Saccharide-Directed Cell Recognition and Molecular Delivery Using Macrocyclic Saccharide Clusters: Masking of Hydrophobicity to Enhance the Saccharide Specificity

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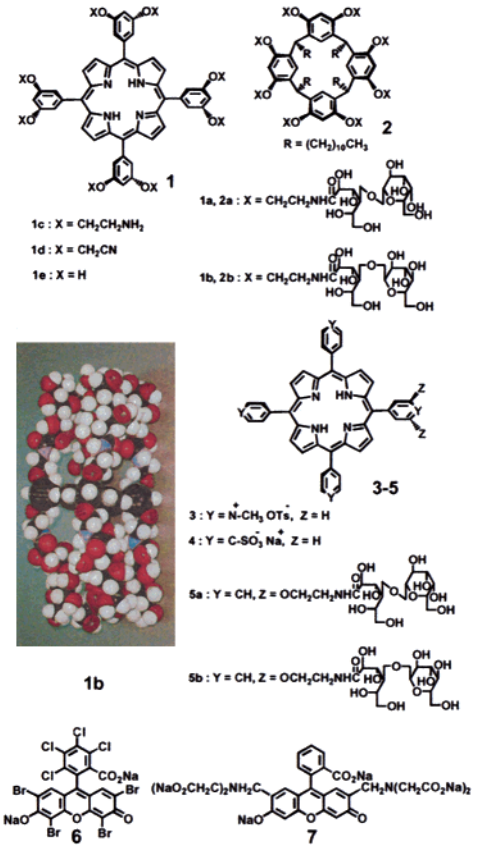
Oligosaccharides play essential roles in various cellular activities as antigens, growth signals, targets of bacterial/viral infection, and glues in cell adhesion and cancer transfer,[1] where the saccharide-receptor interactions are usually specific and multivalent.[2] This specificity suggests a potential utility of synthetic, mostly polymeric, multiantennary saccharide derivatives as carriers in directed drug delivery[3] and as blockers or inhibitors of undesired saccharide-receptor associations.[4] However, saccharide-receptor interactions are by no means the sole access routes to the cells. The cells, especially tumor cells, show nonspecific affinities to hydrophobic molecules. This is the basis of photodynamic therapy of tumors by the use of porphyrin and related sensitizers.[5] For the saccharide-directed cell recognition, however, this is a problem to be overcome. We thought a key solution would be to mask the hydrophobicity upon increase in saccharide multivalency. The present work is concerned with the use of highly saccharide-functionalized porphyrin[6] and calix[4]resorcarene[7] derivatives for the capture of and molecular delivery to hepatocytes (liver cells), which are well-known to have receptors for the terminal galactose residues of asialoglycoproteins.[8,9] We report here that these saccharide-coated macrocycles with masked hydrophobicity exhibit a remarkable saccharide (galactoside/glucoside) specificity.

Chart 1



In a similar manner as for the calix[4]resorcarene analogues **2a** and **2b**,[7,10] amide-linked octa(galactose) and octa(glucose) derivatives of tetraphenylporphyrin **1a** and **1b** (Chart 1) were obtained by the reactions of lactonolactone or maltonolactone with octaamine **1c** derived from octaol **1e**[11] via octanitrile **1d**.[12] These porphyrin glycoconjugates are fluorescent as expected and are remarkably water-soluble. Their interactions with rat hepatoma (liver cancer) cells (RLC-16)[13] were investigated by fluorescence microscopy. Figure 1a (for **1a**) and Figure 1b (for **1b**) clearly show that galactose cluster **1a** is captured by the cells, while glucose cluster **1b** is not.

Control experiments indicate that simple water soluble nonsaccharide cationic (pyridinium) and anionic (sulfonate) porphyrins **3** and **4** as well as scarcely water soluble di(galactose) and di(glucose) derivatives **5a** and **5b** are all bound to the cells under similar conditions (Figure 1c for **4** and 1d for **5b**). The nonspecific adsorption on the cells of relatively hydrophobic reference porphyrins **3**−**5** with an exposed porphyrin plane is undoubtedly driven by hydrophobic forces.[5,6d−f] The lack of cell affinity of

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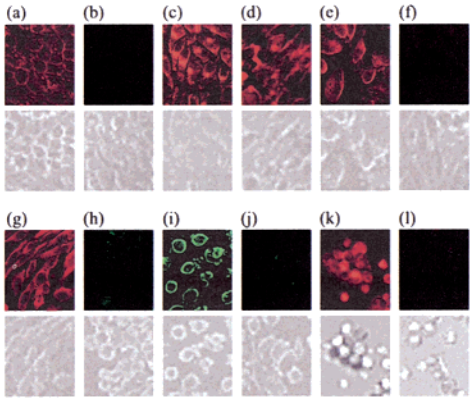

Figure 1. Microphotographs (bottom) and their fluorescence images (top) of rat hepatoma RLC-16 cells (a−j) or mouse spleen LT4Tr cells (k and l) after incubation with an aqueous solution (pH 7.3 with PBS) of (a) **1a** (0.25 mM), (b) **1b** (0.25 mM), (c) **4** (0.25 mM), (d) **5b** (0.25 mM), (e) **2a** (2.5 mM) + **6** (1 mM), (f) **2b** (2.5 mM) + **6** (1 mM), (g) **6** (1 mM), (h) **7** (5 mM), (i) **2a** (15 mM) + **7** (5 mM), (j) **2b** (15 mM) + **7** (5 mM), (k) **6** (1 mM), (l) **2a** (2.5 mM) + **6** (1 mM) at 37 °C, followed by washing twice with the PBS buffer. The incubation time was 30 min (a−d), 1 min (e−g, k, and l), or 3 min (h−j), depending on the fluorophores. The cells were obtained from RIKEN Cell Bank and cultivated according to literature methods.

the glucose cluster **1b** must therefore be a result of hydrophobicity masking or steric inaccessibility to the porphyrin ring, both sides of which are in fact protected by the clustering saccharide moieties as shown in Chart 1.[14] In this context, there is little doubt that the adsorption of galactose cluster **1a** on the cells is *solely* due to specific interaction of the galactose residues in **1a** and the galactoside receptor sites on the cell membranes.

