Ternary Complexation Involving Protein. Molecular Transport to Saccharide-Binding Proteins Using Macrocyclic Saccharide Cluster as Specific Transporter

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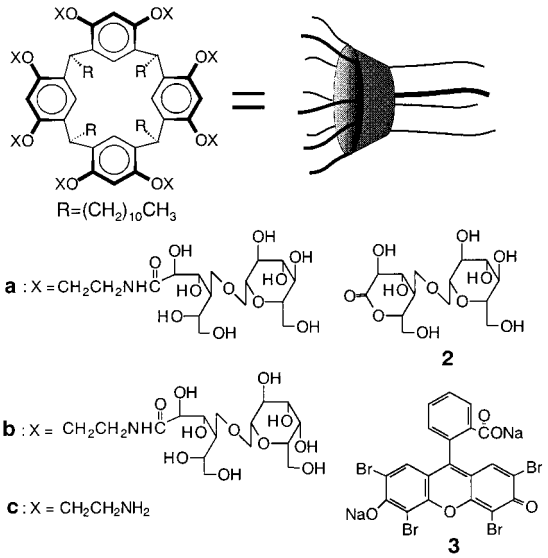
A variety of cellular events are triggered by specific saccharide−receptor interactions,[1] which are often claimed to be multivalent.[2] We have recently reported that macrocyclic saccharide cluster **1b** *in water* can be adsorbed, together with included guest molecules, on the silica surface via multiple hydrogen bonding.[3] The present work is concerned with saccharide−receptor proteins, lectins, as targets. We report here that the saccharide cluster host mediates a novel ternary complexation, thereby transporting the included guest molecules to the lectin.



Compound **1b** with terminal galactose residues was prepared by the reaction of octaamine **1c** with lactonolactone.[3] A similar reaction with maltonolactone (**2**) afforded cluster compound **1a** having terminal glucose moieties.[4] Concanavalin A (ConA)[5] and peanut lectin (PNA)[6] are well-studied glucoside- (and mannoside-) and galactoside-binding lectins, respectively, which are similar in size (∼100 kD) and are composed of four subunits, each having a saccharide-binding site. Consequently, they are cross-linked or aggregated (agglutinated) upon interaction with multiantennal saccharide derivatives, as conveniently monitored by following the turbidity of the solution.[7] Figure 1 shows that a solution[8] of ConA (1.88 μM), but never PNA, becomes turbid with compound **1a** (18.9 μM); deaggregation (deagglutination) occurs upon addition (shown by an arrow a in the figure) of a large excess amount (8.30 mM) of glucose as a competitive inhibitor. In a similar manner, a solution[8] of PNA, but never ConA, is agglutinated with compound **1b**; deagglutination occurs only with galactose in a large excess (arrow b). These results indicate that lectins ConA and PNA specifically interact with the glucose and galactose clusters **1a** and **1b**, respectively, although concurrent precipitation of the resulting adducts precludes determination of the binding constants.

The specific binding of glucose cluster **1a** with ConA ($K_{\mathrm{ConA/1a}} = 1 \times 10^6\ \mathrm{M}^{-1}$, vide infra) can also be directly shown by using an immobilized ConA-Sepharose gel (Pharmacia, containing 10−16 or ∼13 mg of ConA per 1 mL of drained gel) and by monitoring the absorbance change at 283 nm for **1a** in the aqueous phase. The results are graphically shown in Figure 2 (entries 1−6), where percent adsorption of compound **1** on the gel is shown by open bars. Upon addition of 0.25 mL of the gel (containing ∼0.12 μmol (subunit basis) of ConA) to an aqueous solution[8] of **1a** (23.5 μM, 4.25 mL, containing 0.10 μmol of **1a**), 73% of **1a** is adsorbed on the gel (entry 1). The adsorption is suppressed to 15% in the presence of glucose in a large excess (0.13 M, 0.56 mmol) (entry 2). The affinity of the galactose cluster **1b** is even lower (6−8%) and is not affected by glucose (entries 3 and 4). Since adsorption of a similar range (8−9%) occurs for both **1a** and **1b** when ConA-free Sepharose is used (entries 5 and 6), the matrix Sepharose in ConA-Sepharose must provide a principal site of adsorption of **1b** (entries 3 and 4) as well as **1a** when the ConA site is poisoned by glucose (entry 2).

