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Improvement of some pharmaceutical properties of DY-9760e by sulfobutyl ether β-cyclodextrin

Y. Nagase [a], M. Hirata [b], K. Wada [b], H. Arima [b], F. Hirayama [b], T. Irie [b], M. Kikuchi [a], K. Uekama [b,*]

[a] *Tokyo Pharmaceutical Research Center, Daiichi Pharmaceutical Co. Ltd., 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*

[b] *Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862-0973, Japan*

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Abstract

The interaction of DY-9760e, a novel cytoprotective agent, with sulfobutyl ether β-cyclodextrin (SBE-β-CyD) in phosphate buffered saline (PBS) at various pH and ionic-strengths was studied by spectroscopic methods and the solubility method, and the results were compared with that of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD). The circular dichroism (CD) spectroscopic studies suggested that both β-CyDs form the inclusion complexes with DY-9760e in a molar ratio of 1:1, and the interaction of DY-9760e with SBE-β-CyD is much stronger than that with HP-β-CyD at any pH studied, in terms of a synergetic effect of hydrophobic and electrostatic interactions. The different intermolecular interaction between the SBE-and HP-β-CyD complexes was clearly reflected in the stability constant (K'), e.g. the different dependence of K' value on pH and ionic strength of solutions. ^{1}H- and ^{13}C-NMR studies suggested that HP-β-CyD interacts preferably with the benzene ring of DY-9760e, whereas SBE-β-CyD interacts not only with the benzene ring via hydrophobic interaction but also with the piperazine ring of the drug via electrostatic interaction. The solubilizing ability of SBE-β-CyD against DY-9760e was much greater than that of HP-β-CyD at any pH studied. Furthermore, SBE-β-CyD markedly suppressed the photo-degradation of DY-9760e in aqueous solution and reduced the adsorption of DY-9760e from PBS to polyvinyl chloride (PVC) tubes after incubation. The results suggest that SBE-β-CyD is useful in preparing parenteral solutions of poorly water-soluble drugs with positive charge such as DY-9760e.

Keywords: DY-9760e; Sulfobutyl ether β-cyclodextrin; 2-Hydroxypropyl-β-cyclodextrin; Inclusion complexation; Solubility; Photostability; Adsorption

1. Introduction

DY-9760e, 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperazinyl]ethyl]-5, 6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydrochloride 3.5 hydrate, is a novel cytoprotective agent on the basis of a

* Corresponding author. Tel.: +81-96-371-4160; fax: +81-96-371-4160.

E-mail address: uekama@gpo.kumamoto-u.ac.jp (K. Uekama).

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calmodulin inhibitory effect for the treatment of acute ischemic stroke (Sugimura et al., 1997; Sato et al., 1999; Fukunaga et al., 2000). Since DY-9760e is a dicationic compound with pK_a values of 5.38 (pK_{a1}) and 7.00 (pK_{a2}) in imidazole and piperazino groups, respectively, it exists as neutral, monocationic and dicationic species depending on pH of solutions (Fig. 1). In spite of its charged form, DY-9760e is poorly soluble in water (5.7 μM in pH 7.4 phosphate buffer at 25 °C). Therefore, some physicochemical drawbacks of DY-9760e including the marginal solubility and adsorption to plastic tubes and containers should be improved for development of injectable preparations.

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of several glucopyranose units and have been studied extensively in improvements of various physicochemical properties such as solubility and stability of drugs by forming inclusion complexes (Saenger, 1980; Frömming, 1994; Loftsson and Brewster, 1996; Szente and Szejtli, 1999). Recently, a number of chemically modified CyDs (Uekama et al., 1998) have been prepared to improve the inclusion capacity and the physicochemical properties of parent CyDs. Of the CyD derivatives, amorphous 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) and sulfobutyl ether β-cyclodextrin (SBE-β-CyD) have been widely investigated on account of their high solubility in water and minimal toxicity (Frijlink et al., 1990; Pitha, 1993; Albers and Müller, 1995; Stella and Rajewski, 1997; Thompson, 1997). It is noteworthy that oral preparation and parenteral injection for itraconazole, an anti-fungus drug, containing 40% (w/v) of HP-β-CyD, have been recently, commercialized in USA and Europe (Stevens, 1999).

