Tris-bipyridine ruthenium complex-based glyco-clusters: amplified luminescence and enhanced lectin affinities

Teruaki Hasegawa, Takahiro Yonemura, Kazunori Matsuura and Kazukiyo Kobayashi*

Department of Molecular Design and Department of Biotechnology, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan
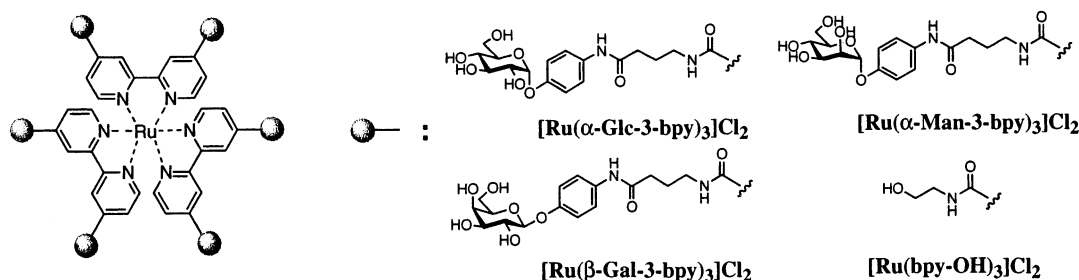
Abstract—Tris-bipyridine ruthenium complexes carrying hexavalent α-glucoside, α-mannoside, and β-galactoside clusters were synthesized. Isomeric Λ- and Δ-complexes were separated, and their optical and conformational characteristics were investigated by UV–vis, CD, luminescence, and NMR spectroscopies as well as molecular dynamics calculation. The complexes exhibited strong luminescence and also strong inhibition of lectin-induced hemagglutination: particularly, the binding of hexavalent mannose cluster complexes to concanavalin A was strong. © 2001 Elsevier Science Ltd. All rights reserved.

A recent development in glycobiology has revealed the importance of the saccharide chains of glycoproteins and glycolipids on cell surfaces as key substances in various cellular recognition events and signal transductions[1]. Furthermore, various viruses and bacteria attach to carbohydrate chains as their main target molecules on host cell surfaces in the first stage of the infection process. Multivalent or clustered saccharide structures have received increasing interest as carrier molecules for inter-cellular signal transduction. Many glycoconjugates based on artificial templates such as linear polymers[2], dendrimers[3], calixarenes[4], cyclodextrins[5], and DNAs[6] have been developed as clustered saccharide models to enhance affinities for lectins. In our series of research on glycoconjugates, we reported the strong affinity of hexavalent glycosylated tris-bipyridine ferrous complexes with lectins[7]. The ferrous complexes could be simply prepared only by mix-

ing glycosylated bipyridine ligands with a ferrous ion in water/ethanol at room temperature. However, we have found that much stronger binding to lectins cannot be detected because the Fe(II)–bipyridine bond is labile and the conjugate is easily dissociated in very diluted aqueous solution ($<10^{-6}$ M). In this paper, we employed more robust ruthenium complexes as the core component and synthesized glycosylated tris-bipyridine ruthenium complexes shown in Fig. 1. In addition to their stability, ruthenium complexes are redox-active as well as luminescent[8], and hence these glycoconjugates may be of promise as biosensors.

The 2,2′-bipyridine ligand (α-Glc-3-bpy) tethering two α-glucoside moieties via C3 alkyl spacer was synthesized from *p*-nitrophenyl α-D-glucopyranoside and 2,2′-bipyridine 4,4′-dicarboxylic acid. When 3 equivalents of α-Glc-3-bpy was treated with $RuCl_3$ in a minimum

**Figure 1.** Structures of tris-bipyridine ruthenium complexes carrying hexavalent α-glucoside, α-mannoside and β-galactoside clusters, together with that carrying no saccharide cluster ([Ru(bpy-OH)₃]Cl₂).

