COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Ruthenium complexes carrying a disialo complex-type oligosaccharide: enzymatic synthesis and its application to a luminescent probe to detect influenza viruses†

Shinji Kojima,[a] Teruaki Hasegawa,[a] Takahiro Yonemura,[a] Ken Sasaki,[b] Kenji Yamamoto,[c] Yutaka Makimura,[c] Tadanobu Takahashi,[de] Takashi Suzuki,[de] Yasuo Suzuki[de] and Kazukiyo Kobayashi*[ae]

[a] *Department of Molecular Design and Department of Biotechnology, Graduate School of Engineering, Nagoya University, Nagoya 464-8603, Japan. E-mail: kobayash@mol.nagoya-u.ac.jp; Fax: +81-52-789-2528*

[b] *Central Research Laboratory, Taiyo Kagaku Co., Ltd., Yokkaichi 510-0844, Japan*

[c] *Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan*

[d] *University of Shizuoka School of Pharmaceutical Sciences, Shizuoka 422-8526, Japan*

[e] *CREST, Japan Science and Technology Corporation (JST), 4-1-8 Hon-cho, Kawaguchi Saitama 332-0012, Japan*

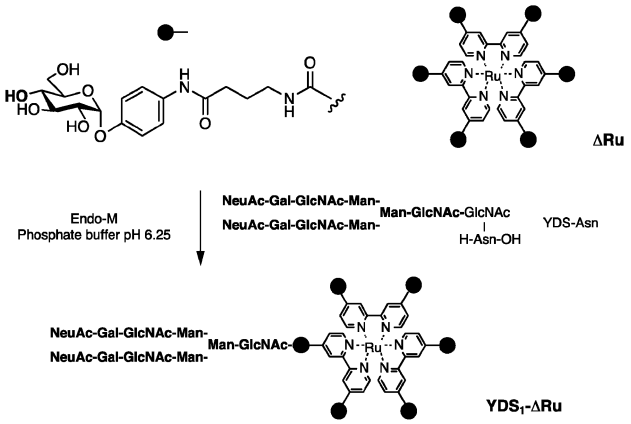
Received (in Cambridge, UK) 31st October 2002, Accepted 24th March 2003
First published as an Advance Article on the web 23rd April 2003

Tris-bipyridine ruthenium-complexes carrying a disialo complex-type oligosaccharide were prepared *via* a one-pot transglycosylation using endo-glycosidase (Endo M); they bind to type-A influenza viruses with excellent affinity (IC_{50} = 8.4 μM), and their luminescence intensity is strongly depressed by virus-binding.

Influenza virus infection is epidemic and sometimes critical for aged patients with respiratory disease.[1] Type-A influenza viruses easily mutate their immunogen structures to evade human immune defence systems and then cause fatal disease. In spite of the frequent mutations, influenza viruses have a highly conserved binding specificity to sialo oligosaccharides on the host-cell surface. Disialo complex-type oligosaccharides, in particular, induce strong binding to the viruses.[2] Polymer-based adsorbents having multivalent sialoside arrays have been reported to capture and neutralize influenza-viruses.[3] Monitoring systems have also received increasing interest from the viewpoint of preventive medicine.[4]

In our researches on artificial glycoconjugates, we reported that tris-bipyridine ruthenium complexes carrying simple monosaccharides exhibited strong luminescence as well as excellent lectin-affinities.[5] The characteristic "saccharide-shell" structure isolates the ruthenium-core from outer solvents and then enhances the luminescence intensity. This structure resembles that of natural proteins having a redox- and luminescent core embedded on the polypeptide shell. Interestingly, binding of lectin to these glycoconjugates reduces their luminescence by disrupting their saccharide shell structure.[5] In this respect, the ruthenium-containing glycoconjugates should be new potent luminescent monitors to detect various carbohydrate-recognition phenomena. Herein, we wish to report 1) the chemo-enzymatic synthesis of ruthenium-complexes having a native disialo complex-type oligosaccharide and 2) the application of the resulting ruthenium-complex to an influenza-sensory system.

A tris-bipyridine ruthenium-complex having six α-glucoside appendages (ΔRu) was synthesized as described previously.[5] Transglycosylation of a yolk disialo complex-type oligosaccharide (YDS) onto ΔRu was achieved in potassium phosphate buffer (pH 6.25) containing 20% v/v acetonitrile (Scheme 1).[6,7] Endo-β-GlcNAc-ase from *Mucor hiemalis* (Endo M) effectively catalyzed one-pot deca-saccharide transfer from the asparagine-bonded disialo complex-type oligosaccharide (YDS-Asn) onto 4-OH of an α-glucoside unit of



Scheme 1 Schematic illustration of transglycosylation of disialo complex-type oligosaccharide onto glyco-appended ruthenium complex.

ΔRu. Two transglycosylation products (retention time = 12.8 and 6.3 min) were detected by HPLC analysis of the reaction mixture (Fig. 1), and they could be assigned to mono- (YDS_1-ΔRu) and bis-adducts (YDS_2-ΔRu) according to their ^{1}H-NMR spectra after purification.[8] The transglycosylation yields were 42 and 12% for mono- and bis-adducts, respectively, after a 4 h reaction.

