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The Chemo-enzymatic Synthesis and Evaluation of Oligosaccharide-Branched Cyclodextrins

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Abstract: The syntheses of oligosaccharide-branched cyclodextrins, which showed potential binding to the saccharide-interacting protein, lectin, were investigated. The transglycosylations to Fmoc-Asn(GlcNAc)-NH-cyclodextrin by endo-β-*N*-acetylglucosaminidase of *Mucor hiemalis* (Endo-M) gave three kinds of natural oligosaccharide-branched cyclodextrins (**7-9**) in satisfactory yields. The association constant of the high-mannose type oligosaccharide-branched CD (**7**) with immobilized concanavalin A was found to be approximately 1.6 x 10^7 M^{-1} using an optical biosensor. © 1997 Elsevier Science Ltd.

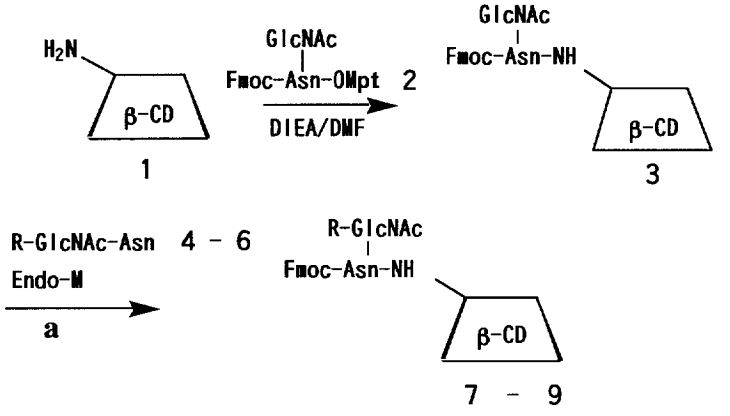
Cyclodextrin (CD) can be used in various drugs as an auxiliary additive such as a carrier, diluent, and solubilizer for tablet ingredients[1-3]. The synthesis of oligosaccharide-branched cyclodextrins which have specificity for binding to lectin protein was previously investigated[4]. This investigation demonstrates oligosaccharide-branched CDs interacted with the lectin protein and is expected as a drug carrier for targeting DDS.

The endo-β-*N*-acetylglucosaminidase (endo-β-GlcNAc-ase) of *Mucor hiemalis*, Endo-M, was found to transfer the Asn-linked sialo complex-type and the high-mannose type oligosaccharides to the *N*-acetylglucosamine (GlcNAc) moieties of GlcNAc-peptide[5]. Takegawa *et al.*[6] and, recently, Lee *et al.*[7] also reported that Endo-A of *Arthrobacter protophormiae* transferred the high-mannose type oligosaccharide to a glycosyl peptide.

In this study, three kinds of natural oligosaccharide-branched CDs (**7-9**) were synthesized and the molecular interaction to immobilized concanavalin A (ConA) was studied using an optical biosensor equipped with resonant mirror detector based on surface plasmon resonance (SPR)[4,8].

On the chemo-enzymatic syntheses of oligosaccharide-branched CDs, the glycosyl acceptor (**3**) for transglycosylation by Endo-M was prepared using 6-*mono*-amino-β-CD (**1**) and *N*-α-Fmoc-*N*-ω-(2-acetamide-2-deoxy-β-D-glucopyranosyl)asparagine dimethylphophinothioic mixed anhydride[9] (**2**) in the presence of *N,N*-diisopropylethylamine in DMF through amide formation with 55 % yield. The glycosyl donors were the high-mannose type oligosaccharide (**4**) from ovalbumin[10], the asialo complex-type oligosaccharide (**5**) and the sialo complex-type oligosaccharide (**6**) from human transferrin[11]. After the

enzymatic reaction with Endo-M for 6 h at 37 °C in the same manner as reported previously[5], the transglycosylation products (**7**, **8** and **9**) were isolated by a preparative HPLC with satisfactory yields and identified by their HPLC[12] and MALDI-TOF MS spectra[13].



a: Glycosyl Donor, 25mM; Glycosyl Acceptor, 10mM; Endo-M, 4mU/ml; Phosphate buffer, pH 6.25, 60 mM; 37°C, 6 h.

