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ORIGINAL ARTICLE

Detection of environmental chemicals by SPR assay using branched cyclodextrin as sensor ligand

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Abstract We obtained the association constants *Ka* of estrogen (E2) and environmental chemicals by the surface plasmon resonance (SPR) assay using the immobilized mono-6-*O*-α-maltosyl-*β*-CD ($G_2\beta$CD) compared with the immobilized *β*-CD and the immobilized estrogen receptor (ER). The association behavior of $G_2\beta$CD was shown as a ER model compound. The calibration curve was determined by the initial rate of association depending on the various concentrations, and the minimum detectable concentrations in the order of parts per billion were calculated. The SPR assay has advantages that the pre-treatment of the sample is not necessary and the immobilized ligand is stable and useful for the repeated measurement.

Keywords Maltosyl cyclodextrin · Surface plasmon resonance · Environmental chemicals · Estrogen receptor

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Introduction

There has been increasing concerns about environmental chemicals that disrupt the endocrine function in wildlife and humans [1]. A number of pollutants including pesticides, polychlorinated biphenyls, alkylphenols, and synthetic steroids have been reported to disrupt the normal hormonal pathways by binding to the ER [2].

Various kinds of assay are used to analyze the environmental chemicals in environmental water. A simple assay with high sensitivity is required. For quantitative determination of environmental chemicals, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), GC-MS, and LC-MS-MS are often used [3–7]. Although these methods have high detection sensitivity, more rapid and simple procedures are required to improve the efficiency of the measurement.

We investigated that cyclodextrin derivatives as well as ER associated with the environmental hormones having similar structure like E2. The association behavior has been analyzed kinetically by optical biosensor SPR assay changing ligand from ER to mono-6-amino-*β*-CD. On the other hand, $G_2\beta$CD was reported as a promising ER model compound among CD derivatives by SPR assay using the immobilized E2 ligand [8].

In this work, we investigated the association behavior between several kinds of environmental chemicals and the aminated-$G_2\beta$CD immobilized as a ligand on the SPR biosensor cuvette. We obtained the results of the association behavior of the environmental chemicals; bisphenol A (BPA), nonylphenol (NP) and nonylphenol ethoxylate (NPE) as well as estrogen (E2) by SPR assay.

Moreover, the calibration curves were determined by plotting the initial association rate depending on the various concentrations, and the minimum detectable concentrations for the environmental chemicals on the order of parts per billion were determined.

Experimental

Materials

Estrogen receptor (β-type, human, recombinant) were purchased from CALBIOCHEM. Nonylphenyl ethoxylate (NPE) has repeating ethylene oxide unit of average ten.

Synthesis

Regioselective mono-2-*O*-sulfonation of $G_2\beta$CD was conveniently achieved by using the combination of *p*-toluenesulfonyl imidazole and molecular sieves 4A in DMF [9]. The $G_2\beta$CD 2-*O*-tosylate was converted with aqueous ammonia at 25 °C for 120 h to 2,3-manno-epoxide according to the known method [10]. The reaction mixture was treated with ion exchanger (CM-Sephadex). The product was characterized by MALDI-TOF MS and TLC. Yield was 8.7%.

Immobilization of the ligands in SPR cuvette

IAsys (Thermo Co.) was used for all SPR measurements. A solution of 10 mM bis(sulfo-succinimidyl)suberate (BS^3) of 200 μL was incubated on the aminosilane type of cuvette for 30 min. Then, the solution of ligands (mono-6-amino-β-CD, mono-aminated-$G_2\beta$CD or ER) was incubated onto the cuvette. Residual carboxyl groups were blocked with 1 M ethanolamine for 5 min. The cuvette was washed with 10 mM phosphate buffer (PB) of pH 7.7. The yield was calculated as 16% of aminosilane group on cuvette surface (Fig. 1).

Association parameters and minimum detectable concentrations for the environmental polluting chemicals

Various kind of environmental polluting chemicals were solved in aqueous solution. The analyte solution of 200 μL was injected into the cuvette. The changes of the response were taken on each sensorgram. Regeneration of the cuvette was achieved by the injection of 20 mM HCl or 8 M urea.

Minimum detection concentration was determined from a calibration curve between the initial rate and the concentration of the analyte. FAST-FIT software (Thermo Co.) was used for the data procedures to determine the association parameters and the initial rate.

Result and discussion

We carried out SPR assay using the immobilized $G_2\beta$CD ligand as well as β-CD and ER. The results of the association parameters are shown in Table 1.