We evaluated the binding of YDS-adducts to type-A influenza virus (A/Memphis/1/71) using an inhibition test of virus-infection to MDCK cells.[9] Although ΔRu did not show any inhibitory potency against virus-infection even at high concentrations (> 100 mM, data not shown), the corresponding YDS-adducts showed strong inhibitory potency (Fig. 2). Their inhibitory potencies were found to be two orders of magnitude higher than that of YDS-Asn. Especially, bis-YDS-adducts showed the highest inhibitory potency (IC_{50} = 8.4 μM),

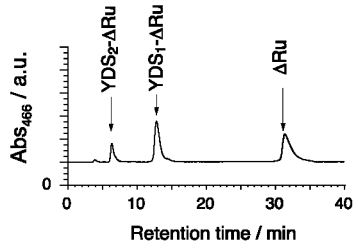


Fig. 1 HPLC of a mixture of transglycosylation products. Isocratic elution on a CrestPak C18T-5 using 14% aqueous acetonitrile containing 0.1 M ammonium acetate. Products were detected by monitoring the absorbance at 466 nm.

† Electronic supplementary information (ESI) available: the molecular conformation of YDS_1-ΔRu obtained by MD calculations. See http://www.rsc.org/suppdata/cc/b2/b210739b/

DOI: 10.1039/b210739b

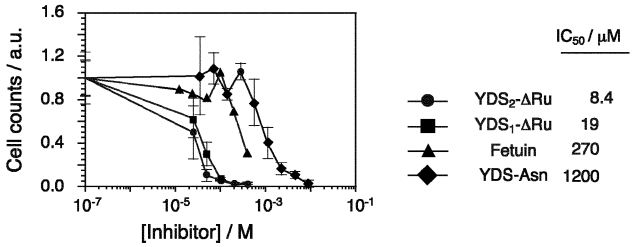


Fig. 2 Inhibition of virus-infection (A/Memphis/1/71) of MDCK cells with ruthenium complexes and fetuin.

probably due to the concurrent binding of its two YDS appendages onto hemagglutinin. Molecular modeling revealed that the maximum distance between two YDS terminals of the expanded bis-adducts is 80 Å which is large enough to cross-link the two binding sites on the adjacent subunits (46 Å) in the hemagglutinin-trimer.

It should be noted that these YDS-adducts showed extremely high virus-affinity in spite of their relatively small molecular size. The YDS-adducts inhibit virus-infection much more strongly than fetuin (IC_{50} = 270 μM) and comparably to the synthetic polymer (IC_{50} = *ca*. 50 μM)[3b] whose high virus-affinities are assigned to multiple interactions between sialoside-arrays and hemagglutinin-trimers. The origin of the excellent virus-affinity of YDS-adducts is still unknown. However, the calculation of a partial charge distribution on the hemagglutinin crystal structure using the InsightII/Delphi program revealed that the saccharide-recognition site of hemagglutinin is negatively charged under neutral conditions. Along with the strong virus-affinity arising from YDS-appendages, some electrostatic and hydrophobic interactions between the cationic and aromatic-rich complex center and the amino acid residue of hemagglutinin should play additional roles in the enhanced affinity.

The YDS-adducts exhibited strong luminescence at around 605 nm (Fig. 3a). Interestingly, there is an increase in the luminescence intensity with an increase of attached YDS residues. The bulky YDS-oligosaccharide should contribute to isolate the luminescence core from the outer aqueous solvents to enhance the luminescence intensity. Molecular dynamics (MD) calculations support the compact packing of all saccharide residues including bulky YDS around the complex core. The average distances from the ruthenium-atom were only 7.0 ± 1.0 and 7.8 ± 1.7 Å for the carboxyl group of 2–3 and 2–6 linked sialic acids, respectively, in spite of the long saccharide linkers. It is likely that the electrostatic interaction between the cationic complex center and anionic sialic acid accounts for the close packing.

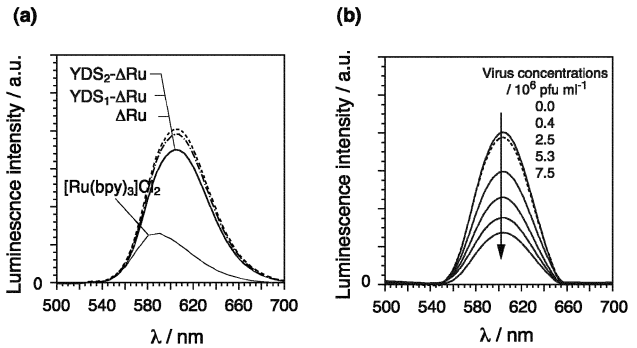


Fig. 3 (a) Luminescence spectra of ruthenium-complexes in water at 25 °C, [complex] = 1.0×10^{-5} M. λ_{ex} = 450 nm. (b) Decrease of luminescence intensity of YDS_1-ΔRu (2.5×10^{-7} M) in the presence of various concentrations of type-A influenza virus. The black dotted line is the luminescence spectrum of YDS_1-ΔRu (2.5×10^{-7} M) in the presence of YDS-Asn (2.5×10^{-3} M) and type-A influenza virus (7.5×10^6 pfu ml^{-1}). Conditions: PBS (pH 7.4) at 4 °C. λ_{ex} = 450 nm.