Glycosyl Donor	Product	R-	Yield %
4	7	Man Man — Man Man - Man Man - GlcNAc -	6.3
5	8	Gal - GlcNAc - Man Gal - GlcNAc - Man Man - GlcNAc -	9.1
6	9	NeuAc-Gal-GlcNAc-Man NeuAc-Gal-GlcNAc-Man Man-GlcNAc-	12.4

Fig. 1 Scheme for the chemo-enzymatic synthesis of natural oligosaccharide-branched cyclodextrins(7-9)

The immobilization of ConA on an cuvette for the optical biosensor, IASys(FAST Co.,Ltd.), was carried out under the same conditions as previously reported[4]. The aminosilane biosensor surfaces were activated with bis(sulfosuccinimidyl) suberate, and then ConA in the acetate buffer was immobilized. Then, its cuvette was blocked with ethanolamine. The cuvette surface was washed with 8 M urea and acetate buffer. Using an acetate buffer of pH 5.3 at 25 °C, ConA was immobilized as a dimer and partly as

a tetramer. The change in the response position, ΔR, after the ConA immobilization was approximately 640 arc sec.

This biosensor was used for the analysis of interaction between the oligosaccharide-branched CDs and ConA. The change in the observed response, R, corresponds to the amount of the associated oligosaccharide-branched CDs with immobilized ConA in the optical biosensor[14]. The specific interaction curve of the newly synthesized oligosaccharide-branched CD (**7**) with ConA was observed. Other oligosaccharides such as maltosyl-β-CD, maltosyl-γ-CD and glucosylgluconoamide-β-CD showed slight interaction, but no interaction for β-CD as they are observed in the same results reported previously[4].

The association rate constant (k_a) and the dissociation rate constant (k_d) were calculated by plotting the slope (k_{on}) between dR / dt and R, changing the concentration of the oligosaccharide-branched CD. From the linear plot between k_{on} and the CD concentration, the k_a as the slope and the k_d as the intercept were obtained. The kinetic linear plots by the optical biosensor for the branched β-CD binding to the immobilized ConA is shown in Fig. 2.

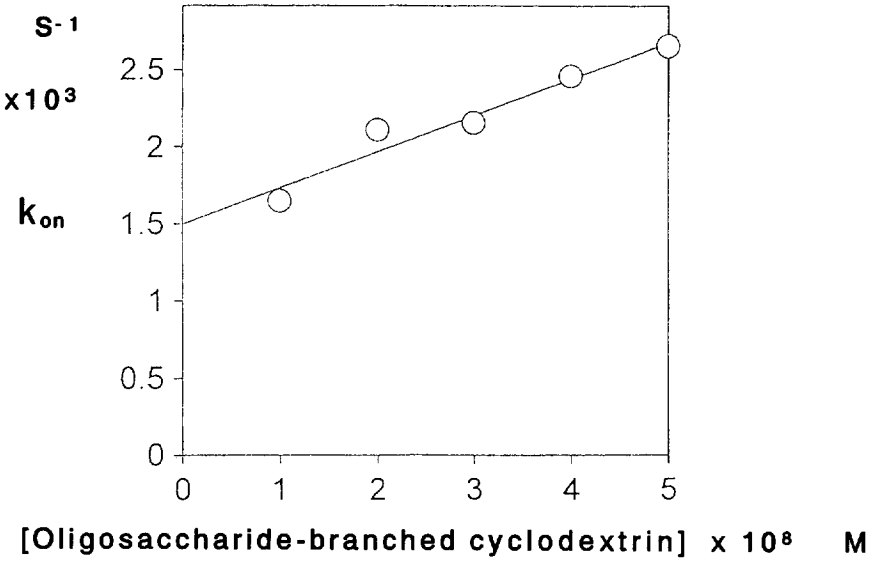


Fig. 2 Kinetic linear plots between k_{on} and CD concentration during the interaction between ConA and oligosaccharide-branched CD with an optical biosensor in acetate buffer, pH 5.3, containing 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 100 mM NaCl at 25.0 °C.

The association rate, k_a, and dissociation rate, k_d, were $(2.36 \pm 0.32) \times 10^4$ M^{-1} s^{-1} and $(1.50 \pm 0.11) \times 10^{-3}$ s^{-1}, respectively. Then, the association constants, K_a, of high-mannose type oligosaccharide branched CD (**7**) was calculated to be $(1.57 \pm 0.36) \times 10^7$ M^{-1}. The values of k_a increased 220 times and k_d decreased 8.4 times in comparison with that for glucosylgluconoamide-β-CD of our previous study[4]. The K_a value was enhanced by 1800 times. The reported K_a values[15] between ConA and natural oligosaccharides of high-mannose type having triantenna were approximately same as the present results in the range of 10^6-10^7 M^{-1} depending on the structure.

