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ORIGINAL ARTICLE

Evaluation of the abilities of γ-cyclodextrin to form complexes by surface plasmon resonance with a Biacore® system

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Abstract Although phase-solubility studies have often been used to evaluate the interaction of cyclodextrins (CDs) with various drugs, hundreds of milligrams of both CD and drug are required to prepare a phase diagram. A method that would require considerably less material for evaluating complex formation between a CD and guest compound is therefore needed. We previously reported the detection of the interactions between β-CD and various drugs using a Biacore® system. In this study, we succeeded in immobilizing 6-monodeoxy-6-monoamino-γ-CD on the gold surface of a sensor chip and in detecting the interactions between the immobilized γ-CD and various drugs. The interaction processes were kinetically analyzed using Biacore®. The surface plasmon resonance sensorgrams indicated that the association and dissociation rates of the interactions between γ-CD and drugs were faster than those between β-CD and drugs. Although the association constants calculated from the sensorgrams were smaller than those calculated from phase-solubility studies, good correlation was shown between these data.

Keywords γ-Cyclodextrin · Inclusion complex · Surface plasmon resonance · Kinetic analysis · Association constant · Biacore®

Introduction

It is well known that cyclodextrins (CDs) can form water-soluble inclusion complexes with many hydrophobic drugs. Therefore, CDs are often used as pharmaceutical excipients to improve the solubility of a drug in water. Currently, however, it is difficult to select the CD with the highest solubilizing ability for each drug. Although phase-solubility studies [1] have often been used to evaluate CD/drug interactions, the preparation of a phase diagram requires hundreds of milligrams of both CD and drug. Surface plasmon resonance (SPR) can detect molecular interactions by monitoring a small change in mass on a gold surface decorated with immobilized ligands [2]. As compared with phase-solubility studies, SPR assays have the advantage that only a small amount of CD is required as the immobilized ligand, and the CD-immobilized sensor chip can be used for multiple measurements. Furthermore, an interaction analysis requires just micrograms of a drug. We previously studied the interactions between β-CD and drugs by SPR using a Biacore® system [3]. In this study, we examined the interactions between γ-CD and drugs using the Biacore® system.

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Experimental

Materials

Reagents and chemicals

γ-CD was purchased from Wacker-Chemie (Tokyo, Japan). Biacore®-specific products, such as Sensor Chip CM5 (research grade), HBS-N (0.01 M HEPES pH 7.4, 0.15 M NaCl), and chemicals required for covalent immobilization, were purchased from GE Healthcare UK Ltd. (Buckinghamshire, England). Other chemicals were analytical grade reagents from commercial sources.

Preparation of 6-monodeoxy-6-monoamino-γ-cyclodextrin [4]

γ-CD was activated to 6-monodeoxy-6-monotosyl-γ-CD (6-OTs-γ-CD) by *p*-toluenesulfonyl chloride in pyridine at 25 °C for 12 h. 6-OTs-γ-CD was modified to 6-monodeoxy-6-monoazido-γ-CD (6-N_3-γ-CD) by sodium azide in water at 80 °C for 5 h. After 6-N_3-γ-CD was reacted with triphenylphosphine in *N,N*-dimethylformamide at 25 °C for 1 h, the product was treated with 28% aqueous NH_3 at 25 °C for 3 h to produce 6-monodeoxy-6-monoamino-γ-CD (6-NH_2-γ-CD) with a final yield of 4.4%.

Immobilization of γ-CD on the sensor chip

The sensorgram obtained from the γ-CD-immobilized flow cell is shown in Fig. 1. γ-CD was immobilized on the CM5 sensor chip by flowing 6-NH_2-γ-CD in HBS-N solution following activation of carboxymethyl dextran by *N*-hydroxysuccinimide (NHS)/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Residual activated carboxy groups were blocked using ethanolamine. The amount of immobilized 6-NH_2-γ-CD was 645 RU (645 pg/mm^2). The gold surface of the reference flow cell was immediately blocked by ethanolamine following activation by NHS/EDC.

Methods

Selection of running buffer

Generally, HBS-N buffer is used as a running buffer during the experiment to keep the baseline steady. However, it is possible that HEPES buffer might interfere with the interaction between CD and drugs. The SPR signal is usually poor in the case of small molecule assays. Furthermore, SPR signals are particularly small for lipophilic drugs due to the low solubility of these drugs in the running buffer. Generally, the solubility of lipophilic drugs increases upon adding ethanol (EtOH) to the solvent.

