New Supramolecular Approach for Saccharide-directed Chemical Modification of Concanavalin A

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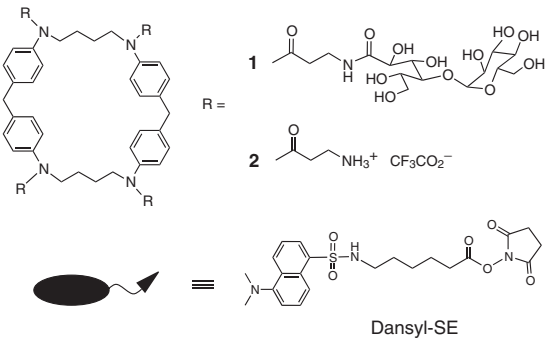
A new saccharide-directed chemical modification method toward a protein surface was developed. Hydrophobic guests such as Dansyl-SE were effectively incorporated by the cyclophane (**1**) having terminal glucose moieties. The amine-coupling fluorescent dye was carried from the aqueous phase to the glucoside-binding site of Con A by **1** and reacted at the proximal binding site. The modified Con A acts as a fluorescent saccharide biosensor.

Currently, much attention has been focused on the development of site-selective chemical modification methods on protein surfaces for novel engineered proteins.[1] We describe here a new supramolecular modification method for a protein surface using a macrocyclic carrier. A tetramaltose derivative of cyclophane (**1**) composed of tetraaza[6.1.6.1]paracyclophane and four branches with a terminal glucose group was designed. The cyclophane moiety provides a cavity for guest-binding, while terminal glucoside moieties act as a tag directed toward carbohydrate-binding proteins[2] (lectins). The guest-binding ability of **1** for hydrophobic guests such as amine-reactive fluorescent dye and its saccharide-directed chemical modification toward a lectin were examined from a viewpoint of fluorescent biosensor.



Cyclophane with terminal glucose groups **1**[3] was prepared by aminolysis of maltonolactone[4] with tetra-ammonium derivative **2**[5] in the presence of triethylamine. Cyclophane **1** is soluble in neutral aqueous media and behaves as a water-soluble host toward hydrophobic guest molecules. The guest-binding behavior of **1** for various hydrophobic guests such as 6-(5-dimethylaminonaphthalene-1-sulfonylamino)hexanoic acid succinimidyl ester (Dansyl-SE), 6-*p*-toluidinonaphthalene-2-sulfonate (TNS), and 8-anilinonaphthalene-1-sufonate (ANS) was examined by fluorescence spectroscopy in an aqueous HEPES buffer (0.01 mol dm^{-3}, pH 7.0, μ 0.10 with KCl) at 298 K. Upon addition of **1** to aqueous solutions containing each of the guests, fluorescence intensity originated from the guest molecules was increased along with a concomitant blue shift of the fluorescence maximum, showing typical saturation behavior (Figure 1 for TNS). Job plots applied to the host–guest complexation revealed 1:1 host:guest stoichiometry for the complexes. The binding constants (K) of **1** for the guests were evaluated on the basis of Benesi–Hildebrand relationship: K, 1.2×10^4, 3.1×10^3, and 1.0×10^3 dm^3 mol^{-1} for complexation with Dansyl-SE, TNS, and ANS, respectively. The spectroscopic evidences strongly indicate that the guest is incorporated into **1** through hydrophobic interaction, as schematically shown in Scheme 1.

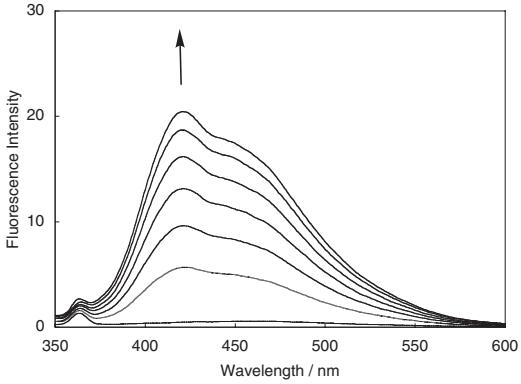
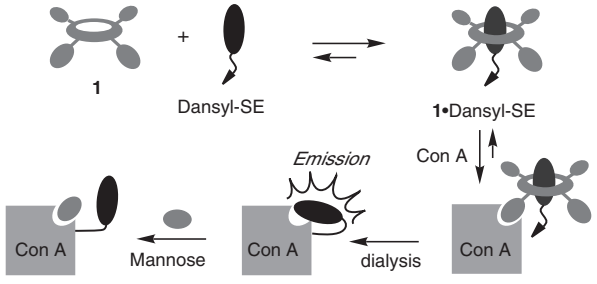


Figure 1. Fluorescence spectral changes for aqueous solution of TNS (1.0 μmol dm^{-3}) upon addition of **1** in HEPES buffer. [**1**] = 0, 33, 67, 100, 133, 167, and 200 μmol dm^{-3} (from bottom to top).

