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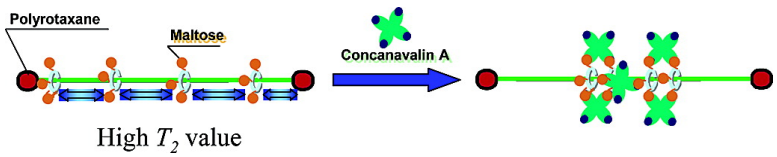

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Supramolecular Design for Multivalent Interaction: Maltose Mobility along Polyrotaxane Enhanced Binding with Concanavalin A

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The supramolecular architecture of interlocked molecules such as rotaxanes and polyrotaxanes is one of the key nanoscale molecular designs for biological applications.[1] Rotaxanes and polyrotaxanes are defined as a molecular assembly, in which one or many cyclic molecules are threaded onto a linear chain capped with bulky end-groups. Cyclodextrins (CDs) have the ability to encapsulate guest molecules within their hydrophobic cavities and have been used as a building-block for supramolecular nanostructures.[2] In the 1990s, a new type of polyrotaxane consisting of α-CDs and a poly(ethylene glycol) (PEG) has been reported by Harada et al.[3] The most characteristic structure is that all of the α-CDs are mechanically locked by the PEG chain, and, thus, each α-CD is expected to slide[4] and rotate along the PEG chain. This feature spurred us toward CD movement-based molecular recognition using polyrotaxanes. Recently, we found that ligand-polyrotaxane conjugates, in which many ligands were covalently bound to α-CDs, recognized those binding proteins in a multivalent manner.[5] However, the contribution of α-CD movements to the multivalent recognition has been unclear, although the dynamic feature of the polyrotaxanes provides us with the potential for its use as a molecular machine in biomedical fields.

Here, we investigate how α-CDs and ligand mobility in ligand-polyrotaxane conjugates affect the multivalent interaction with a binding protein. Maltose and concanavalin A (Con A) were selected as a ligand and a binding protein, respectively, because Con A recognizes maltose,[6] and Con A-glycopolymer systems have been extensively studied as a model of multivalent interaction.[7] A series of maltose-polyrotaxane conjugates (Mal-α/E20-TYR-Zs, **1**−**3**) (Figure 1) were synthesized by a condensation reaction between β-maltosylamine and carboxyethylester-polyrotaxanes[8] in the presence of BOP reagent and HOBt.[9] Because the stoichiometric number is ca. 227, the threading % values of α-CDs were 22%, 38%, and 53%, respectively (Table 1). As a reference, maltose-α-CD (Mal-α-CD, **4**) and maltose-poly(acrylic acid) (Mal-PAA, **5**) conjugates with a varying number of maltose groups were synthesized (Table 1).

The effect of the mechanically locked structure in the maltose-polyrotaxane conjugates on multivalent interaction was assessed using the Con A-induced hemagglutination inhibition assay (Figure 2). The minimum inhibitory concentration (MIC) of maltose unit was determined. Relative potency was calculated from the ratio of MICs of the maltose-polyrotaxane conjugate and the maltose itself. The relative potency of Mal-α/E20-TYR-Zs (**1**−**3**) and Mal-PAA (**5**) increased with the number of maltose groups up to around 120, although the absolute values were varied. On the other hand, the relative potency of Mal-α-CD (**4**) was very small, and the number of maltose groups per α-CD is the same as that in **1c** and **2d**. The potency increase in **1**−**3** and **5** can be attributed to the chelate effect[10] and was consistent with the multivalent effects in terms of increasing the number of saccharide groups conjugated with the

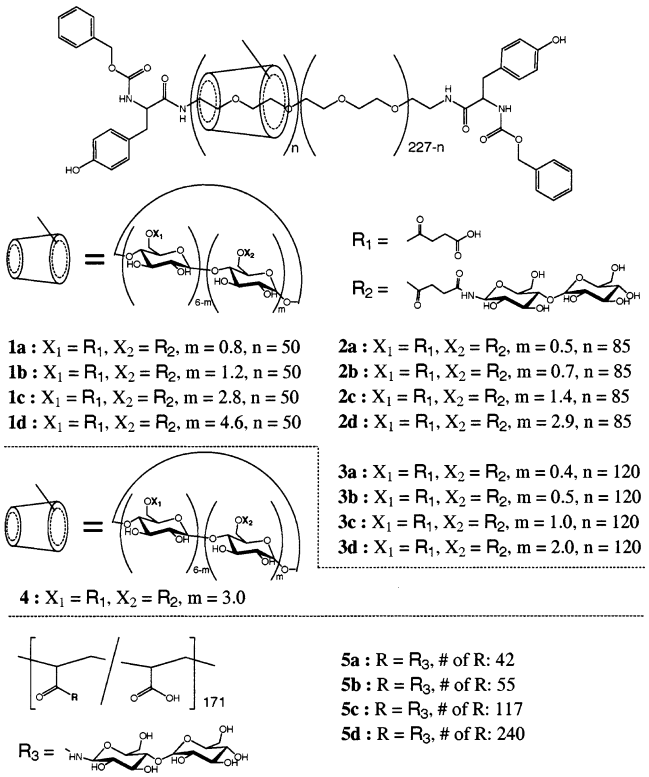

Figure 1. Chemical structure of maltose-polyrotaxane conjugates consisting of α-CDs, PEG, benzyloxycarbonyl-tyrosine and maltose (Mal-α/E20-TYR-Zs, **1**−**3**), maltose-α-CD (**4**), and maltose-poly(acrylic acid) (**5**) conjugates.

polymer backbone.[7b] The relative potencies of **3** and **5** decreased with a further increase in the number of maltose groups (**3d** and **5d**). This result is well consistent with previous glycopolymer systems:[11] all of the maltose groups conjugated with the polymer backbone cannot necessarily bind to the binding sites of Con A, and hence unavailable maltose groups are buried in **3d** and **5d**. However, the relative potency of **2d** was significantly higher than those in **1d**, **3d**, and **5d** despite a similar number of maltose groups (Figure 2).

