A General Semisynthetic Method for Fluorescent Saccharide-Biosensors Based on a Lectin

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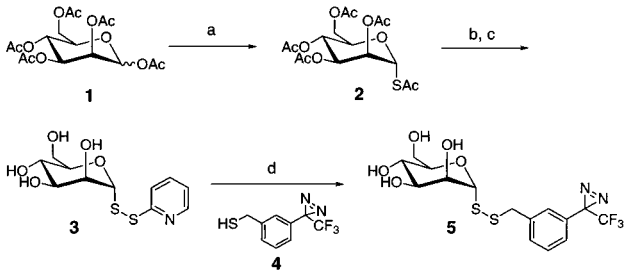
Recent advances in glycoscience have revealed that many saccharides play crucial roles in biological phenomena. Therefore, it is desired to measure the concentrations of various saccharides for clinical use as well as for the development of glycobiochemistry. Although electrochemistry-based amperometric sensors for glucose are now widely used for diabetes, an optical sensing system for other saccharides is expected to be more flexible and convenient. The fluorescent biosensor is one of the ideal systems to monitor ions and molecules of biological importance both in vivo and in vitro,[1] while chemosensors based on small organic molecules are now actively investigated.[2] Since a protein framework can provide a highly selective molecular recognition site, rational coupling of a signal transduction device with the molecular recognition event is an essential key to construct sophisticated biosensors. In contrast to small synthetic molecules, however, there are limited methodologies for the rational manipulation of biomacromolecules, most of which are based on genetic protein engineering or total- or semisynthesis of proteins.[3]

We propose herein a new method (post-photoaffinity labeling modification) to construct a fluorescent saccharide-biosensor based on a naturally occurring saccharide-binding protein, a lectin (concanavalin A: Con A).[4] The active site-directed incorporation of a potentially reactive group is conducted using photoaffinity labeling, followed by chemical modification to yield a fluorescent Con A. The emission intensity and maximum of the fluorophore attached to the proximity of the sugar-binding site of Con A are sensitively changed by specific saccharides.

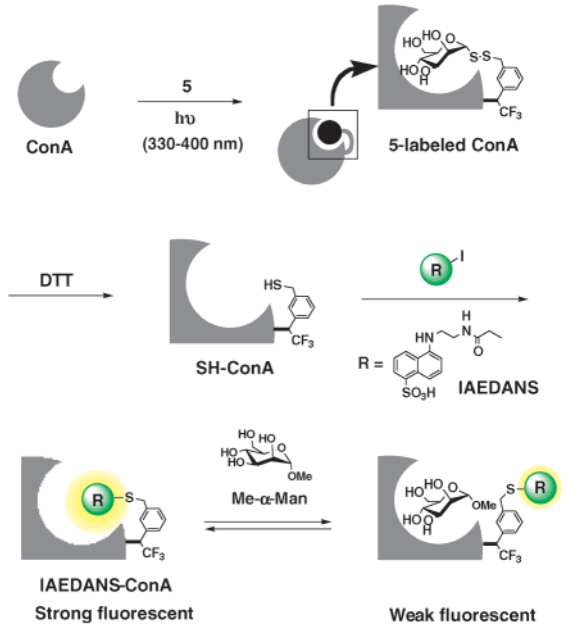
The photoaffinity labeling reagent (**5**), which bears a dithiomannoside group as an affinity ligand and 3-(trifluoromethyl)-3-phenyldiazirine unit[5] as a labeling moiety, was designed and synthesized as outlined in Scheme 1. The glycosylation of peracetyl-D-mannose **1** with thioacetic acid gave peracetyl-α-D-1-acetylthiomannoside **2**.[6] The deacetylation of **2** by LiOH followed by the reaction with 2,2′-dithiodipyridine gave pyridyldithio-mannose **3**.[7] The disulfide exchange reaction of **3** with 3-(3-mercaptomethyl-phenyl)-3-trifluoromethyl-3*H*-diazirine **4**[8] afforded the labeling reagent **5**.[9]

Scheme 1. Synthetic Scheme for the Photoaffinity Ligand **5**[a]



[a] **Reaction conditions**: (a) AcSH, $BF_3{\cdot}Et_2O$, dry CH_2Cl_2, rt, 20h (75%); (b) LiOH, MeOH, H_2O, rt, 10 min; (c) 2,2′-Dithiodipyridine, AcOH, MeOH, H_2O, rt, 2h (66%); (d) 3-(3-Mercaptomethylphenyl)-3-trifluoromethyl-3*H*-diazirine (**4**), AcOH, MeOH, H_2O, rt, 1h (18%).

