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THE SPECIFICITY OF ASSOCIATION BETWEEN CONCANAVALIN A AND OLIGOSACCHARIDE-BRANCHED CYCLODEXTRINS WITH AN OPTICAL BIOSENSOR

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Abstract: The immobilized concanavalin A on aminosilane-hydrogel surface interacted specifically with a newly synthesized oligosaccharide-branched cyclodextrin, α-glucosyl-glucono-amide-β-CD(**1**) in the association constant, K_a of 8730 M^{-1}, using an optical biosensor on the principle of surface plasmon resonance.

Cyclodextrin(CD) can be used in various drugs as auxiliary additives such as carriers, diluents and solubilizers of tablet ingredients[1,2]. Application of saccharide-binding specificity of lectin proteins has become important connected with the biological recognition and adhesion processes[3]. Recently, the interaction between concanavalin A(ConA) and glucose-derivatized polymer was investigated by hemagglutination inhibition assay[4]. In order to construct the targeting drug delivery system, the synthesis of oligosaccharide-branched cyclodextrins which have potential binding to the protein were initially investigated[5].

In this study, the molecular interactions between immobilized ConA and newly synthesized oligosaccharide-branched CDs were studied using an optical biosensor IAsys (FAST Co., Ltd.) equipped with a resonant mirror detector through the change in reflected laser light based on surface plasmon resonance(SPR)[6], which corresponds to the mass on the surface of the metal. The advantages of this method are the conventional techniques includes high sensitivity, real time monitoring, and no requirement for the use of labeled materials. The number of publications in molecular interaction analysis using optical biosensor technology has rapidly increased[7-12].

Lactones of maltose, lactose and glucose were prepared according to the literature[13]. These lactones were connected with 6-*mono*amino-β-CD in the same manner as previously reported[5]. The crude products were purified through an ion exchange column and preparative HPLC in the same manner as formerly report[4]. The obtained α-glucosyl-glucono-amide-β-CD(**1**), β-galactosyl-glucono-amide-β-CD(**2**) and glucono-amide-β-CD(**3**) were identified from NMR and MS spectra[14]. Glucosyl-α, β, γ-CD, maltosyl-α, β, γ-CD, and α, β, γ -CD were available from Ensuiko Co.,Ltd. and ConA was purchased from Wako Co.,Ltd. A IAsys cuvette of 200 μL volume coated with aminosilane was supplied by FAST Co.,Ltd. Bissulfosuccinimidylsuberate(BS3) was purchased from Pierce & Warriner.

The typical immobilization conditions of ConA on a IAsys cuvette were described as follows; the aminosilane biosensor surfaces were activated with 200 μL of 10 mM phosphate buffered saline(pH 6.5)(PBS) for 8 min. The BS3(1 mM) of the crosslinker solution was injected into the aminosilane cuvette for 10 min. It was washed with PBS for 8 min. This crosslinker attachment was repeated 3 times. After it was washed with PBS(8 min) and acetate buffer(pH 5.3, 10 mM)(2 min), ConA(2 mg/mL in the acetate buffer) was immobilized for 45 min by a reaction with the amino group. Moreover, the cuvette was washed with acetate buffer(2 min), blocked with 1 M ethanolamine(5 min). The cuvette surface was washed with NaOH(pH 8.90) for 5 min and with acetate buffer(2 min). The change in the response position at the immobilization using the biosensor was showed in Fig. 1. There is a reversible dimer-tetramer equilibrium of ConA[15]. In this experiment, ConA was immobilized as a dimer using acetate buffer of pH 5.3 at 25.0 °C.