Compounds **1a** and **1b** function as excellent hosts for various guests in homogeneous aqueous solutions. For example, they form a stable 1:1 (from Job plot) complex with eosin Y (**3**), which thereby undergoes a red-shift by 13−14 nm in λ_{max}.[9] Spectrophotometric titration shows an excellent isosbestic point at 521 nm; the binding constants are $K_{\mathbf{1a/3}} = 7.5 \times 10^5\ \mathrm{M}^{-1}$ and $K_{\mathbf{1b/3}} = 1.8 \times 10^5\ \mathrm{M}^{-1}$ for **1a** and **1b**, respectively.[8]

The adsorption behavior of the gel/host/guest ternary system is readily evaluated by analyzing the change in absorption spectra for the aqueous phase upon addition of the gel.[10] The results are shown by open bars for the host and filled bars for the guest in Figure 2 (entries 7−12).[11] The host−guest complexation is 77% for an equimolar solution[8] of the glucose host **1a** and guest **3** (23.5 μM, 4.25 mL, containing 0.10 μmol of both host and guest). Addition of the lectin gel (0.25 mL, containing ∼0.12 μmol (subunit basis) of ConA) to this solution results in adsorption of 92% of the host and 78% of the guest (entry 7). In the presence of glucose (0.13 M, 0.56 mmol), adsorption is suppressed to similar extents for both the host (17%) and the guest (15%) (entry 8). The enhanced adsorption (entry 7) and glucose-induced desorption (entry 8) of host **1a** and guest **3** are thus coupled. When the galactose host **1b** is used, the adsorption behavior, irrespective of the absence (entry 9) or presence (entry 10) of glucose, is very

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(4) Compound **1b** was fully characterized by spectroscopy (IR, ^{1}H and ^{13}C NMR, and TOF-MS) and elemental analysis.
(5) Williams, T. J.; Plessas, N. R.; Goldstein, I. J.; Lönngren, J. *Arch. Biochem. Biophys*. **1979**, *195*, 145−151.
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(8) At pH 7.2 (0.01 M phosphate) with [NaCl] = 0.5 M, [$MnCl_2$] = 0.1 mM, and [$CaCl_2$] = 0.1 mM at 25 °C.
(9) λ_{max} (ϵ) = 517 nm (8.0×10^4) for **3**, 531 nm (7.6×10^4) for **1a·3**, and 530 nm (7.4×10^4) for **1b·3**.
(10) The concentrations of free host **1**, free guest **3**, and complex **1·3** are readily known from the absorbance at the isosbestic point (521 nm) for the total guest concentration ([**3**] + [**1·3**]), shape analysis of the spectrum for the complexed-to-free guest ratio ([**1·3**]/[**3**]), and the solution equilibrium (K = [**1·3**]/[**1**][**3**]) for [**1**]).
(11) The adsorption percentages shown in Figure 2 are "equilibrium" values, independent of the order of addition of the components.

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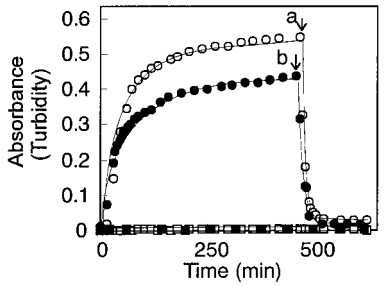

Figure 1. Time courses of the absorbance (turbidity) change at 25 °C for a solution of **1a** (18.9 μM) and ConA (1.88 μM) (○), **1a** (18.9 μM) and PNA (1.88 μM) (□), **1b** (53.0 μM) and PNA (3.83 μM) (●), and **1b** (53.0 μM) and ConA (3.83 μM) (■) in water.[8] Arrows a and b indicate addition of glucose (8.30 mM) and galactose (8.48 mM), respectively.

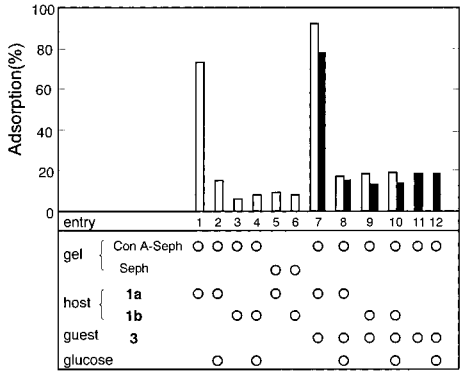

Figure 2. Adsorption percentages of host **1a** or **1b** (open bars) and guest 3 (filled bars) on the gel (ConA-Sepharose (ConA-Seph) or ConA-free Sepharose (Seph)) after shaking for 1 h at 25 °C of a mixture of 0.25 mL of the gel (~0.12 μmol (subunit basis) of ConA in case of ConA-Seph) and 4.25 mL of an aqueous solution[8] of host (23.5 μM, 0.10 μmol) and/or guest (23.5 μM, 0.10 μmol) in the presence (0.13 M, 0.56 mmol) or absence of glucose.

similar to that in entry 8, i.e., with glucose host **1a** on the glucose-poisoned gel. In the absence of any host, 18% of guest **3** is adsorbed (entry 11), while glucose has again no effect (entry 12).

