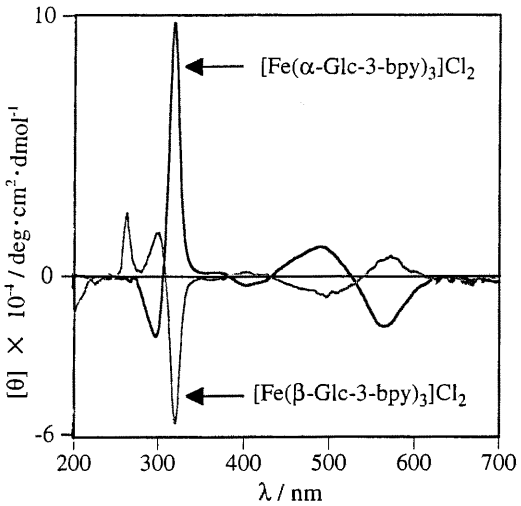

Figure 1. CD spectra of glucosylated tris-bipyridine ferrous complexes in water at 20 °C.

tio-pure $[Fe(bpy)_3]^{2+}$,[11,13] which indicated that the glycoconjugate metal complexes were mixtures of Λ- and Δ-stereoisomers.

The two stereoisomers in each glycoconjugate were separated by reverse-phase HPLC on a CrestPak C18T-5 analytical column using a linear gradient of acetonitrile and an aqueous 0.1 M ammonium acetate solution. The major and minor peaks of $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$ were assigned, respectively, to Λ- and Δ-isomer on the basis of their CD spectra. The Λ-Δ ratio estimated from their integration was 73:27. The rather high diastereo-excess (46% de) was in accordance with that estimated by ^{1}H-NMR spectrum. Nuclear Overhauser effect (NOE) was detected between the phenyl and bipyridyl protons in ^{1}H-NMR spectrum of $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$ in D_2O. We assume that the hydrophobic interaction between the phenyl and bipyridyl moieties separating with the flexible alkyl spacer may result in a compactly packed conformation of the complex in water. The resultant proximity of the chiral saccharide units to the complex center may account for the high diastereo-selectivity of the complexes.

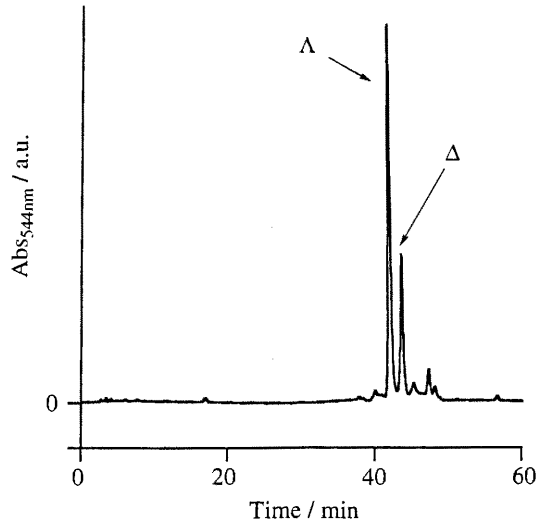

Figure 2. HPLC separation of the two diastereomers of $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$. The chromatogram was monitored at 544 nm, a maximum adsorption wave length of the complex. CrestPak C18T-5 analytical column. a linear gradient of acetonitrile / aqueous 0.1 M ammonium acetate solution.

Binding affinity of the conjugates was investigated by inhibition of lectin-induced hemagglutination[14] using ConA (concanavalin A from jack bean, α-Glc specific) and RCA_{120} (*Ricinus communis* agglutinin from castor bean, β-Gal specific). Table 1 summarizes the minimum inhibition concentrations per saccharide residue (IC_{min}). $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$ was a stronger inhibitor for ConA-induced hemagglutination than D-glucose and α–Glc-*p*NP by about 1000- and 100-fold, respectively. $[Fe(\beta\text{-Glc-3-bpy})_3]Cl_2$ was less potent inhibitor. RCA_{120}-induced hemagglutination was not inhibited by these conjugates

Table 1. Inhibition of lectin-induced hemagglutination by glycoconjugates[a]

Inhibitor	IC_{min}[b] / M[c]	
	ConA	RCA_{120}
$[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$	9.9×10^{-6}	n.i.[d]
$[Fe(\beta\text{-Glc-3-bpy})_3]Cl_2$	1.9×10^{-3}	n.i.[d]
α–Glc-pNP	8.0×10^{-4}	n.i.[d]
β–Glc-pNP	2.0×10^{-3}	n.i.[d]
glucose	1.4×10^{-2}	n.i.[d]

[a][Lectin]= 4 x [Minimum concentration required for hemagglutination]. [b]Minimum inhibition concentration. [c]Molarity of saccharide unit. [d]Not inhibited by 0.1 M.

($IC_{min} \geq 1 \times 10^{-1}$ M). The enhanced specific interaction of $[Fe(\alpha\text{-Glc-3-bpy})_3]Cl_2$ for ConA may arise from hexavalent α-glucoside assembly and hydrophobic phenyl aglycon. In addition, their saccharide moieties may be induced-fit to the binding site of ConA, because of the flexibility of alkyl spacer.

In conclusion, hexavalency, hydrophobicity, and flexibility of the glycosignals on metal complexes play a substantial role in enhancement of diastereo-selectivities and affinities for lectins. These properties will be advantageous for high-sensitive monitoring of various saccharide recognition phenomena.

References and Notes

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