Surface Plasmon Resonance Study on the Interaction of Immobilized Macrocyclic Sugar Clusters with Lectins and Water-soluble Polymers

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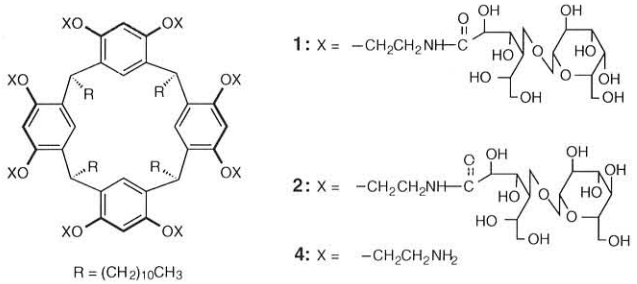
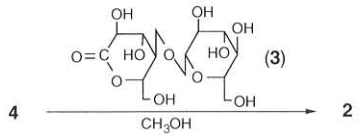
A surface plasmon resonance study indicates that an amphiphilic calix[4]resorcarene derivative having eight polar side chains with terminal galactose or glucose residues and four long alkyl chains are immobilized on a hydrophobic sensor chip; the resulting monolayer of the sugar moieties, exposed to the bulk aqueous phase, binds lectin polysaccharides, and ploy(vinyl-alcohol) in water.

Glycoside cluster effect by naturally occurring cell-surface oligosaccharides provides an important key for understanding of cellular recognition mechanisms in various biological processes such as differentiation, proliferation, immunological responses, cell adhesion, cancer transfer, and so on.[1] In order to perform a functional simulation of the cell-surface oligosaccharides, we have recently developed an amphiphilic calix[4]resorcarene derivative having eight polar side chains with terminal galactose residues and four hydrocarbon branches as hydrophobic arms (**1**).[2] Compound **1** exhibits the following unique functions in aqueous media. (1) As a host, it strongly binds anionic and hydrophobic guests such as 1-anilinonaphthalene-8-sulfonate and methyl orange with binding constants of 2.2×10^5 and 6.4×10^5 dm^3 mol^{-1}, respectively. (2) As a sugar cluster, it can be adsorbed, not only as such but also as a host-guest complex, onto polar silica surfaces from an aqueous phase through multivalent hydrogen bonding interactions. In order to get further insight into polar interactions of clustering sugar moieties, we prepared an analogous derivative with terminal glucose residues (**2**). We report here on the application of the surface plasmon resonance (SPR)[3] technique to prove adsorption of lectins, polysaccharides, and water-soluble polymers on the monolayer assembly of the sugar cluster.





The octa(glucose) derivative of calix[4]resorcarene **2** was obtained by the reaction of maltonolactone (**3**) with octaamine **4**,[2] which was prepared in four steps from the parent macrocycle in a manner similar to that applied to the preparation of **1**. Compound **2** was fully characterized by means of spectroscopy (IR, ^1H and ^{13}C NMR, and TOF-MS) and elemental analysis.

In SPR, a change in the refractive index of light and hence in the concentration of a solution in vicinity of a sensor chip is detected as a change in the intensity of light. Adsorption of substance(s) on the chip can thus be read out as a change in resonance[4] (1000 resonance units (RU) corresponds to a surface concentration change of ~1ng/mm^2).[5] Throughout this work was used a hydrophobic sensor chip (HPA, research grade; Figure 1)[6] set in BIAcore X (Pharmacia Biotech); the chip consists of an alkanethiol-coated thin gold film on a thin glass plate.

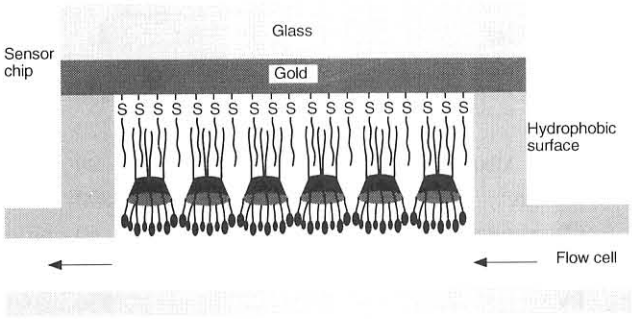


Figure 1. Schematic representation of immobilization of cluster **1** or **2** on the hydrophobic sensor chip.

