Per-C-6 Oligosaccharide-Branched Cyclodextrin Interacting with Both the Lectin and Drug

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The improved dual interactions with both the lectin (PNA, a cellular receptor model) and an anticancer drug (DXR) have been observed in *per*-C-6 oligosaccharide-branched cyclodextrin (**2**) using an optical biosensor based on SPR.

Conjugated oligosaccharides in biological events have been known to act a variety of roles in recognition phenomena.[1] This concept has attracted considerable attention in the receptor-binding properties of a variety of multi-antennary saccharide-conjugates such as polymers,[2] dendrimers,[3] calixarenes,[4] and cyclodextrins.[5] We already reported natural oligosaccharide-branched cyclodextrins[6a] which showed potential binding to lectin protein. We have been studied in the development of drug carriers for targeting drug delivery systems.

In the present research, the branch component, galactosyl-glucono-amide-ethanethiol was synthesized in the reaction between the lactonolactone[7] and aminoethanethiol to combine in the amide linkage. Galactosyl-glucono-amide-ethanethiol was introduced at the *mono*- and *per*-C-6 position of halogeno-β-cyclodextrin.[8] Purification by preparative HPLC was made until the product of a single peak was obtained. MS (FAB^+): *m/z* 1532 [$M+H^+$] for **1**; 3933 [$M+H^+$] for **2.**

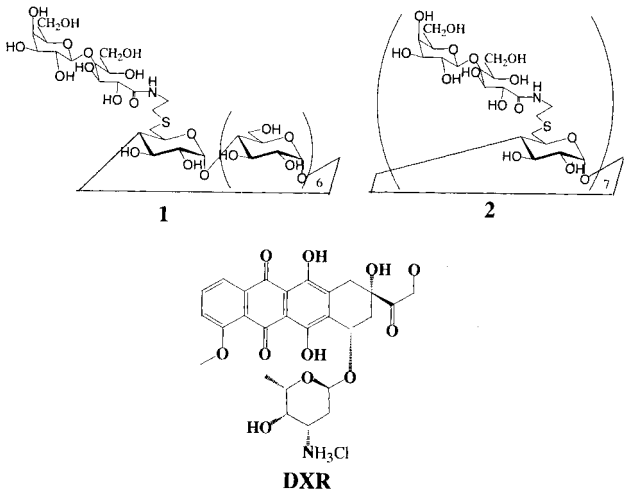

Figure 1. Structure of **1**, **2** and **DXR.**

The saccharide-interaction of **1** and **2** with peanut lectin (PNA)[9] was confirmed with the competitive inhibition assay by addition of lactose as an inhibitor using optical biosensor based on SPR(IAsys, Biosensor Laboratory) as shown in Figure 2 (A for **1**, B for **2)**. PNA lectin was immobilized on metal surface in aminosilane cuvette intervening suberate diamide as a linker group in the same manner of the previous report.[6b]

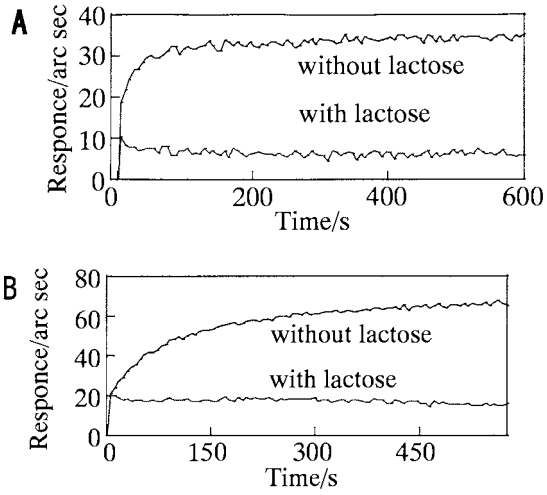

Figure 2. Confirmation of saccharide-interaction association by competitive inhibition with lactose addition using SPR. A: [**1**] = 10^{-3} M + [lactose] = 5×10^{-3} M , B: [**2**] = 10^{-3} M + [lactose] = 2×10^{-2} M in [acetate buffer] = 10^{-2} M (pH 5.3) + [$MgCl_2$] = 10^{-3} M + [$CaCl_2$] = 10^{-3} M (1M=1 mol dm^{-3}). Y-axis represents response in arc sec unit, the change of reflect angle, which is proportional to the associated amount on the sensor metal.

The association equilibrium constants (K_a), association rate constants (k_{ass}), and dissociation rate constants (k_{diss}) of **1** and **2** with immobilized PNA were obtained. The results are shown in Table 1.