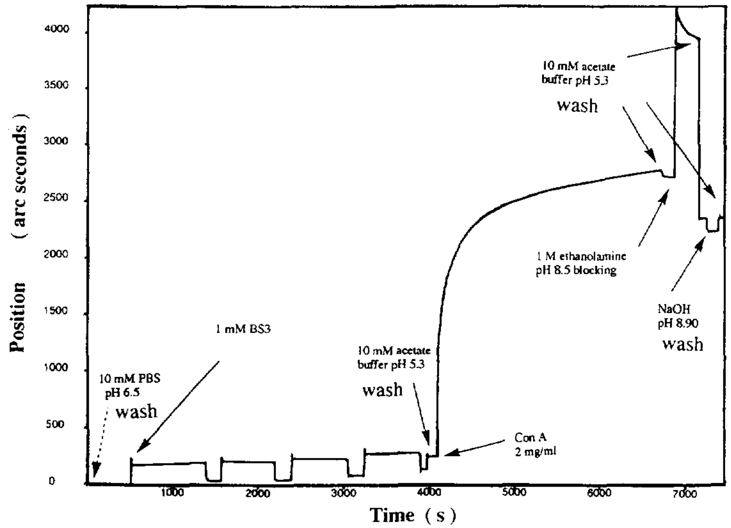


Fig. 1 Immobilization of ConA on aminosilane surface of optical biosensor

This biosensor was used for the interaction analysis between the various oligosaccharide-branched CDs and ConA. The response of optical biosensor for the branched β-CD binding to the immobilized ConA was shown in Fig. 2 that presents the specific interaction curve of the newly synthesized oligosaccharide-branched CD(**1**). Only α-glucosyl-glucono-amide-β-CD(**1**) showed an interaction but not β-galactosyl-glucono-amide-β-CD(**2**), glucono-amide-β-CD(**3**) or β-CD.

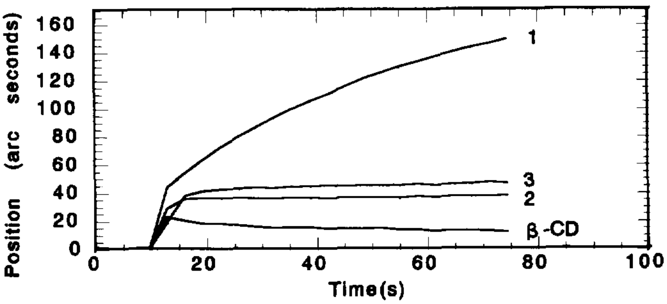


Fig.2 Interaction between ConA and oligosaccharide-branched CDs in acetate buffer pH 5.3, containing 1mM $CaCl_2$, 1mM $MnCl_2$ and 100 mM NaCl at 25.0±0.1 °C and [CDs] = 17 μM.

Using the IAsys apparatus, the association rate constant (k_a) and the dissociation rate constant (k_d) can be calculated from the relation $dR/dt=k_aR_{max}C - (k_aC+k_d)R$, where R is the response in reflect light(arc sec), C is the concentration of CD(**1**), and R_{max} is the maximum binding response. If $k_a >> k_d$ is satisfied, the value k_a and k_d can be obtained by plotting $(dR/dt)R^{-1}$(on rate constant, k_{on}) with changing C. The result of plotting is illustrated in Fig. 3.

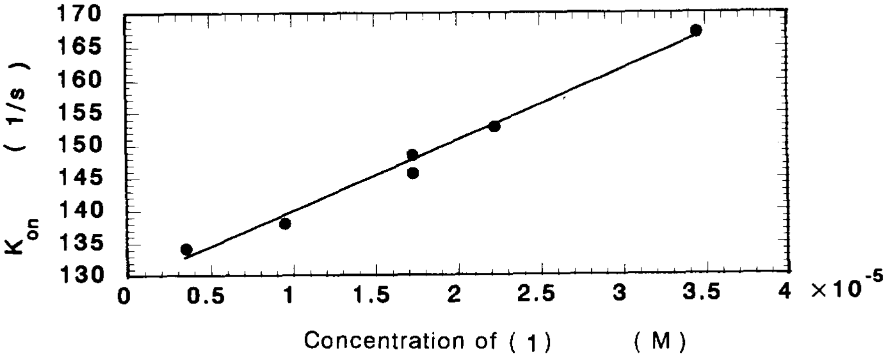


Fig.3 Plotting for the interaction analysis betweenConA and 1

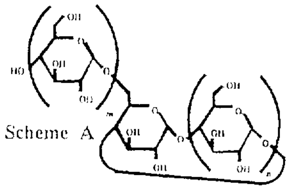
The association rate k_a and dissociation rate k_d were $(1.08\pm0.10)\times10^{2}$ $M^{-1}s^{-1}$ and $(1.26\pm0.02)\times10^{-2}$ s^{-1}, respectively. The association constants, K_a, of glucosyl-glucono-amide-β-CD(1) was calculated to be 8730±880 M^{-1}. The reported K_a values [12,16] between ConA and the oligomannoses were approximately the same as the present results in the range of 10^{3}~10^{5} M^{-1}. However, oligosaccharides of the triantena type having mannosyl units to ConA using microequilibrium dialysis coupled with HPLC[16], and also sialyl units to the recombinant macropharge lectin using optical biosensor BIAcore based on SPR[12] showed more than 10^{7} M^{-1} for the K_a values. The present results can be summarized that the CD derivatives having a glucose unit at the non-reducing end interacted with ConA at a relatively high association constant.