The addition of type-A influenza virus to the YDS-adducts in PBS resulted in a marked decrease in its luminescence intensity (Fig. 3b). It is clear that the specific interactions between the YDS-appendages of ruthenium complex and the type-A influenza virus is responsible for the luminescence quenching, since virus-induced quenching could be inhibited by YDS-Asn itself. Although further investigation of the mechanism of quenching is needed, disruption of the saccharide-shell and the subsequent exposure of the luminescence core to an aqueous environment should be responsible for the quenching.[5]

In conclusion, we have succeeded in a one-pot trans-glycosylation of disialo complex-type oligosaccharides onto an α-glucose-functionalized tris-bipyridine ruthenium complex. The resulting glycoconjugates exhibited excellent lectin-affinities comparable to that of polymers presenting multiple sialoside arrays. Also, they have a strong luminescence which is markedly depressed by addition of virus, probably due to disruption of their saccharide-shell structures. The molecular design of saccharide-shell/luminescent-core structures will be a widely applicable strategy for the construction of sensitive sensory systems for various toxins, viruses, and bacteria. Investigations into the origin of their excellent binding properties and change in luminescence intensity, along with their application, are now in progress.

Notes and references

1 Y. Suzuki, *Prog. Lipid Res.*, 1994, **33**, 429.

2 S. Sabesan, J. Duus, P. Domaille, S. Kelm and J. C. Paulson, *J. Am. Chem. Soc.*, 1991, **113**, 5865.

3 (*a*) S.-K. Choi, M. Mammen and G. M. Whiteside, *J. Am. Chem. Soc.*, 1997, **119**, 4103; (*b*) A. Tsuchida, K. Kobayashi, N. Matsubara, T. Muramatsu, T. Suzuki and Y. Suzuki, *Glycoconjugate J.*, 1998, **15**, 1047; (*c*) J. D. Reuter, A. Myc, M. M. Hayes, Z. Gan, R. Roy, D. Qin, R. Rui, L. T. Piehler, R. Esfand, D. A. Tomalia and J. R. Baker Jr., *Bioconjugate Chem.*, 1999, **10**, 271; (*d*) R. Roy, D. Zanini, S. J. Meunier and A. Romanowska, *J. Chem. Soc., Chem. Commun.*, 1993, **24**, 1869.

4 (*a*) M.-G. Beak, R. C. Stevens and D. H. Charych, *Bioconjugate Chem.*, 2000, **11**, 777; (*b*) A. Reichert, J. O. Nagy, W. Spevak and D. H. Charych, *J. Am. Chem. Soc.*, 1995, **117**, 829.

5 (*a*) T. Hasegawa, T. Yonemura, K. Matsuura and K. Kobayashi, *Bioconjugate Chem.*, in press; (*b*) T. Hasegawa, T. Yonemura, K. Matsuura and K. Kobayashi, *Tetrahedron Lett.*, 2001, **42**, 3989.

6 (*a*) K. Yamamoto, *J. Biosci. Bioeng.*, 2001, **92**, 493; (*b*) K. Matsuda, T. Inazu, K. Haneda, M. Mizuno, T. Yamanoi, K. Hattori, K. Yamamoto and H. Kumagai, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2353.

7 Transglycosylation conditions: [YDS-Asn] = 70 mM. [ΔRu] = 14 mM. [Endo M] = 60 mU ml^{-1} in potassium phosphate buffer containing 20% acetonitrile at 37 °C. YDS-Asn is a glycosyl donor.

8 The number of YDS-appendages was determined by ^{1}H-NMR based on the peak intensity ratio between H4 of mannose moieties (δ 4.1 ~ 4.3 ppm) in YDS residues and the bipyridine group (δ 7.8 ~ 8.3 ppm). The regiochemistry of the bis-YDS-adduct is still unknown, however, molecular mechanics calculations demonstrated that YDS_2-ΔRu bearing two YDS-appendages on the antipodes has a lower minimum-energy (402.1 kcal mol^{-1}) compared to the other regioisomers (414.0 ~ 454.9 kcal mol^{-1}), due to their steric and electrostatic repulsion.

9 Various concentrations of the glycoconjugates were pre-incubated with influenza virus for 1 h and then MDCK cells were added. Incubation (34 °C, 17 h) and washing were followed by the labeling of infected cells with monoclonal antibody that binded to virus-antigen. After treatments with the enzyme-linked secondary antibody and the subsequent H_2O_2-DEPDA-4-CN color producing reagents, blue-colored infected cells were counted by direct observation using a microscope.