Concanavalin A (Con A),[2] a glucoside- and mannoside-binding lectin, and Dansyl-SE, an amine-reactive fluorescent dye for protein labeling,[6] were used in a proof of principle experiment. It is expected that Dansyl-SE reacts randomly with amino residues of Con A surface in the absence of host **1**, but was effectively approached to saccharide-binding site of Con A to react with an ε-amino group of lysine residues (e.g. K101)[2] located near the binding site in the presence of host **1**, as shown in Scheme 1. Con A (0.1 mmol dm^{-3} (subunit basis))



Scheme 1. Schematic representation for saccharide-directed chemical modification of Con A surface.

was added to an aqueous HEPES buffer of **1** (0.5 mmol dm^{-3}) and Dansyl-SE[7] (0.1 mmol dm^{-3}), and the mixture was stirred for 2 h at 278 K. After dialysis (5 *k*Da cut-off) for 1 day, MALDI-TOF MS spectroscopy clearly showed that the dansyl group was covalently bound to Con A (m/z 25930: Dansyl-labeled Con A). Sensing behavior of the resulting Dansyl-labeled Con A for monosaccharides such as mannose, glucose, and galactose was evaluated from the fluorescence spectroscopy. Upon addition of mannose to an aqueous solution containing the resulting Dansyl-labeled Con A, the fluorescence intensity originated from the dansyl decreased with saturation behavior (Figure 2), which is similar to that in our previous reports.[8]

The lessened emission intensity which is sensitive to a microenvironmental polarity,[9] indicates that the dansyl group which was originally located in the near hydrophobic saccharide binding sites was turned out and exposed to bulk aqueous phase upon mannose-binding, as schematically shown in Scheme 1. Similar spectroscopic behavior was observed for glucose, but the fluorescence intensity did not change for galactose (Figure 2). The binding affinity[2] of Con A was clearly retained (Man (410 dm^3 mol^{-1}) > Glc (80 dm^3 mol^{-1}) ≫ Gal (ca. 0 dm^3 mol^{-1})). It is noteworthy that randomly labeled Con A, which was prepared in the absence of **1**, did not show detectable changes in fluorescence spectra when these monosaccharides were added. In addition, negligible fluorescent change was observed using the labeled Con A which was prepared under the former reaction conditions, but in the presence of a large excess of mannose as a competitive inhibitor. These results suggest that dansyl group was attached to the proximity of the saccharide-binding site of Con A by direction of the macrocyclic carrier **1**.[10]

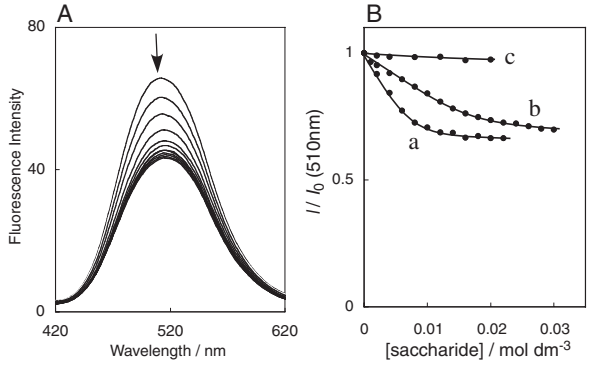


Figure 2. (A) Fluorescence spectral changes for dansyl-labeled Con A on addition of mannose in HEPES buffer. [mannose] = 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 mmol dm^{-3} (from top to bottom). (B) Titration profiles obtained adding mannose (a), glucose (b), and galactose (c) to a solution of dansyl-labeled Con A.

In conclusion, the present study demonstrates a new convenient strategy for the active site-directed chemical modification of a lectin surface by using a saccharide-branched macrocyclic host. Cyclophanes with other saccharides are expected to be utilized as a mediator/transporter toward lectins, which produce many sorts of saccharide biosensors with distinct selectivity. Further studies are currently in progress along this line.

References and Notes

1 S. Gordon and S. Rabinowitz, *Adv. Drug. Delivery Rev.*, **4**, 27 (1989).
2 T. K. Dam and C. F. Brewer, *Chem. Rev.*, **102**, 387 (2002).
3 600 MHz ^{1}H NMR (DMSO-d_6, 363 K) δ 1.3 (NCH_2CH_2), 2.1 ($NCOCH_2$), 3.2 ($NCOCH_2CH_2$), 3.5 (NCH_2CH_2), 3.95 ($ArCH_2Ar$), 3.1-5.1 (carbohydrate), 7.1 (ArH(ortho)), 7.3 (ArH(meta)), 7.4 (NH). MS (FAB) m/z 2151 $(M + H)^+$. Found: C, 51.61; H, 6.69; N, 5.34%. Calcd for $C_{94}H_{140}N_8O_{48}\cdot 2H_2O$: C, 51.64; H, 6.64; N, 5.13%.
4 O. Hayashida, K. Mizuki, K. Akagi, A. Matsuo, T. Kanamori, T. Nakai, S. Sando, and Y. Aoyama, *J. Am. Chem. Soc.*, **125**, 594 (2003).
5 O. Hayashida and I. Hamachi, *Chem. Lett.*, **32**, 288 (2003).
6 C. M. Yengo, L. R. Chrin, and C. L. Berger, *J. Struct. Biol.*, **131**, 187 (2000).
7 The host–guest complexation was estimated to be 85% for Dansyl-SE under such conditions.
8 a) I. Hamachi, T. Nagase, and S. Shinkai, *J. Am. Chem. Soc.*, **122**, 12065 (2000). b) T. Nagase, S. Shinkai, and I. Hamachi, *Chem. Commun.*, **2001**, 229. c) T. Nagase, E. Nakata, S. Shinkai, and I. Hamachi, *Chem.—Eur. J.*, in press.
9 M. Onodera and K. Yagi, *Biochem. Biophys. Acta*, **253**, 254 (1971).
10 Digestion of the dansyl-labeled Con A by trypsin coupled with MALDI-TOF MS experiments suggested that ε-amino group of K101 was modified with dansyl-SE.

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