The results showed relatively high efficiency of CD derivatives to associate with the undesirable and harmful environmental chemicals as well as estrogen. The relative ratios of *Ka* showed that the association constants of mono-aminated $G_2\beta$CD as the immobilized ligand were 35–45 times larger than the immobilized βCD. However, the association constants by using ER ligand were 25–35 times larger than $G_2\beta$CD as ligand. In order to approach better ER model, the structure of the CD derivatives are still necessary to improve. The effect of maltose branch seems to take advantage in the association constant *Ka*, with the increasing contribution of the association rate constant k_a.

The results of minimum detectable concentrations are shown in Table 2.

The calibration curve was determined by the initial rate of association depending on the various concentrations, and the minimum detectable concentrations were obtained in the order of parts per billion.

Conclusion

We immobilized $G_2\beta$CD as a ligand to detect environmental chemicals. $G_2\beta$CD was found as the promising ER model compound by SPR assay. Relative ratio of *Ka* showed that the association constant of mono-aminated-$G_2\beta$CD as ligand was 35–45 times larger than βCD. However, the association constant by using ER ligand was 25–35 times larger than $G_2\beta$CD as ligand. In order to approach better ER model, the structure of the CD derivatives are still necessary to improve.

The minimum detectable concentrations in the order of parts per billion were calculated. The SPR assay has advantages that any pre-treatment of the sample was not necessary and that the immobilized ligands are stable and useful for the repeated measurements.

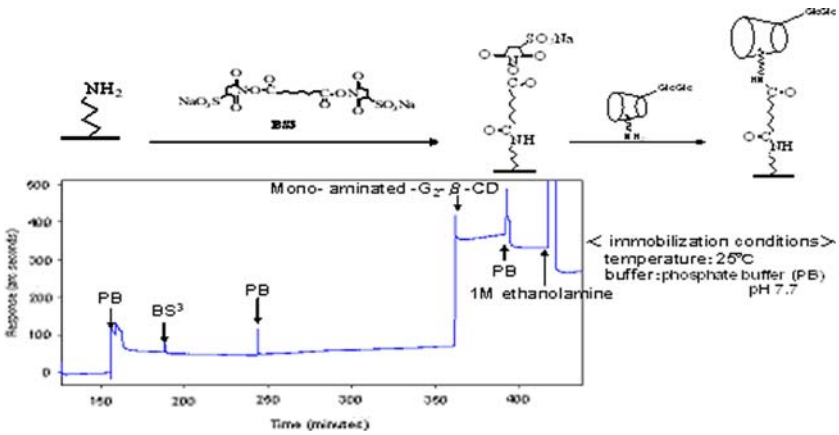

Fig. 1 Immobilization of mono-aminated-$G_2\beta$CD on SPR optical biosensor

Table 1. Comparison of association parameters between the immobilized ligands and various analytes

Association parameters are measured in aqueous solution for βCD and $G_2\beta$CD in phosphate buffer of pH 7.7 for ER. E2: estrogen, BPA: bisphenol A, NP: nonylphenol, NPE: nonylphenyl ethoxylate (repeating units of ethylene oxide = 10), $G_2\beta$CD: maltosyl-β-cyclodextrin, ER; estrogen receptor-β

Analyte	Immobilized ligand	Association rate constant k_a ($10^5 M^{-1}s^{-1}$)	Dissociation rate constant k_d ($10^{-2}s^{-1}$)	Association constant Ka ($10^6 M^{-1}$)	Rate of Ka
E2	βCD	0.106	3.23	0.33	1
	$G_2\beta$CD	2.26	1.52	14.8	45
	ER	69.2	1.34	520	1575
BPA	βCD	0.485	18.4	0.264	1
	$G_2\beta$CD	2.3	2.48	9.30	35
	ER	100	4.1	240	909
NP	βCD	0.30	1.63	1.85	1
	$G_2\beta$CD	1.70	3.15	5.4	3
	ER	15.5	1.91	81.2	44
NPE	βCD	0.803	0.869	9.24	1
	$G_2\beta$CD	1.99	1.11	18	1.9
	ER	No association	No association	No association	–

Table 2 Minimum detectable concentrations of the combinations between analytes and ligands in SPR assay

Analyte	Immobilized ER			Immobilized $G_2\beta$CD		
	Slope of calibration curve (are seconds)	Minimum detectable concentration (ppb)	Association constant ($10^6 M^{-1}$)	Slope of calibration curve (are seconds)	Minimum detectable concentration (ppb)	Association constant ($10^6 M^{-1}$)
E2	2.09×10^{-7}	4	241	6.95×10^{-7}	16	14.8
BPA	5.97×10^{-6}	22	13.6	5.03×10^{-5}	222	9.3
NP	1.74×10^{-7}	12	81.2	4.02×10^{-6}	58	5.4
NPE	No association	–	–	2.57×10^{-6}	28	18

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