We then moved to the calix[4]resorcarene-based octa(galactose) and octa(glucose) clusters **2a** and **2b**. While they are highly hydrophilic, they form 1:1 complexes with a variety of hydrophobic dyes.[7,10] We used here a fluorescent dye phloxine B (**6**, Chart 1). The isosbestic spectral change with a saturation behavior (Figure 2 for **2a**), coupled with continuous-variation Job analysis (not shown), indicates a 1:1 host−guest complexation with $K_{2a}(\mathbf{6}) = 2.1 \times 10^5\ M^{-1}$ and $K_{2b}(\mathbf{6}) = 2.0 \times 10^5\ M^{-1}$ (pH 7.3 with PBS at 25 °C) being evaluated from least-squares curve fitting. Fluorescence microscopy now can be used to monitor the adsorption of fluorescent guest **6** on the hepatoma cells, as controlled by nonfluorescent saccharide cluster hosts.

Here is again a marked contrast between galactose cluster **2a** and glucose cluster **2b**, which, respectively, lead to guest-on and guest-off (Figure 1e and 1f). In the absence of any host, guest **6** is, not surprisingly, bound to the cells (Figure 1g) in a manner similar to that of reference porphyrins **3**−**5**. This spontaneous guest adsorption is almost completely suppressed by glucose cluster **2b** (Figure 1f) at [**2b**] = 2.5 mM ([**6**] = 1.0 mM), where ∼100% (judging from $K_{2b}(\mathbf{6})$) of the guest is bound to the host in solution; the resulting complex **2b**·**6** must be inert to the cells. With the galactose analogue **2a** under similar conditions, the guest finds itself on the cells (Figure 1e). This is most likely a result of host−guest-cell ternary complexation mediated by the dual roles of the galactose cluster host **2a** acting as a hydrophobic guest

(14) The CPK model in Chart 1 is only schematic. Octa(saccharide) clusters **1** and **2** in water are actually highly aggregated to give extensive ^{1}H NMR line broadening. Details will be reported shortly.

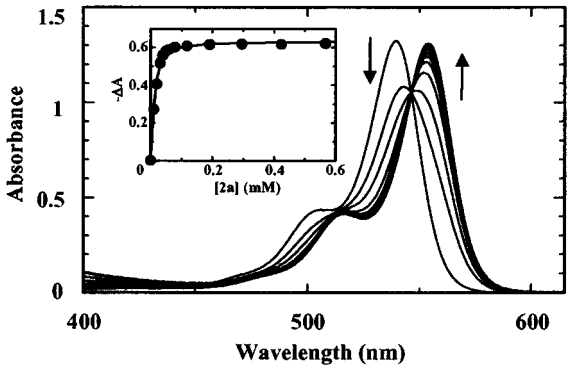

Figure 2. Electronic spectra for a series of solutions of guest **6** (1.45×10^{-2} mM) and varying amounts of host **2a** ($0-5.69 \times 10^{-1}$ mM) in water (pH 7.3 with PBS) at 25 °C. Inset: change in absorbance at 540 nm with increasing host concentrations.

binder (note that $K_{2a}(\mathbf{6}) \cong K_{2b}(\mathbf{6})$) as well as a specific oligosaccharide cell binder.

The significance of host−guest coadsorption or delivery of included guest molecules becomes clearer when a less hydrophobic guest is used. Calcein (**7**) is a pentacarboxylate fluorescent dye, which is also bound to the present hosts in a 1:1 manner with good spectral behaviors; the binding affinities of $K_{2a}(\mathbf{7}) = 8.2 \times 10^2\ M^{-1}$ and $K_{2b}(\mathbf{7}) = 1.6 \times 10^3\ M^{-1}$ are much lower than those for guest **6** in the order of $10^5\ M^{-1}$. When alone, guest **7** shows little affinity to the hepatoma cells (Figure 1h). However, it can be *delivered* to the cells by the galactose cluster **2a** (Figure 1i) as a transporter under conditions ([**2a**] = 15 mM and [**7**] = 5 mM) where 90% of the guest would otherwise be bound to the host in solution. This is not the case with the glucose analogue **2b** (Figure 1j). The **2a**-mediated guest delivery is also cell-specific. When mouse spleen LT4Tr cells[15] lacking in characteristic galactoside receptors are used in place of hepatoma cells, the galactose cluster **2a** inhibits the otherwise ready adsorption (Figure 1k) of guest **6** on the cells (Figure 1l).

This work demonstrates the importance of hydrophobicity masking for the saccharide-directed cell recognition. The macrocyclic saccharide clusters **1** and **2** are electrically neutral and highly hydrophilic, where neither the hydrophobic nor the electrostatic force for nonspecific incorporation into the cells works effectively. Under these circumstances, the identity of the saccharide moieties plays a crucial role; the right one (galactoside) undergoes specific saccharide receptor interactions with the right (hepatic) cells, while the wrong one (glucoside) is completely rejected by the cells. The included guest molecules are thereby either delivered to the target cells or protected in solution away from the cells. Since the saccharide-receptor interactions are ubiquitous, well-defined/well-designed synthetic saccharide clusters of the present type may serve as a new tool in glycoscience and glycotechnology of cell communication.

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