Pure & Appl. Chem., Vol. 70, No. 12, pp. 2379–2384, 1998.
Printed in Great Britain.

Molecular delivery systems using macrocyclic sugar clusters

Yasuhiro Aoyama,*,† Yusuke Matsuda, Jirarut Chuleeraruk, Kouichi Nishiyama, Kazuhisa Fujimoto, T. Fujimoto, T. Shimizu, and Osamu Hayashida†

Institute for Fundamental Research of Organic Chemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

† *CREST, Japan Science and Technology Corporation (JST)*

Abstract: Sugar cluster compounds obtained by use of a macrocyclic calix[4]resorcarene framework exhibit remarkable cluster effects. As hosts, they form stable host-guest complexes in water. As adsorbates, they are strongly adsorbed on quartz surfaces from aqueous solution. They bind to lectin proteins not only as such but also as host-guest complexes, thereby delivering or transporting included guest molecules to the specific sugar-binding sites. Surface prasmon resonance (SPR) is utilized to charakterize the polar interactions involving clustering sugar moieties at interfaces. Evidence is also presented for the strong interactions between sugar clusters in homogeneous aqueous solutions.

INTRODUCTION

Biological sugar-receptor interactions are often claimed to be multivalent. This led many investigators to prepare multiantennal sugar derivatives such as polymers and oligomers (ref. 1), surfactant aggregates (ref. 2), metal complexes (ref. 3), and dendrimers (ref. 4). In our workwe take advantage of a well-defined calix[4]resorcarene macrocyclic skeleton to assemble sugar moieties. An obvious merit of sugar clusters is that they allow multiple polar interactions, a common feature for the maintenance of structure and function of informative biopolymers such, as nucleic acids and proteins. An essential question here is to what extent otherwise weak polar or hydrogen-bonding interaction s of sugar moieties in water becomes efficient upon cluster modification.

Another characteristic of cyclophane-type macrocycles is their functioning as hydrophobic hosts. Sugar clusters may therefore form receptor•(sugar cluster)•guest ternary complexes via simultaneous host-guest complexation at the aromatic cavity and sugar-receptor interaction at the clustering sugar moieties. In this way, otherwise inert guest molecules may be delivered or transported to a particular sugar-receptor site by the action of a sugar cluster as a specific carrier. We report here that this is in fact the case.