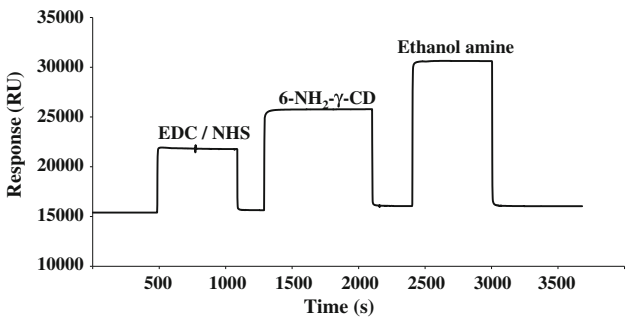

Fig. 1 Immobilization of γ-CD on a CM5 sensor chip

Therefore, the interaction between immobilized γ-CD and drugs in HBS-N, phosphate buffer (pH 7.4), and HBS-N containing 5% (v/v) EtOH (HBS-N + EtOH) was examined using Biacore®. Hydrocortisone was dissolved in the running buffer in five concentrations and injected in the γ-CD-immobilized flow cell and reference flow cell. Association constants were calculated by the following equation using BIAevaluation®,

$$R_{eq} = \frac{K_A C R_{\max}}{1 + K_A C n}$$

where K_A and C represent the association constant and concentration of analyte, respectively. The factor n is a steric interference factor specifying how many binding sites on average are blocked by binding one analyte molecule. R_{max} is the theoretical value of the response in association of drugs with all immobilized γ-CD. R_{eq} is the response at equilibrium.

Interaction analysis

Dexamethasone [5] and spironolactone [6] were used as model drugs because their interactions with γ-CD had previously been studied using the phase-solubility method. Hydrocortisone was tested by the phase-solubility method in HBS-N. The structures of these drugs are shown in Fig. 2. Each drug was dissolved in the running buffer in five concentrations and injected into both the γ-CD-immobilized and reference flow cells. Sensorgrams were evaluated using BIAevaluation®.

Results and discussion

Selection of running buffer

Association constants between immobilized γ-CD and hydrocortisone are shown in Table 1. The association constants calculated from SPR sensorgrams were 2180 M^{-1}, 2110 M^{-1} and 2130 M^{-1} in HBS-N, phosphate buffer and HBS-N + EtOH, respectively. Because these results suggested that HEPES and 5% (v/v) EtOH had no effect on CD/drug interaction, HBS-N + EtOH was used for subsequent experiments.

Interaction analysis

The SPR sensorgrams obtained by the interaction of immobilized γ-CD with hydrocortisone, dexamethasone and spironolactone are shown in Fig. 3. The SPR sensorgrams of the interaction of immobilized β-CD with quetiapine, amoxapine and trazodone previously reported by Kobayashi et al. are shown in Fig. 4 [3]. Compared with

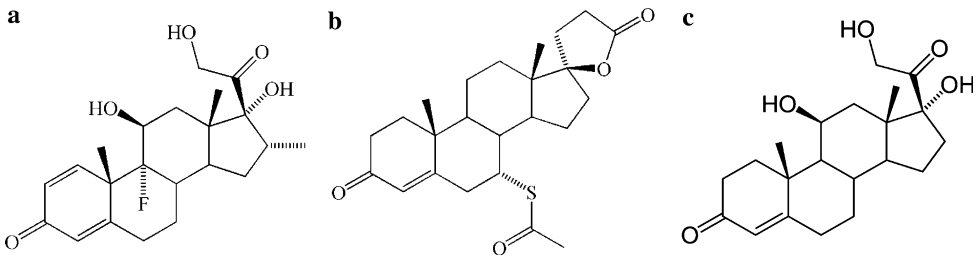

Fig. 2 Structures of **a** dexamethasone, **b** spironolactone and **c** hydrocortisone

Table 1 Association constants between immobilized γ-CD and hydrocortisone calculated from SPR data

Running buffer	Association constant (M^{-1})
HBS-N	2180
Phosphate buffer (pH 7.4)	2110
HBS-N + EtOH 5%	2130

phase-solubility studies, very small amounts of the drugs were used, and it was possible to obtain the interaction data in a period of 2 h, making this screening method appropriate for rapidly evaluating the suitability of a CD for each drug. A CD covalently attached to the surface of a sensor chip can withstand several repeated measurements. In the case of β-CD, the association and dissociation regions of the sensorgrams tend to gradually change. On the other hand, in the case of γ-CD, the *box type* sensorgrams obtained indicate that the association and dissociation rates tend to be fast. The SPR sensorgrams indicated that the association and dissociation rates of the interactions between γ-CD and drugs (hydrocortisone, dexamethasone and spironolactone) were faster than those between β-CD and drugs (quetiapine, amoxapine and trazodone) (Figs. 3, 4). This difference may be due to deformation of the β-CD macrocycle induced by the guest molecule [7]. If so, it is possible that the shape of the sensorgrams would reflect the kinetic characteristics of the formation and dissociation of these complexes. Although the association constants calculated from the SPR sensorgrams were smaller than those obtained from phase-solubility studies, there was good overall correlation between these data (Fig. 5). These results suggest that the interactions between CD and drugs might be hampered by steric hindrance arising from the direct immobilization of γ-CD to a dextran layer. If this is the case, then an improved method for immobilizing CD will be required, for example, by increasing the amount of immobilized 6-NH_2-γ-CD in order to enhance sensitivity, and by introducing a chemical linker to separate γ-CD from dextran layer and improve the mobility of γ-CD [8].

