THE SYNTHESIS OF OLIGOSACCHARIDE-BRANCHED CYCLODEXTRINS AND THEIR INTERACTION WITH CONCANAVALIN A

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ABSTRACT

New oligosaccharide-branched β-cyclodextrins having a various oligosaccharides in a primary hydroxyl group of β-cyclodextrin(CD) were synthesized and examined for the interaction with the immobilized concanavalin A(ConA) compared with 6-*O*-glucosyl and maltosyl CD. Oligosaccharides were converted to lactones at the reducing end, which was connected with 6-*mono*amino-β-CD forming an amide bond. For the analysis of the interaction between ConA immobilized on an aminosilane-hydrogel surface and various oligosaccharide CDs, a biosensor of the FISONS IAsys apparatus based on a resonant mirror detector (RMD) was used. As a result, it interacted with immobilized ConA and both maltosyl-β-CD(**3**) and maltosyl-γ-CD(**6**), and glucosylglucono-amide-β-CD(**7**) with the association constants, K_a, of 134, 833 and 8730 M^{-1}, respectively.

1. INTRODUCTION

Biological recognition and adhesion processes often involve the formation of saccharide-protein complexes. Elucidation of the carbohydrate-binding specificity of lectins has become important in connection with the structure analysis of carbohydrates and the biological roles of the sugar chains and lectins. ConA is widely used as a biological tool to investigate the membrane properties of both normal and transformed cells[1)]. In this study, the molecular interaction between immobilized ConA and oligosaccharide-branched CDs were measured using a resonant mirror detector through the change in reflected light(arc sec) *vs.* time.

2. MATERIALS AND METHODS

2.1 Materials

Glucosylgluconolacone, galactosylgluconolactone, and gluconolactone were prepared according to the literature[2)]. These lactones were connected with 6-amino-β-CD.

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The crude products were purified through an ion exchange column and preparative HPLC. The obtained glucosylglucono-amide-β-CD(**7**), galactosylglucono-amide-β-CD(**8**) and gluconoamide-β-CD(**9**) were identified from NMR and MS spectra[3]. Glucosyl-β, γ-CD(**3,4**), maltosyl-β, γ-CD(**5,6**), and β, γ-CD(**1,2**) were available from Ensuiko Co., Ltd. and ConA (Wako Co., Ltd.) was dissolved in *p*H 6.5 phosphate buffer solution (PBS, SIGMA), giving a 10 mM solution of *p*H 7.4 in ultrahigh purity water (filtered by Mill-Q, Millipore) with 2.7 mM KCl and 0.14 M NaCl. An IAsys cuvette coated with aminosilane was supplied by FAST. Bissulfosuccinimidyl suberate(BS3) was purchased from Pierce & Warriner. One mg/ml BS3 in PBS was made with 0.05 % v/v Tween 20(Pierce & Warriner). Acetate buffer (*p*H 5.4, 10 mM) was made by titrating sodium acetate with 2 M acetic acid.

2.2 Immobilization of ConA on the optical sensor

To immobilize ConA, aminosilane biosensor surfaces were activated with 10 mM PBS (*p*H 6.5). BS3(1 mM, 0.2 ml) of the crosslinker solution was injected into the aminosilane cuvette. After it was washed with 10 mM PBS (*p*H 6.5) and 10 mM acetate buffer (*p*H 5.4), ConA was immobilized by a reaction with the amino group. Moreover, its cuvette was washed with NaOH (*p*H 8.90) and 10 mM acetate buffer, blocking ConA with 1 M ethanolamine.

2.3 Determination of the association constant K_a

Using the IAsys apparatus (FISONS) with a resonant mirror detector.[4,5,7], the association rate constant (k_a) and the dissociation rate constant (k_d) can be calculated in the elapsed time from the intercept of a plot of dR/dt *vs.* R through the response in reflected light, R (arc sec), at the concentration of saccharide-branched CD as C if the maximum binding response R_{max}, was known and the interactions were carried out under pseudo first order conditions ($k_a >> k_d$) for which the relation is:

$$dR/dt = k_a R_{max} C - (k_a C + k_d) R$$

However, if R_{max} is not known, the value of k_a and k_d can be obtained by plotting the slope of the plot dR/dt *vs.* R changing C.

3. RESULTS AND DISCUSSION

3.1 Immobilization of ConA on the optical sensor

The conditions of the immobilization for ConA were investigated based on the *p*H, the presence of epitope and metal ion, recycle condition, and the reproducibility. The typical immobilization conditions of ConA on an IAsys cuvette were described in the of Materials and Methods section.

3.2 The interaction analysis of the CDs with ConA.

The biosensor was applicable for investigating for the interaction analysis, such as the association constant between the various oligosaccharide-branched CDs and ConA. Fig. 1 shows the response of the optical sensor for the oligosaccharide-branched CD binding to the immobilized ConA. Fig. 2 presents the interaction curve of the newly synthesized oligosaccharide-branched CDs with ConA using the optical biosensor IAsys. From the result of Fig. 2, only glucosylglucono-amide-β-CD(**7**) shows an interaction but not galactosylglucono-amide-β-CD(**8**), gluconoamide-β-CD or β-CD(**1**). The results of the association constants are summarized in Table 1. As a result, it interacted with immobilized ConA and both the maltosyl-β-CD(**3**) and maltosyl-γ-CD(**6**).