Scheme 2. Schematic Illustration for Semisynthesis of IAEDANS-ConA



Our method for the fluorescent Con A is briefly outlined in Scheme 2. When the affinity ligand **5** was bound to the sugar-binding pocket of Con A, photolabeling was carried out by UV light irradiation (330 nm < λ < 400 nm). The labeled Con A was purified by gel chromatography (Biogel P-30), followed by affinity chromatography (Sephadex G-100).[10] The yield of this step was about 10%. Subsequent treatment of the labeled Con A

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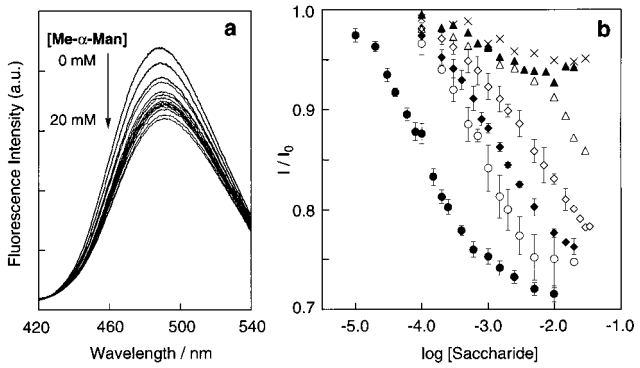

Figure 1. (a) Fluorescence spectral changes of IAEDANS-ConA upon the addition of Me-α-Man (0−20 mM). [IAEDANS-ConA] = 5 μM, 50 mM HEPES buffer (pH 7.0), 1 mM $CaCl_2$, 1 mM $MnCl_2$, 0.1 M NaCl, temp = 15 ± 1 °C, λ_{ex} = 340 nm. The experimental details are described in the Supporting Information. (b) Fluorescence titration profile of the relative intensity (I/I_0) versus the saccharide concentration (log [saccharide]): Me-α-Man (●), Me-α-Glc (○), D-Man (◆), D-Glc (◇), L-Glc (▲), Me-β-Glc (△), D-Gal (×).

with DTT gave a uniquely produced mercaptobenzyl site (SH-Con A) which was modified with an iodoacetylated dansyl group (IAEDANS-Con A). These two steps proceeded almost quantitatively. The modified Con As in all of the steps of this procedure were characterized by MALDI-TOF mass spectroscopy. The mass peaks (m/z) at 25 990 ± 10 for the labeled Con A, at 25 796 ± 10 for SH-Con A, and at 26 097 ± 10 for IAEDANS-Con A, were observed. The data show good agreement with the sum of the MW of the native Con A and the corresponding residues.[11] The secondary structure of the modified Con A was monitored by CD spectroscopy. A negative Cotton peak at 218 nm, characteristic of the typical β-sheet, in all of the modified Con As (the labeled, SH-, and IAEDANS-Con A), is comparable to that of the native Con A,[12] suggesting that the secondary structure of Con A is not perturbed by the present chemical modification.

IAEDANS-Con A showed a strong fluorescence due to the dansyl unit, an environmentally sensitive fluorophore.[13] We monitored the emission spectral changes of IAEDANS-Con A by the addition of methyl-α-D-mannoside (Me-α-Man), the strongest monosaccharide ligand for native Con A (Figure 1a). With an increase in the Me-α-Man concentration, the emission intensity at 484 nm is gradually lessened and slightly red-shifted (488 nm), implying that the dansyl unit moves into the more polar microenvironment from the original position upon complexation of IAEDANS-Con A with Me-α-Man.[14] These results clearly indicate that the binding process of IAEDANS-Con A with Me-α-Man can be directly monitored by the fluorescence signal change. The Benesi−Hildebrandt plot of the saturation curve obtained by the fluorescent titration gave the binding constant of 7.8×10^3 M^{-1} for Me-α-Man, which is almost comparable to that of native Con A (1.1×10^4 M^{-1}).[15]

We conducted similar fluorescence titrations for other types of saccharides using IAEDANS-Con A. The titration curves summarized in Figure 1b shows several definite features: (i) mannose derivatives are stronger ligands than the glucose derivatives (Me-α-Man > Me-α-Glc, Man > Glc), (ii) D-galactose, D-ribose, and D-arabinose show negligible affinity, (iii) the α-linked glycoside bond is more preferable to the β-linkage (Me-α-Glc > Me-β-Glc), and (iv) L-glucose, a mirror image of the native D-glucose, is not practically bound. It is clear that the binding selectivity of IAEDANS-Con A obtained here is completely identical to that of the native Con A. On the basis of these titration curves, the binding constants for various saccharides were also found to be 7.8×10^3 M^{-1} for Me-α-Man, 1.6×10^3 M^{-1} for Me-α-Glc, 1.3×10^3 M^{-1} for Man, 1.0×10^3 M^{-1} for Glc, and $< 0.2 \times 10^3$ M^{-1} for Me-β-Glc. These are only slightly lower than the literature values of native ConA,[15] revealing that the binding affinity of the native Con A for the saccharide derivatives is also retained in IAEDANS-Con A.

In conclusion, we successfully developed a simple method to convert a lectin protein into a fluorescent saccharide sensor. The generality of the present strategy may allow us to apply this method to other saccharide-binding proteins. Exchanging the sugar part of the photoaffinity reagent **5** for other lectins can produce distinct fluorescent biosensors with different saccharide specificities. Photoaffinity labeling has already been widely used in structural and functional biology.[5] Our results expand this technique into the field of protein engineering. The high reactivity and the active-site direction of a photoaffinity-labeling reagent are demonstrated to be unique when this method is coupled to post-modification.[16]

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Supporting Information Available: Experimental details including characterization of all products for the labeling reagent synthesis and protein-labeling (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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