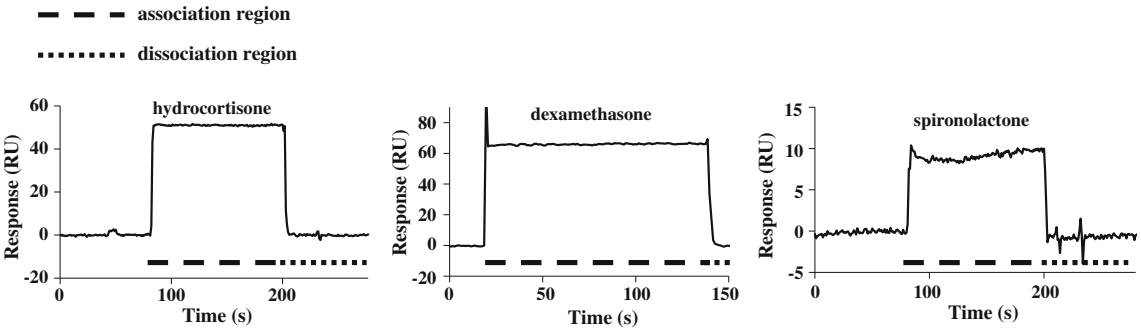

Fig. 3 SPR sensorgrams obtained by interactions between immobilized γ-CD and drugs

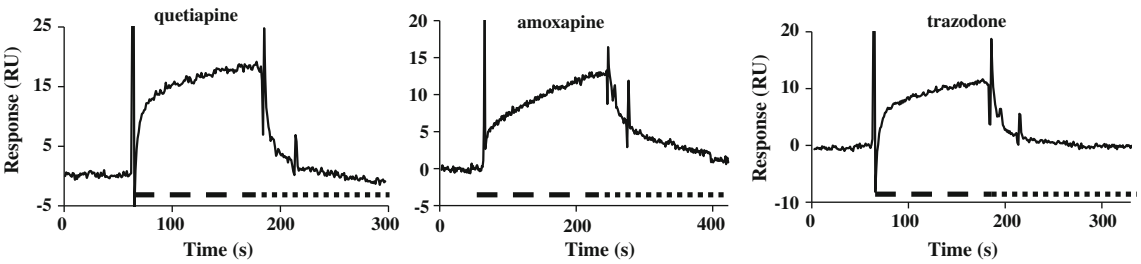

Fig. 4 SPR sensorgrams obtained by interactions between immobilized β-CD and drugs

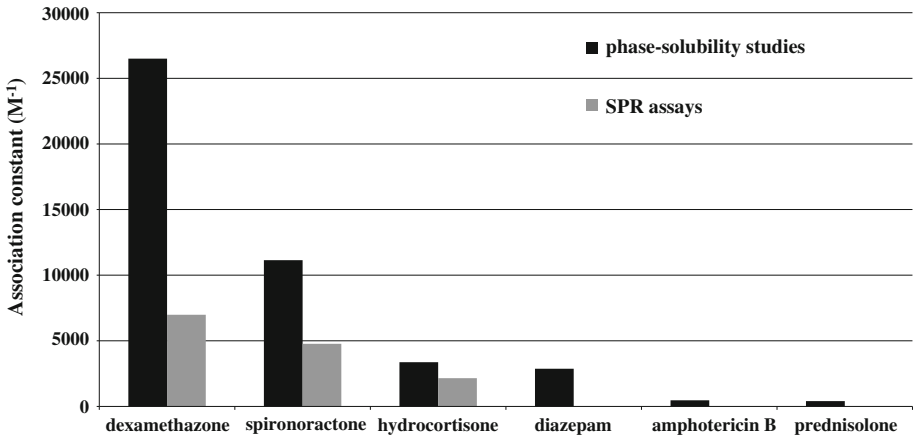

Fig. 5 Association constants calculated by phase-solubility studies and SPR assays

Conclusions

Immobilization of 6-NH_2-γ-CD (645 RU) on a Biacore® sensor chip was successfully accomplished using an amine coupling approach. Immobilized γ-CD on the sensor chip was stable and could be used for repeated measurements because no change in the SPR baseline was observed throughout the measurements. It may be possible to further apply the Biacore® assay to rare CDs such as modified CD or large-ring CDs [9]. The interactions between immobilized γ-CD and drugs (hydrocortisone, dexamethasone and spironolactone) were readily detected by SPR. The SPR studies required only very small amounts of drugs compared to phase-solubility studies, and full data sets could be obtained in 2 h. The sensorgrams indicated that the association and dissociation rates of the interaction between γ-CD and drugs (hydrocortisone, dexamethasone and spironolactone) were faster than those of β-CD and drugs (quetiapine, amoxapine and trazodone). The association constants calculated by SPR analysis were smaller than those obtained from phase-solubility studies, suggesting that improvements in the methodology for immobilizing CD are required.

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