* Corresponding author.
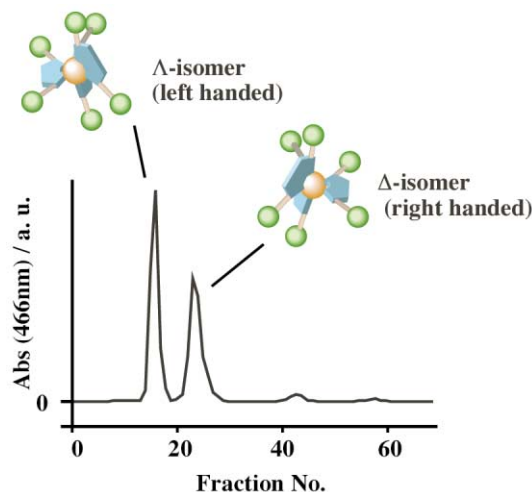
Figure 2. Separation of the two diastereomers of [Ru(α-Glc-3-bpy)$_3$]Cl$_2$. The chromatogram was monitored at 466 nm. Toyo-Pearl HW40-S and 0.1 M ammonium acetate aqueous solution.
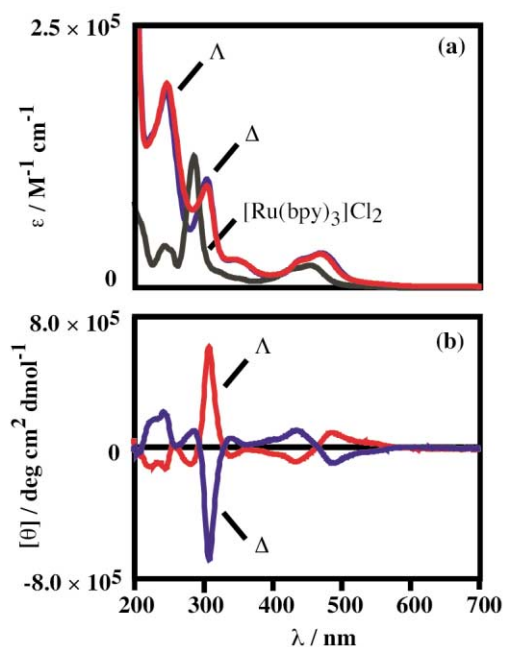

Figure 3. (a) UV and (b) CD spectra of Λ- and Δ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$ in water at 25°C, together with [Ru(bpy)$_3$]Cl$_2$.

amount of boiling water/ethanol mixture, the solution turned orange, suggesting the formation of the corresponding tris-bipyridine ruthenium complex. After 18 h heating, the solution was concentrated and the water-soluble fraction was chromatographed through Toyo-Pearl HW40-S gel by using aqueous 0.1 M ammonium acetate as eluent. As shown in Fig. 2, the two isomeric products were obtained in a total yield of 53% and assigned to Λ- and Δ-isomers of [Ru(α-Glc-3-bpy)$_3$]Cl$_2$ (Λ:Δ = 51:49) as follows. They gave similar UV–vis spectra (Fig. 3(a)) with MLCT (metal-to-ligand charge transfer) absorption band at 466 nm characteristic for tris-bipyridine ruthenium complex. The MLCT absorption bands of α-glucosylated complexes as well as [Ru(bpy-OH)$_3$]Cl$_2$ carrying no sugar unit were observed at 466 nm which are shifted to a longer wavelength ($\sim$16 nm) with a slightly larger extinction coefficient than that of [Ru(bpy)$_3$]Cl$_2$ itself. The shift may be attributable to the electron-withdrawing amide linkage of these complexes. CD spectra of these two isomers in Fig. 3(b) were almost symmetrical at bipyridine π–π* (ca. 300 nm) and MLCT absorption bands (ca. 460 nm) characteristic for Λ- and Δ-configurations. The formation of the α-glucosylated tris-bipyridine ruthenium complex was also shown in ESI-TOF-MS, in which a dicationic molecular ion peak (M/2) was observed at 1431.93 for each isomer (calcd 1431.97).

The corresponding hexavalent α-mannoside clusters Λ- and Δ-[Ru(α-Man-3-bpy)$_3$]Cl$_2$ were obtained via similar synthetic and separation procedures, whereas the β-galactoside cluster, [Ru(β-Gal-3-bpy)$_3$]Cl$_2$ could not be separated into the diastereomeric isomers. These complexes also exhibited similar absorption spectra. These ruthenium-based glycoconjugates were stable: the Ru(II)–bipyridine bond was locked at room temperature and no isomerization or dissociation was detected in diluted aqueous solution.