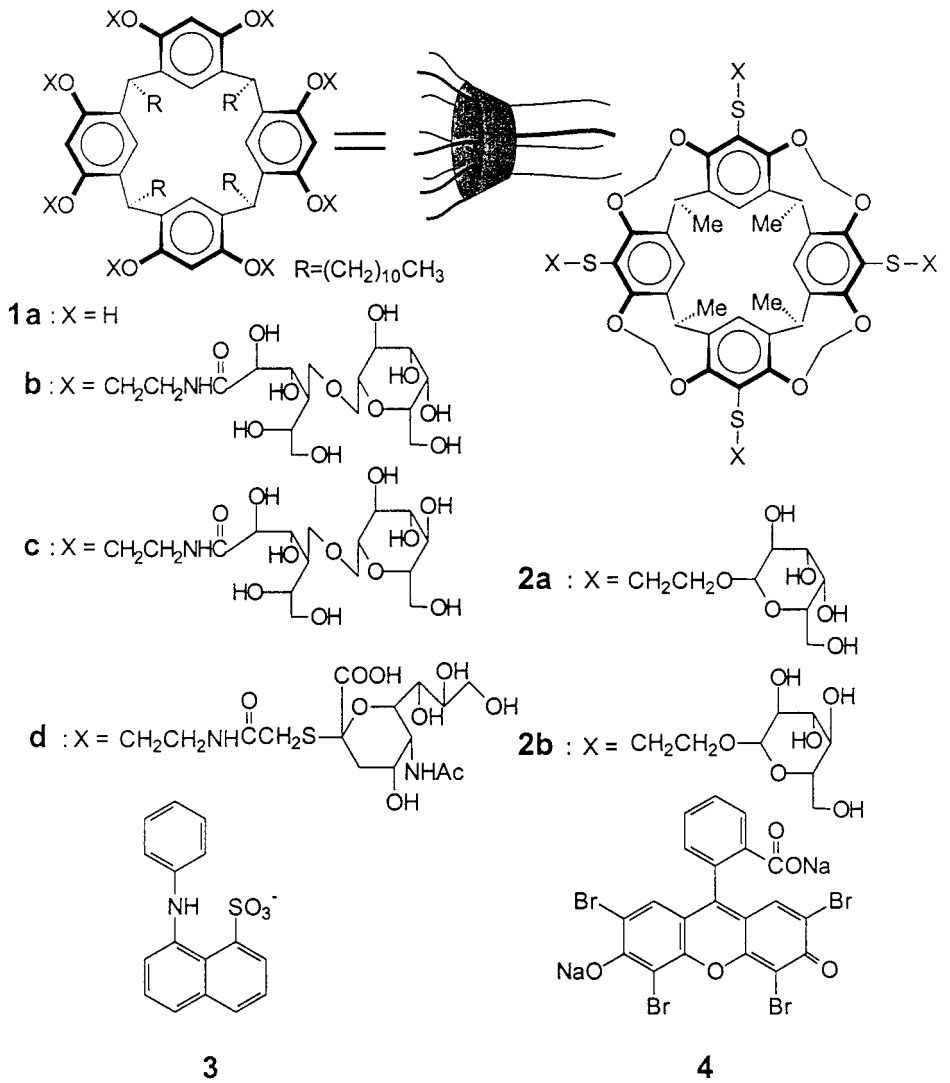
DELIVERY TO POLAR SOLID SURFACE

We have so far prepared two types of sugar cluster compounds which utilizie the calix[4]resorcarene skeleton. One is amide-linked octa(sugar) derivatives **1b-d**, where the sugar moieties are assembled on the eight OH groups of the parent macrocycle **1a**. The other is S-linked tetra(sugar) derivatives **2a** and **2b**, in which the sugar moieties are attached to the aromatic rings of the OH-protected macrocycle.

The octa(gaalactose) and octa(glucose) cluster compounds **1b** and **1c** can be obtained readily by reaction of the octa(amine) derivative of the parent macrocycle with lactonolactone or maltonolactone, respectively, in aqueous alcohol solution. They are highly soluble in water. Nevertheless, they are strongly adsorbed on a quartz surface from dilute aqueous solution (ref. 5). The absorbance data indicate that the galactose cluster **1b** forms a closely packed monolayer on the quartz surface. Absorbed compound **1b** cannot be desorbed easily by repeated washing with water. Desorption occurs only when rinsed with an aqueous amine solution with an efficiency which depends on the basicity of the amine used. This is taken as evidence that polar interaction of the hydrogen-bonding type provides the primary driving force for the adsorption of compound **1b** on a quartz surface. The adsorption is not inhibited at all by a large excess of the corresponding monosugar derivative; the

*Lecture presented at the 1st International Conference on Supramolecular Science and Technology, Zakopane, Poland, 27 September–3 October 1998.
Other presentations are published in this issue, pp. 2337–2408.

cyclic/linear or the octamer/monomer selectivity factor is ≥ 10^4. These results suggest a remarkable cluster effect on polar interactions involving sugar moieties in water (ref. 5).



Compounds **1b** and **1c** are also good hosts and form 1:1 complexes with various dye stuffs, such as ANS (**3**) and eosin Y (**4**), in water. The binding constants for host **1b** are $K_{\mathbf{1b}\cdot\mathbf{3}}$ = 2.2 x 10^5 M^{-1} and $K_{\mathbf{1b}\cdot\mathbf{4}}$ = 2.2 x 10^5 M^{-1} (ref. 6). Guest **3** alone in water shows little affinity for quartz. It is, however, efficiently adsorbed thereon in the presence of cluster host **1b**. Detailed analysis of the absorbance data indicate that complex **1b•3** is adsorbed in a similar manner as free host **1b**, resulting in a delivered or enforced coadsorption of guest **3** on the quartz surface, as schematically shown in Fig. 1 (ref. 5).