The behavior of the interaction of commercially available oligosaccharide-branched β-CD derivatives with immobilized dimeric ConA was also examined at pH 5.3 and 7.0 using the biosensor IAsys. The summarized results are shown in Table I.

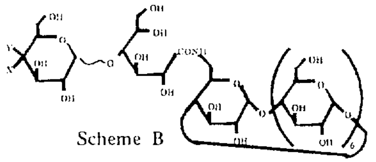
Table I Interaction between immobilized ConA and branched CDs using optical biosensor

Structure		Compound	pH 5.3	pH 7.0
Scheme 1.				
n=5, m=0	**1**	α-CD	0	0
1	**2**	glucosyl- α-CD	0	+++
2	**3**	maltosyl- α-CD	0	0
n=6, m=0	**4**	β-CD	0	0
1	**5**	glucosyl- β-CD	-	+
2	**6**	maltosyl- β-CD	+	-
n=7, m=0	**7**	γ-CD	0	-
1	**8**	glucosyl- γ-CD	0	0
2	**9**	maltosyl- γ-CD	+	+
Scheme 2.				
X=-OH,Y= -H	**10**	α-glucosyl-glucono-amide- β-CD	+++++	+++++
X= -H, Y=-OH	**11**	β-galactosyl-glucono-amide- β-CD	0	0
	12	glucono-amide- β-CD	0	0

Acetate buffer of pH 5.3 was used at 10 mM and tris-HCl buffer of pH 7.0 at 10 mM containing 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 100 mM NaCl at 25.0± 0.1 °C with [CDs] = 20 mM. Sign(+), (-) and 0 show the increase, decrease or no change of the response by using optical biosensor.



Scheme A



Scheme B

Some of the branched CDs (maltosyl-β, γ-CD at pH 5.3 and glucosyl-α, β-CD at pH 7.0) showed a slight association with ConA. Also, another (glucosyl-β, γ-CD at pH 5.3 and maltosyl-β-CD at pH 7.0) showed a slight lowering of the response position. Though they have a glucose unit at the non-reducing end, the conformational structure would not be properly fitted for recognizing the receptor site of ConA. We now assumed the three modes of the interaction as follows: 1) there should be a stereospecific molecular recognition between the oligosaccharide-modified CD and the ConA. These showed saccharide recognition: 2)others showed the cavity inclusion of CD: and 3) it accompanied stripping of a part of ConA subunit.

The recognition of ConA for the oligosaccharides showed a strictly sharp dependency on the type of saccharides. ConA was associated with glucosyl derivative (**1**) but not with galactosyl derivative(**2**). The length and flexibility of the spacer between the glucosyl unit and CD cavity also seems to be important conditions for the interaction with ConA. The non-reducing end of the newly synthesized oligosaccharide-branched CD(**1**) would behave more flexible movement than that of the commercially available 6-*O*-glucosyl and 6-*O*-maltosyl CDs. There might be caused by these 6-*O*-glucosyl, 6-*O*-maltosyl CDs in various modes of interaction beside saccharide recognition, such as CD cavity inclusion and stripping of subunits as well as no interaction towards ConA protein.

This investigation has demonstrated the convenient applications of a optical biosensor IAsys on the principle of SPR to determine the kinetics for the interaction of the oligosaccharide-branched CDs with immobilized ConA on the surface of the aminosilane cuvette. By using this method, the binding assay for the interaction between ConA and various oligosaccharide derivatives of CD is under investigation.

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14. Characterization for compound **1**: 100.4 MHz ^{13}C-NMR(D_2O) δ 173(C=O), 36(C-N); MS(FAB): m/z 1472.7(M-H), for **2**: 100.4 MHz ^{13}C-NMR(D_2O) δ 173(C=O), 38(C-N); MS(FAB): m/z 1473.5(M-H), for **3**: 100.4 MHz ^{13}C-NMR(D_2O) δ 173; MS(FAB): m/z 1310.5(M-H)
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