Nuclear Overhauser effect was observed between the bipyridine and the anomeric protons of these glycoconjugates in NOESY spectra in D$_2$O at room temperature. We assume that these glycoconjugates may take compact or shrunk conformations owing to hydrophobic interaction between the bipyridine ring of the complex core and the phenyl ring of the aglycon. The compact or shrunk conformations of these conjugates
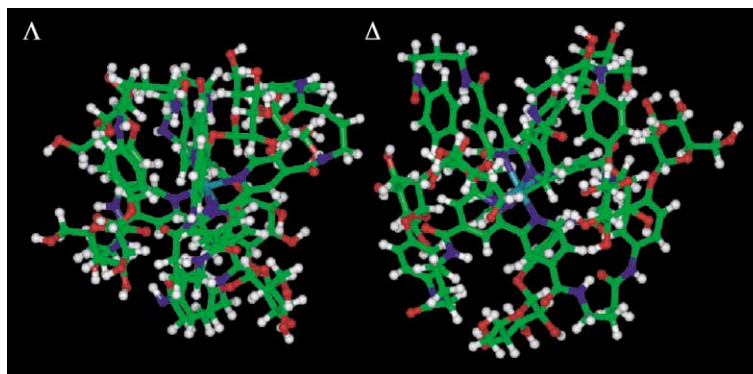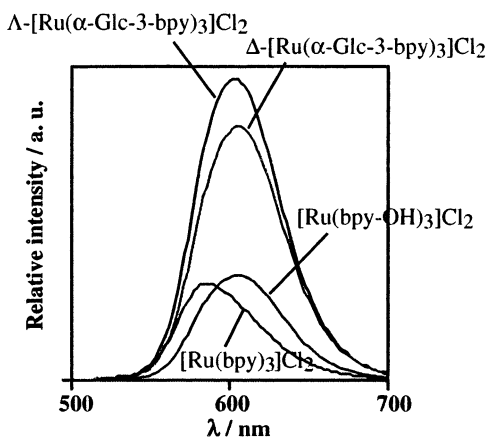

Figure 4. Most stable conformation of (a) Λ- and (b) Δ-isomer of α-glucosylated complexes given by molecular dynamics calculations (InsightII/Discover, esff, 300 K, ε = 80.0, 400 ps equilibrium, 600 ps dynamics).

were also supported by molecular dynamics calculations at 300 K and $\varepsilon = 80.0$ using InsightII/Discover program and esff force field, as shown in Fig. 4. The average distance between the anomeric carbons and the ruthenium center of Λ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$ (8.7 Å) was slightly shorter than that of the corresponding Δ-isomer (9.2 Å). This difference between the two isomers was clearly reflected in the anomeric proton signals in ^{1}H NMR spectra: Λ-isomers of glucosylated and mannosylated complexes exhibited broad and upper field resonances at 4.4 and 4.8 ppm, respectively; their corresponding Δ-isomers resonated at 5.1 ppm. These upper field resonances may be probably due to the anisotropy provided by the aromatic rings of the complex core.

These glycoconjugates exhibited intense emission by excitation of the MLCT band in water as shown in Fig. 5. As compared with [Ru(bpy)$_3$]Cl$_2$, their maximum emission bands were red-shifted and the intensities were increased, and their luminescence quantum yields (Φ_{em}) were estimated to be 0.105 and 0.086, respectively, for the Λ- and Δ-isomers of the α-glucosylated complexes. Since [Ru(bpy-OH)$_3$]Cl$_2$ induced the red-shift of MLCT but no enhancement of luminescence, the enhancement of luminescence of the glycoconjugates may be caused by the closely packed saccharide clusters surrounding the complex core. We assume that the 'carbohydrate-shell' may isolate the luminescence core from the outer aqueous circumstance to obstruct the thermal degradation, which results in enhancement of emission intensity.