The CD derivatives having a high-mannose type oligosaccharide (**7**) interacted with ConA with a strong association constant. The recognition of ConA for the oligosaccharide-CD showed a high dependency on the structure of the saccharides, *i.e.*, ConA associated selectively with the branched oligosaccharides on CD moiety in the order; the *hexa*-mannosyl > *mono*-glucosylgluconoamide > *mono*-maltosyl > *mono*-glucosyl[4]. With *mono*-glucosyl branch or without branch on CD, ConA showed no association. Multiple interacting sites in the triantennary high mannosyl branch may be effective for the strong interaction. The length between the terminal mannose unit and CD cavity also seems to be an important factor for the interaction with ConA considering the steric hindrance. The present oligosaccharides attached on CD have flexible movement enough to interact with the receptor site of lectin.

References and Notes

1. Szejtli, J. in *Cyclodextrin Technology,* 1988, Kluwer Academic Publishers.
2. Froming, K. H.;.Szejtli, J. in *Cyclodextrin in Pharmacy,* 1993, Kluwer Academic Publishers.
3. Uekama, K.; Hirayama, F.; Irie, T. in *Modification of Drug Release by Cyclodextrin Derivatives, "New Trends in Cyclodextrins and Derivatives",* Ed. by D. Duchene, Editions de Sante, Paris, 1991, pp. 409-446.
4. Imata, H.; Kubota, K; Hattori, K.; Aoyagi, M.; Jindoh, C. *Bio. Med. Chem. Lett.,* 1997, **7**, 109; *Polymer J.*, **1997**, *29*, 563.
5. Haneda, K.; Inazu, T.; Yamamoto, K.; Kumagai, H.; Nakahara, Y.; Kobata, A. *Carbohydr. Res.*, **1996**, *292*, 61.
6. Takegawa, K.; Tabuchi, M.; Yamaguchi, S.; Kondo, A.; Kato, I.; Iwahara, K. *J. Biol. Chem.,* **1995**, *270*, 3094.
7. Wang, L. X.; Fan, J. Q.; Lee, Y. C. *Tetrahedron Lett.*, **1996**, *37*, 1975.
8. Shuster, S. C.; Swanson, R. V.; Alex, L. A.; Bourret, R. B.; Simon, M. I. *Nature*, **1993**, *365*, 343.
9. Inazu, T; Mizuno, M.; Kohda, Y.; Kobayashi, K.; Yaginuma, H. "Peptide Chemistry 1995", Ed. by Nishi, N., Protein Research Foundation, Osaka (1996), pp. 61-64.
10. Kadowaki, S.; Yamamoto, K.; Fujisaki, M.; Izumi, K.; Tochikura, T. *Agric. Biol. Chem.*, **1990**, *54*, 97.
11. Tai, T.; Yamashita, K.; Ogata-Arakawa, M.; Koide, N.; Muramatsu, T.; Iwashita, S.; Inoue, Y.; Kobata, A. *J. Biol. Chem.*, **1975**, *250*, 8569.
12. HPLC was carried out using a Mightysil RD-18 column of size ϕ 6 × 250 mm by linear increase of acetonitrile from 15 to 25 in 0.1 % trifluoroacetic acid over 30 min at a flow rate of 1.2 ml/min with detection of 254 nm due to the Fmoc group. Retention time for **3**: 25.8 min (single); for **7**: 17.8 min; for **8**: 17.6 min.; for **9**: 15.5 min. There was no peaks found in the products beside residual **3** at 25.0 min. The yield of transglycosylation with Endo-M in Fig. 1 was obtained from these spectra.
13. Characterization of compound **3**: MALDI-TOF MS: found *m/z* 1699, calcd. 1696, for **7**: MALDI-TOF MS: found *m/z* 2870, calcd. 2871, for **8**: MALDI-TOF MS; found *m/z* 3121, calcd. 3115, for **9**: MALDI-TOF MS: found *m/z* 3695, calcd. 3698.
14. Edwards, P. R.; Gill, A.; Pollard-Knight, D. V.; Hoare, M.; Buckle, P. E.; Lowe, P. A.; Leatherbarrow, R. J.; *Anal. Biochem.*, **1995**, *231*, 210.
15. Mega, T.; Oku, H; Hase, S. *J. Biochem.,* **1992**, *111*, 396.

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