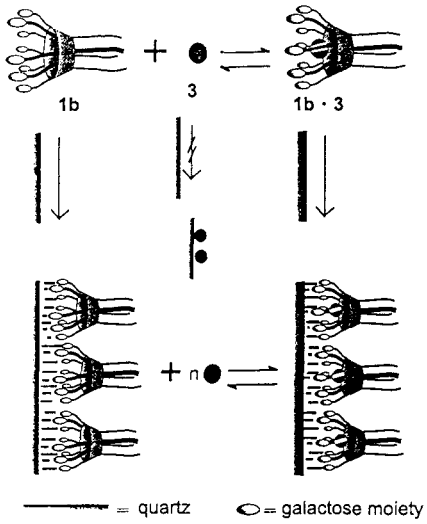


Fig. 1. Schematic representation of the ternary complexation between quartz, host **1b**, and guest **3**.

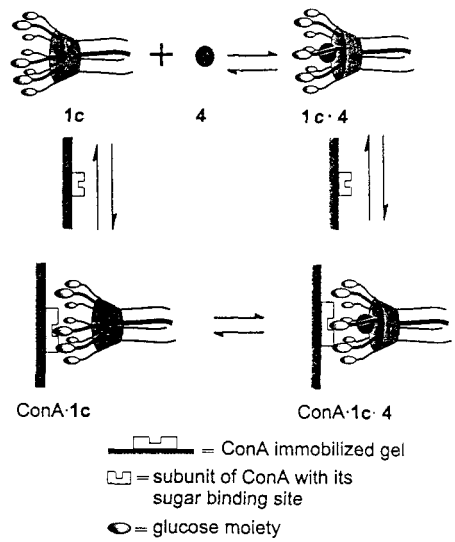


Fig. 2. Schematic representation of the ternary complexation between ConA, host **1c**, and guest **4**.

DELIVERY TO BIOLOGICAL SUGAR-RECEPTOR SITES

Lectines are a typical class of sugar binding proteins. As for other sugar-receptor interactions, the lectin-sugar complexations is very specific. Concanavalin A (ConA), for example, is specific for mannose and glucose, while peanut lectin (PNA) is specific for galactose. Both consist of four subunits, each of which contains a sugar-binding site. Consequently, they are cross-linked or aggregated (agglutinated) upon interaction with multiantennal sugar derivatives, as conveniently monitored by following the change in turbidity of the solution. Glucose and galactose clusters **1c** and **1b** specifically interact with ConA and PNA, respectively, as demonstrated in this way. The inhibitory effect of glucose and galactose on turbidity generation further convincing evidence for specific lectin-cluster interaction (ref. 7)

The specific **1c**-ConA interaction can also be demonstrated by the observation that glucose cluster **1c** in solution is strongly adsorbed on immobilized ConA gel and this process is competitively inhibited by glucose. Eosin Y (**4**) is a guest which is strongly bound to host **1c** with $K_{1c\cdot4} = 7.5 \times 10^5\ M^{-1}$ (ref. 6). In the absence of host, only weaky Eosin Y binds to ConA gel in a nonspecific manner. In the presence of host **1c**, it is again strongly coadsorbed on the gel in a specific manner as a result of ConA•**1c**•**4** ternary complexation. The entire scheme is shown in Fig. 2 (ref. 7). The respective binding constants have also been determined by a frontal chromatography technique.

Specific sugar-receptor interactions are believed to trigger a variety of cell events including infection by viruses and bacteria. The terminal sialic acid residues in cell-surface oligosaccharides are well known to serve as receptor sites for influenza viruses. A variety of synthetic poly(sialic acid) derivatives have been prepared and shown to strongly bind to influenza viruses (ref. 2); they are expected to block or inhibit undesired natural cell-virus interactions (infection). This is the basis of their application as a new type of antiviral glycodrug. We have also prepared a sialic acid cluster compound **1d** (ref. 8). It is again highly soluble in water and forms complexes with a variety of guestmilecules in water; K=2.1 x $10^4\ M^{-1}$ for rose bengal for example. Evidence for its strong binding to a sialic acid-specific lectin comes from SPR measurements (vide infra). Furthermore, it turns out to be an excellent virus-binder (ref. 9). Cluster host **1d** may not only block cell infection but also deliver included guest molecules as either drugs or probes to the viruses. This possibility is now under investigation in our laboratories.