The most dominant parameter to enhance the relative potency observed in **2d** should be the threading % of α-CDs. A ^{1}H NMR signal of **2d** was very sharp, although those of **1d** and **3d** were broadened.[12] The order of sharpening the signals in terms of α-CD threading was 38% (**2d**) ≫ 22% (**1d**) > 53% (**3d**). One of the possible reasons for the sharpening could be the high mobility of Mal-α-CDs in the mechanically locked structure of the polyrotaxane backbone, which has shorter correlation times. The spin−spin relaxation time (T_2) of C(1)H (δ: 5.1 ppm) of maltose groups in **2d** was much longer than those of **1d** and **3d** (Table 2). In addition, **2d** exhibited almost the same T_2 as Mal-α-CD (**4**). These results

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Table 1. Synthesis of Maltose-Polyrotaxane Conjugates and the Reference Samples

sample code[a]	no. of α-CD[b]	α-CD threading (%)[c]	total no. of Mal[b]	no. of Mal/α-CD[d]
1a	50	22	40	0.8
1b			60	1.2
1c			140	2.8
1d			230	4.6
2a	85	38	44	0.5
2b			58	0.7
2c			122	1.4
2d			244	2.9
3a	120	53	42	0.4
3b			64	0.5
3c			117	1.0
3d			240	2.0
4			3	3.0
5a			42	
5b			55	
5c			117	
5d			240	

[a] M_n of PEG for **1**−**3**, 20 000; M_n of poly(acrylic acid) for **5**, 25 000. [b] Calculated from ^{1}H NMR spectra. [c] Calculated by the ratio of the found and stoichiometric numbers of α-CD. If α-CDs are thread stoichiometrically onto a PEG chain, two ethylene glycol units should be included in each α-CD cavity. α-CD threading (%) = [no. of α-CD]/[stoichiometric no. of α-CD] × 100 (see ref 3a). [d] Number of Mal/α-CD was calculated from the integral ratio of the C(1)H and C(1′) of maltose and C(1)H of α-CD on ^{1}H NMR spectra.

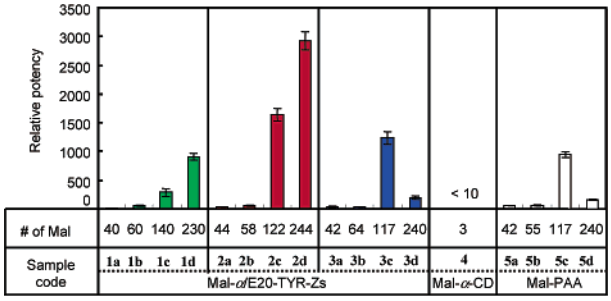

Figure 2. Relative potency of Con-A-induced hemagglutination inhibition based on the minimum inhibitory concentration (MIC) of the maltose unit (concentration of Con A: 1.96 mg/mL, n = 3, mean ± S.E.M.). The hemagglutination experiments were carried out in a 0.1 M PBS buffer (pH 7.4) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. The sample codes are consistent with those in Table 1.

Table 2. T_2 of the C(1)H of Maltose Groups in Each Conjugate[a]

sample code	α-CD threading (%)	total no. of Mal	T_2 [s]
1d	22	230	0.116
2d	38	244	0.230
3d	53	240	0.083
4		3	0.237
5d		240	0.035

[a] 0.1 M phosphate buffer (using D_2O, pD 7.4) containing 1 mM $CaCl_2$ and 0.1 mM $MgCl_2$ was used.

indicate that the maltose groups in **2d** maintain a mobility similar to that in **4**. On the other hand, the maltose mobilities of **3d** and Mal-PAA (**5d**) were lower than the others. Taking these results into account, it is considered that the high mobility of the maltose groups in the polyrotaxane with the appropriate threading % of α-CDs contributes to the enhanced Con A binding. Of course, the high mobility was not the only dominant factor. Even with almost the same values of T_2 and number of maltose groups per α-CD, the mechanically locked structure of the polyrotaxane (**2d**) exhibited an inhibitory effect far superior to that of α-CD (**4**). So far, synthetic multivalent ligands have been designed so as to increase enthalpy gain using the flexible linker of saccharides.[7f,13] However, with an increase in the valency, those ligands are thermodynamically unfavorable due to spatial mismatches between the saccharides and binding protein during clustering.[13] The mechanically locked structure of Mal-α/E20-TYR-Z with the typical α-CD threading % can have favorable thermodynamic parameters in the multivalent interaction. Presumably, the high mobility of Mal-α-CDs reduces the special mismatches between maltose and Con A binding sites, resulting in preventing the entropic loss and gaining the enthalpy during binding. Therefore, it is concluded that the combination of (i) multiple copies of ligands and (ii) their supramolecular mobility along the mechanically locked structure should contribute to significant enhancement of the multivalent interaction due to a reduction of the special mismatches of binding. This will provide quite a new idea on using polyrotaxanes as a biosensing probe in the field of drug delivery, diagnosis, and tissue engineering.

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Supporting Information Available: Synthetic procedure of the conjugates, ^{1}H NMR data, GPC results, and procedure for hemagglutination experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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