Binding of the glycoconjugates to lectins was investigated by a hemagglutination inhibition assay using α-glucoside-specific concanavalin A (ConA) and β-galactoside-specific *Ricinus communis* agglutinin (RCA$_{120}$) (Table 1). These glycoconjugates specifically inhibited lectin-induced hemagglutination more strongly than the corresponding monomeric p-nitrophenyl derivatives. Saccharide clusters play essential roles in their specific binding. It is of interest to note that both Λ- and Δ-isomers of [Ru(α-Man-3-bpy)$_3$]Cl$_2$ functioned even at the low concentration of 9×10^{-8} M

Table 1. Inhibition of ConA- and RCA$_{120}$-induced hemagglutination by glyco-cluster materials based on tris-bipyridine ruthenium complexes[a]

Inhibitor	IC$_{min}$[b]/M[c]	
	ConA	RCA$_{120}$
Λ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$	1.4×10^{-5}	n.i.[d]
Δ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$	1.1×10^{-5}	n.i.[d]
α-Glc-pNP	1.9×10^{-4}	n.i.[d]
Λ-[Ru(α-Man-3-bpy)$_3$]Cl$_2$	9.0×10^{-8}	n.i.[d]
Δ-[Ru(α-Man-3-bpy)$_3$]Cl$_2$	1.5×10^{-7}	n.i.[d]
α-Man-pNP	2.1×10^{-5}	n.i.[d]
[Ru(β-Gal-3-bpy)$_3$]Cl$_2$[e]	n.i.[d]	2.5×10^{-6}
β-Gal-pNP	n.i.[d]	2.8×10^{-4}
[Ru(bpy)$_3$]Cl$_2$	n.i.[d]	n.i.[d]

[a] [Lectin] = 8×[minimum concentration required for hemagglutination].
[b] Minimum inhibition concentration.
[c] Molarity of saccharide unit.
[d] Not inhibited by 0.1 M.
[e] Δ–Λ mixture.

without decomposition and the strong affinity was comparable to that (4×10^{-7} M) of poly(p-acryloylamidophenyl α-mannoside). The glycosylated polymer is capable of cross-linking the multiple binding sites on lectins, but the diameter (~ 3.8 nm) of the present ruthenium complex-based glyco-conjugates is smaller than the distance (ca. 6.8 nm) between the two binding sites on the adjacent subunits in ConA. However, the glycoconjugates are capable of embedding the mannose moieties to one binding site which strongly recognizes 3,6-branched tri-mannosides. We assume that the densely packed mannose arrays of the glycoconjugates are suitable to induce strong binding affinity comparable to those of glycopolymers.[9] Binding of the present glycoconjugates to lectins was also investigated by surface plasmon resonance assay, in which α-mannoside clusters bound to ConA-immobilized surfaces with higher affinities (K_a = ca. 3×10^6 M^{-1}) than α-glucoside clusters (K_a = ca. 5×10^5 M^{-1}).

In conclusion, hexavalent saccharide clusters on tris-bipyridine ruthenium complex have been prepared with glycosylated bipyridines and ruthenium chloride. These glycoconjugates were found to exhibit strong luminescence as well as strong binding to lectins. These properties are advantageous for a sensitive and convenient monitoring system for saccharide binding phenomena. Preliminary experiments revealed that addition of specific lectins influenced the luminescence intensities of the glycoconjugates. Application of the glycoconjugates to sensory or monitoring systems is now under study.

Figure 5. Luminescence spectra of Λ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$, Δ-[Ru(α-Glc-3-bpy)$_3$]Cl$_2$, [Ru(bpy-OH)$_3$]Cl$_2$, and [Ru(bpy)$_3$]-Cl$_2$ in water at 25°C. Ex. = 450 nm.

Acknowledgements

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9. The *p*-butanamidophenyl spacer was indispensable for the strong binding to lectins. The ferrous complex of bipyridine carrying α-glucopyranose without the spacer group exhibited no inhibition under similar conditions probably owing to their dispersed and octahedrally fixed saccharide arrays. The spacer groups in the present glycoconjugates are assumed to enhance the flexibility and hydrophobicity for lectin binding as well as to induce densely packed saccharide arrays.