POLAR INTERACTION OF CLUSTERING SUGARS AT INTERFACE AND IN SOLUTION

A general problem associated with assesing polar interactions in water is that spectroscopic methods, such as IR and ^{1}H NMR, which may be used to detect hydrogen-bonds in apolar organic media, are almost useless for aqueous media, where highly competitive water molecules participate in the hydrogen bonding. A unique technique for this purpose is surface plasmon resonance (SPR). 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 light intensity. Adsorption of substance(s) on the chip can thus be read out as a change in resonance [1000 resonance units (RU) corresponds to a surface concentration change of ~1 ng/mm^2)]

We have to demonstrated that galactose and glucose cluster compounds **1b** and **1c** with long alkyl chains can be irreversibly bound to a hydrophobic sensor chip consisting of an alkanethiol-coated thin gold film on a thin glass plate (ref. 10). The RU data obtained (1520 for **1b** and 1430 for **1c**) indicate that amphiphiles **1b** and **1c** 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, as schematically shown in Fig. 3.

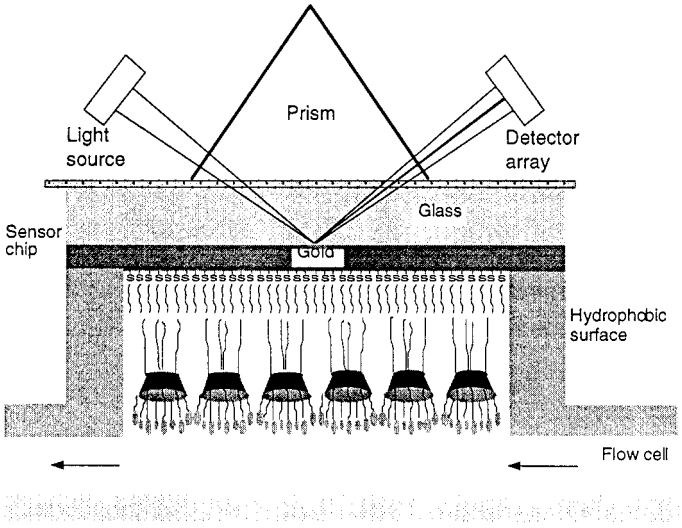


Fig. 3. Schematic representation of immobilization of sugar cluster **1b** or **1c** on a hydrophobic sensor chip.

TABLE 1 shows how lectins, polysaccharides, and water-soluble polymers interact with thus-formed monolayers of sugar clusters (ref. 10). Concanavaline A (ConA), a glucose- (and mannose-) binding lectin, is more strongly bound to immobilized **1c** (RU = 1120) than to **1b** (RU = 110). Peanut lectin (PNA), on the other hand, is a galactose-binding lectin, which is selectively bound to immobilized **1b** (RU =1760) than to **1c** (RU = 120). Thus, ConA and PNA recognize the terminal glucose and galactose residues of sugar clusters **1c** and **1b**, respectively, although there are alsomuch weaker nonspecific ConA-**1b** and PNA-**1c** interactions (RU ≅ 100). The specific interaction between sialic acid cluster **1d** and a sialic acid-specific lectin has also been demonstrated in this manner (ref. 8). Bovin albumin, an inert protein with no carbohydrate binding sites, show similar affinities again with RU ≅ 100 to both **1b** and **1c**.

Polysaccharides dextrin [neutral, RU = 300-400), chitosan (cationic, ~300), and condroitin (anionic, ~60)] are moderately to weakly bound to immobilized sugar clusters **1b** and **1c**. 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. PVA should be more hydrophilic than PEG. In this context, it must be polar interactions involving the OH groups and not a hydrophobic effect that 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 **1a** and **1b**

	Saturated RU changes / RU[c]	
Adsorbate[b]	**1b**	**1c**
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.