However, β-CD(**1**), γ-CD(**4**), glucosyl-β-CD(**2**) and glucosyl-γ-CD(**5**) did not interact with ConA. The association constants, K_a, of maltosyl-β, γ-CD(**3,6**) and glucosylglucono-amide-β-CD(**7**) were measured to be 134, 833 and 8730 M^{-1}, respectively. The reported K_a values between ConA and glucose or maltose were approximately the same as the present results in the range of 10^3-10^4 M^{-1}. However oligosaccharides of triantena type having sialyl unit[5] or mannose unit[6] showed more than 10^7 M^{-1} of K_a values. It was suggested that only CD derivatives that have a glucose unit at the non-reducing end interacted with ConA.

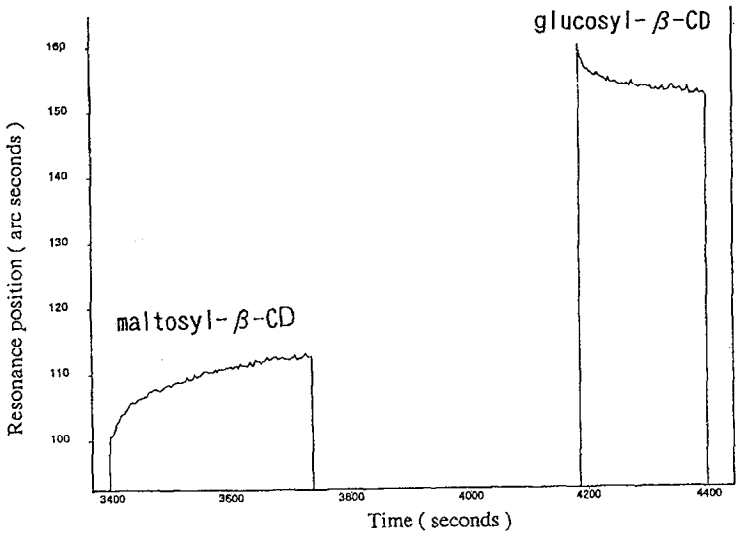


Fig. 1 Profiles of ConA binding to glucosyl-β-CD and maltosyl-β-CD by Iasys
25°C, *p*H 5.3, in acetate buffer, [maltosyl-β-CD]= [glucosyl-β-CD]= 2.0 x 10^{-2} M

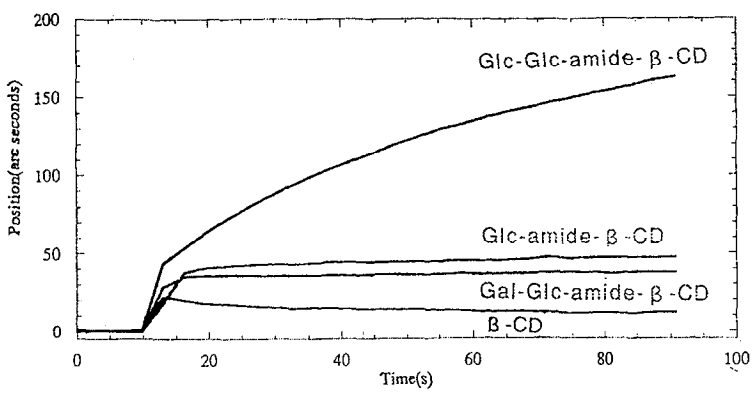


Fig. 2 Interaction between ConA and oligosaccharide-branched CDs
25°C, pH 7.02 in Tris-HCl buffer with 1 mM $CaCl_2$,,100 mM NaCl, [CDs]= 1.7 x 10^{-3} M

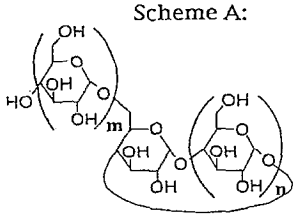
The length of the spacer between the non-reducing end and CD cavity seems vitally important for the interaction with ConA. The non-reducing end of these newly synthesized oligosaccharide-branching CDs(**7**) would have freer movement than that of the commercially available 6-*O*-glucosyl and maltosyl CDs(**3,6**). The behavior of the

interaction of various oligosaccharide-branched α-CD derivatives was also examined and found to be different in complex formation from the derivatives of β- and γ-CD. It is suggested that the ring size of the CD cavity was related to the recognition for ConA. This investigation has demonstrated the applications of IAsys using a resonant mirror detector to determine the association constants for the interaction of the oligosaccharides-branched CD with immobilized ConA on the surface of the aminosilane cuvette.

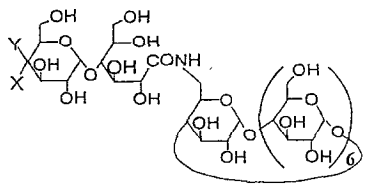
Table 1 Association constant for the oligosaccharide-branched CDs with optical biosensor IAsys

Scheme A,	n= 6, m= 0	(1) β-CD	no complex
	1	(2) Glucosyl-β-CD	no complex
	2	(3) Maltosyl-β-CD	134
	n= 7, m= 0	(4) γ-CD	no complex
	1	(5) Glucosyl-γ-CD	no complex
	2	(6) Maltosyl-γ-CD	833
Scheme B,	X= -OH, Y= -H	(7) Glucosyl-glucono-amide-β-CD	8730
	-H, -OH	(8) Galctosyl-glucono-amide-β-CD	no complex

Scheme A:



Scheme B:



CONCLUSION

The behavior of the interaction of various oligosaccharide CD derivatives was summarized as follows: β- and γ-CD derivatives that have a glucosyl unit at the non-reducing end on the oligosaccharide branch, which is longer than the maltosyl group, show association with immobilized ConA using the optical biosensor. The larger the spacer between CD and the glucose unit, the larger association constant for the oligosaccharide-branched CDs. By using this method, it will be possible to obtain the association constant for the precise investigation of the interaction of ConA and various oligosaccharide derivatives of CD.

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