Table 1. Association parameters of **1** and **2** with immobilized PNA

Products	K_a ($\times 10^3$ M^{-1})	k_{ass} ($\times 10$ $M^{-1}s^{-1}$)	k_{diss} ($\times 10^{-3}$ s^{-1})
1	8.1 ±0.2	2.1 ±0.2	2.6 ±0.2
2	130 ±10	14 ±0.1	1.1 ±0.8

Solvent: [acetate buffer] = 10^{-2} M (pH 5.3) + [$MgCl_2$] = 10^{-3} M + [$CaCl_2$] = 10^{-3} M.

The ratio of the association equilibrium constant K_a (**2** / **1**) in Table 1 was about 16. This may be regarded as a part of the oligosaccharide clustered effect which Y. C. Lee proposed.[10]

An inclusion-interaction of **1** and **2** with doxorubicin (DXR) was confirmed with competitive inhibition assay by addition of cyclohexanol as an inhibitor using SPR. (Figure 3, A for **1**, B for **2**).

The association equilibrium constants (K_a), association rate constants (k_{ass}), and dissociation rate constants (k_{diss}) of **1** and **2** with immobilized DXR were obtained. The results are shown in Table 2.

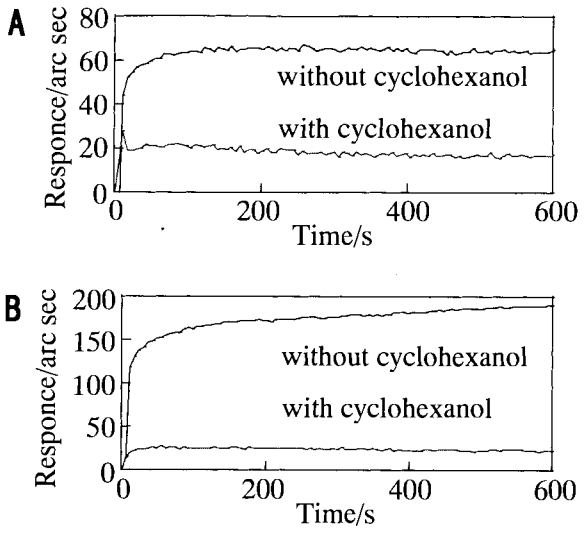

Figure 3. Confirmation of inclusion association by competitive inhibition by cyclohexanol addition.
A: [**1**] = 10^{-3} M + [cyclohexanol] = 5×10^{-3} M , B: [**2**] = 10^{-3} M + [cyclohexanol] = 5×10^{-3} M in [acetate buffer] = 10^{-2} M(pH 5.3) + [$MgCl_2$] 10^{-3} M + [$CaCl_2$]10^{-3} M. DXR was immobilized on aminosilane cuvette using suberate as a linker group according to the previous report.

Table 2. Association parameters of **1** and **2** with immobilized DXR.

Products	K_a ($\times 10^3 M^{-1}$)	k_{ass} ($\times 10^2 M^{-1}s^{-1}$)	k_{diss} ($\times 10^{-2} s^{-1}$)
1	3.1 ±0.2	1.2 ±0.05	4.1 ±0.3
2	62 ±1.0	1.1 ±0.1	0.19 ±0.4

Solvent: [acetate buffer] = 10^{-2} M (pH 5.3)+[$MgCl_2$] = 10^{-3} M + [$CaCl_2$] = 10^{-3} M.

The ratio of association equilibrium constant K_a (**2** / **1**) in Table 2 was about 21.[11] The ratio was mainly attributed to the k_{diss} ratio (**2** / **1**). It is thought to form a complex like the scheme in Figure 4 in the inclusion association of **2** and DXR. In this case, the sugar-clustered cyclodextrin (**2**) is assumed to behave as a kind of induced-fit phenomena.

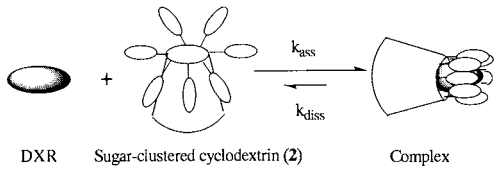

Figure 4. Scheme of complex structure of **2** with DXR.

In summary, the sugar-clustered cyclodextrin, *per*-C-6 oligosaccharide-branched cyclodextrin (**2**) was prepared in this study. It showed some effectiveness in measurements using SPR: the ratio of association constant with PNA and with DXR became about 16 times and about 21 times larger, respectively, in comparison to the corresponding parameters of the *mono*-C-6 oligosaccharide-branched cyclodextrin (**1**). This association behavior of sugar-clustered cyclodextrin will be important factors for application to a targeting drug-delivery system.

References and Notes

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11 The ratio 21 was sustained by the observed association constant for **1** and **2** with DXR to be 2.1×10^3 M and 45×10^3 M, respectively, by Benesi-Hildebrand plots at UV 230 nm. Job plots between **2** and DXR also showed 1:1 complex formation.