These results indicate that the monolayer assembly of sugar clusters exhibits potent binding abilities toward OH-functionalized polymers as well as lectins in water. Now the question is if the polymeric nature of the adsorbates is essential or not. The answer is likely no, since tetra(sugar) cluster compounds **2a** and **2b** are bound to the monolayer of octa(sugar) clusters **1b** and **1c** (ref. 11). Furthermore, there is selectivity here; the monolayer of octa(galactose) compound **1b** binds guests **2a** and **2b** more strongly than that of octa(glucose) derivative **1c** does. If guest **2a** or **2b** behaves as a single molecule, then what is the significance of the monolayer assembly of adsorbent **1b** and **1c** ? We may expect a similar interaction between isolated molecules **1b** or **1c** and **2** in an aqueous *solution*. This is in fact the case. Sugar clusters **1** and **2** form complexes in water, where again the galactose compound **1b** is the stronger binder as compared with the glucose counterpart **1c** (ref. 11). Galactose is slightly more hydrophobic than glucose and this aspect may come into play.

A single polar interaction cannot usualycompete with solvent water. However, multiple polar interactions in water are sizable. Nature utilizes this concept for maintenance of charakteristic structures and functions of nucleic acids and proteins. The present work demonstrates that this may also be the case of sugars.

CONCLUDING REMARKS

Except for complexation with boronic acids (ref. 12), molecular recognition of sugars has so far been mostly concerned with polar or hydrogen-bonding interactions in noncompetitive apolar organic media, a situation far from analogous to actual biological systems. While an isolated single sugar molecule may be too hydrophilic to be captured by polar interaction in water, sugar clusters manifest their remarkable capability of being recognized as polar entities by such interactions. They can be adsorbed on abiological polar solid surfaces, as well as on the biological sugar receptor sites. A variety of OH-functionalized molecules can in turn be adsorbed on the monolayer assembly of sugar clusters. Evidence is also presented for complexation between clustering sugar moieties in free aqueous solution. These observations are relevant to the fact that biological oligosaccharides occur as clusters. Synthetic macrocyclic sugar clusters may thus provide a new and unique strategy in rapidly developing glycoscience and -technology.

ACKNOWLEDGMENT

This work was supported by a grant-in-aid for COE Research "Design and Control of Advanced Molecular Assembly System" (no. 08CE2005) from the Ministry of Education, Science, and Culture of the Japanese Government and also by CREST (Core Research for Evolutional Science and Technology) of the Japan Science and Technology Corporation.

REFERENCES

1. A. Spaltenstein, G. M. Whitesides, *J. Am. Chem. Soc.*, 113, 686 (1991)
2. J. E. Kingery-Wood, K. W. Williams, G. B. Sigal, G. M. Whitesides. *J. Am. Chem. Soc.*, 114, 7303 (1992).
3. S. Sasaki, T. Sasaki. *J. Am. Chem. Soc.*, 116, 1587 (1994).
4. K. Aoi, K. Itoh, M. Okada. *Macromolecules*, 28, 5391 (1995).
5. T. Fujimoto, T. Shimizu, O. Hayashida, Y. Aoyama. *J. Am. Chem. Soc.*, 119, 6676 (1997).
6. T. Fujimoto, T. Shimizu, O. Hayashida, Y. Aoyama. *Gazzetta Chim. Ital.*, 127, 749 (1997).
7. T. Fujimoto, T. Shimizu, O. Hayashida, Y. Aoyama. *J. Am. Chem. Soc.*, 120, 601 (1998).
8. K. Fujimoto, Y. Aoyama, unpublished results.
9. K. Fujimoto, Y. Aoyama, Y. Suzuki, unpublished results.
10. O. Hayashida, C. Shimizu, T. Fujimoto, Y. Aoyama. *Chem. Lett.*, 13-14 (1998).
11. O. Hayashida, Y. Aoyama, unpublished results.
12. Y. Shiomi, M. Saisho, T. Harada, S. Shinkai. *Tetrahedron*, 48, 8239 (1992).