Clearly, there are nonspecific and glucose-insensitive host−gel and guest−gel interactions. A small fraction (10−20%) of host and guest is adsorbed in this manner when the host is **1b** or when the ConA sites on the gel are blocked by glucose as a competitive inhibitor. In its absence, there is a specific ConA−**1a** interaction (entries 1 and 7), by which host **1a** not only as such ($K_{\mathrm{ConA/1a}} = 1 \times 10^6$ M^{-1} in Scheme 1, as compared with $K = 4.5 \times 10^3$ M^{-1} for methyl α-glucopyranoside[12]) but also slightly more strongly as its complex ($K_{\mathrm{ConA/1a\cdot3}} = 2 \times 10^6$ M^{-1}) is bound to the saccharide-binding site of ConA. In a similar manner, guest

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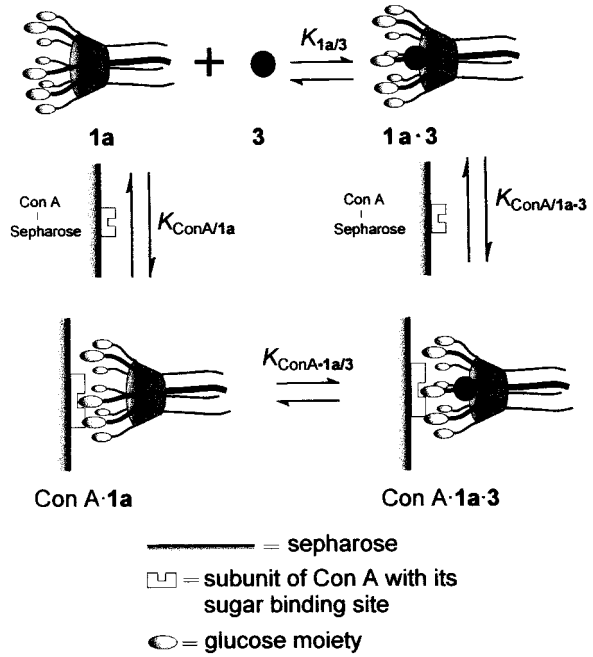
Scheme 1



3 in solution shows a slightly higher affinity to immobilized host ConA·**1a** ($K_{\mathrm{ConA\cdot1a/3}} = 2 \times 10^6$ M^{-1}) than to **1a** in solution ($K_{\mathrm{1a/3}} = 7.5 \times 10^5$ M^{-1}). The respective binding constants for ConA species were obtained from the isocratic elution (frontal chromatography)[13] experiments.[14]

To summarize, the macrocyclic glucose cluster host **1a** mediates otherwise inert protein−guest complexation. Delivery of probes or drugs as guests to various biological saccharide−receptor sites is one of future concerns of this work.

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(13) Ohyama, Y.; Kasai, K.; Nomoto, H.; Inoue, Y. *J. Biol. Chem.* **1985**, *11*, 6882−6887.

(14) Definitions are $K_{\mathrm{ConA/1a}}$ = (ConA·**1a**)/(ConA)[**1a**], $K_{\mathrm{ConA/1a\cdot3}}$ = (ConA·**1a·3**)/(ConA)[**1a·3**], and $K_{\mathrm{ConA\cdot1a/3}}$ = (ConA·**1a·3**)/(ConA·**1a**)[**3**]. At equilibrium with an equimolar (1.0 μM) solution of host **1a** and guest **3**,[8] the gel containing 0.40 μmol of ConA adsorbs 0.22 μmol of the host (ConA·**1a** + ConA·**1a·3**) and 0.11 μmol of the guest (ConA·**1a·3**). The equilibrium composition (Scheme 1) is [**1a**] = [**3**] = 0.66 μM and [**1a·3**] = 0.34 μM ([**1a**] + [**3**] + [**1a·3**] = 1.0 μM and $K_{\mathrm{1a/3}}$ = [**1a·3**]/[**1a**][**3**] = 7.5×10^5 M^{-1}) and (ConA) = 0.18 μmol, (ConA·**1a**) = 0.11 μmol, and (ConA·**1a·3**) = 0.11 μmol ((ConA) + (ConA·**1a**) + (ConA·**1a·3**) = 0.40 μmol). Elution with a 1.0 μM **1a** solution[8] results in adsorption of 0.24 μmol of **1a** on the gel; [**1a**] = 1.0 μM, (ConA) = 0.16 μmol, and (ConA·**1a**) = 0.24 μmol. Guest **3** or galactose cluster **1b** (1.0 μM), on the other hand, is hardly adsorbed (<10^{-3} μmol), leading to an upper limit of $K_{\mathrm{ConA/3}} < 3 \times 10^3$ M^{-1} and $K_{\mathrm{ConA/1b}} < 3 \times 10^3$ M^{-1}.