Treatment several times of a chip with 50 µl of an aqueous solution (pH 7.2 with 0.01 mol dm^{-3} phosphate and µ 0.5 with NaCl) of compound **1** (2.5×10^{-5} mol dm^{-3}) in a flow cell resulted in immobilization of **1**, having a saturation resonance signal of RU = 1520. This value corresponds to a packing density of 0.22 molecules/nm^2 or an occupation area of 4.6 nm^2/molecule for **1** on the chip. Compound **2** behaved similarly; RU = 1430, 0.21 molecules/nm^2, and 4.8 nm^2/molecule. The occupation area found is very similar to the cross sectional area of the sugar-cluster part of compounds **1** and **2**, having a diameter of ~2 nm for the most folded conformation. These results suggest that amphiphiles **1** and **2** form a closely-packed monolayer with their sugar-cluster moieties exposed to the bulk aqueous phase and their hydrophobic alkyl chains embedded in the hydrophobic surface of the chip (Figure 1). The immobilization is practically irreversible, adsorbed **1** can not be washed off by the buffer, although desorption occurs when washed with a mixture of 30% aqueous ethanolamine and 1 mol dm^{-3} hydrochloric acids.

The interaction of thus-formed monolayer of sugar clusters

with lectins, polysaccharides, and water-soluble polymers was evaluated in a similar manner, by using an aqueous solution (1 mg/ml) of an adsorbate. The saturated changes in resonance units (RU) observed are summarized in Table 1. Concanavaline A (Con A), a glucose- (and mannose-) binding lectin, is more strongly bound to immobilized **2** (RU = 1120[7]) than to **1** (RU = 110). In addition, the adsorption of Con A on **2** is inhibited (RU = 510), although partially, in the presence of a large excess amount (1.0 mol dm^{-3}) of methyl-α-D-glucopyranoside. Peanut lectin (PNA), on the other hand, is a galactose-binding lectin, which is selectively bound to immobilized **1** (RU =1760[7]) than to **2** (RU = 120). Thus, Con A and PNA recognize the terminal glucose and galactose residues of the present sugar clusters **2** and **1**, respectively, although there are much weaker Con A-**1** and PNA-**2** interactions (RU ≅ 100). Bovin albumin, an inert protein having no carbohydrate binding sites, show similar affinities again with RU ≅ 100 to both **1** and **2**. This, coupled with the above results, suggests that nonspecific protein-sugar cluster interaction occurs to an extent of RU ≅ 100.

Polysaccharides dextrin (neutral, RU = 300-400), chitosan (cationic, ~300), and condroitin (anionic, ~60) are moderately to weakly bound to immobilized sugar clusters **1** and **2**. Poly(vinyl alcohol) (PVA, with an average molecular weight of M_w = 20,000) is more strongly bound (RU ≅ 900), while poly(ethylene glycol) (PEG, M_w =10,000) is hardly adsorbed. These results suggest that polar interaction involving the OH groups is responsible for the adsorption of PVA as well as polysaccharides on the present monolayer of sugar clusters.

Table 1. Saturated resonance unit (RU) changes upon adsorption of various adsorbates on surface-immobilized **1** and **2**[a]

Adsorbate[b]	Saturated RU changes / RU[c]	
	1	**2**
Con A	110	1120
PNA	1760	120
Bovin Albumin	100	90
Dextrin	280	390
Chondroitin	60	60
Chitosan	330	310
PVA	890	930
PEG	20	0

[a] Running buffer; pH 7.2 with phospahte (0.01 mol dm^{-3}) and μ 0.5 with NaCl, containing $MnCl_2$ (0.1 mmol dm^{-3}) and $CaCl_2$ (0.1 mmol dm^{-3}).

[b] Each adosorbate solution (1 mg/ml, 50 μl) was injected several times in a flow cell to achieve saturation binding.

[c] Only RU values more than 50 RU are reliable because of a limited performance of the sensor chip.[8]

In conclusion, the present macrocycles can be assembled on a hydrophobic sensor chip and the resulting monolayer of the sugar clusters exhibits potent binding abilities toward respective lectins and OH-functionalized polymers in water.

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Reference and Notes

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