Since DY-9760e partly carries a positive charge at physiological pH conditions, CyD derivatives with negative charge may be preferable over neutral CyD derivatives for improvement of the aforementioned drawbacks. In this study, therefore, the inclusion complexation of DY-9760e with SBE-β-CyD having negative charge in phosphate buffered saline (PBS) was investigated by the spectroscopic methods and the phase solubility method, and its inclusion capacity was compared with that of neutral HP-β-CyD. In addition, effects of SBE-β-CyD on the adsorption of DY-9760e from PBS to polyvinyl chloride (PVC) tubes and on the photo-stability of DY-9760e in aqueous solution were studied.

2. Materials and methods

2.1. Materials

DY-9760e was prepared in Daiichi Pharmaceutical Co (Tokyo, Japan). SBE-β-CyD and HP-β-CyD were kindly received from CyDex (Overland Park, KS) and Japan Mainz Co (Tokyo Japan), respectively. The average degrees of substitution (D.S.) of sulfobutyl ether and 2-hydroxypropyl groups in SBE-β-CyD and HP-β-CyD were 6.2 and 4.8, respectively. Deuterium oxide (D_2O), deuterium chloride (DCl) and sodium deuteroxide (NaOD) were purchased from Euriso-Top (GIF-SUR-YVETTE, France), Sigma Chemicals (St Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan), respectively. All other chemicals and solvents were of analytical reagent grade, and deionized, double distilled was used throughout the study.

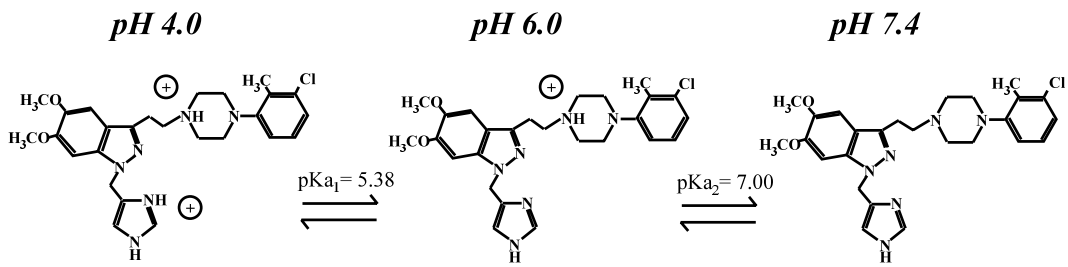

Fig. 1. Acid dissociation of DY-9760e in aqueous solution.

2.2. Spectroscopic studies

Circular dichroism (CD) spectra were recorded with a Jasco J-600 Circular Dichroic spectropolarimeter (Tokyo, Japan). Ionic strength of solutions was adjusted with sodium chloride. ^{1}H- and ^{13}C-NMR spectra were measured with a Jeol JNM-α 500 instrument (Tokyo, Japan) operating at 500 MHz and a sweep width of 10 000 Hz, at 25 °C. ^{1}H-NMR and ^{13}C-NMR chemical shifts were given as parts per million (ppm) downfield from that of tetramethylsilane, by referring to the HOD signal (4.65 ppm) and that of the dioxane signal (67.4 ppm), respectively. In ^{1}H-NMR and ^{13}C-NMR spectroscopic studies, DY-9760e (20.0 mM) and β-CyDs (20.0 mM) were dissolved in D_2O adjusted to pD 4.0 by adding DCl and NaOD solutions.

2.3. Solubility studies

The solubility method was employed according to the method of Higuchi and Connors (Higuchi and Connors, 1965). The excess amount of DY-9760e was added in the test tubes containing β-CyDs solutions at various concentrations in 10 mM PBS (pH 4.0, 6.0 and 7.4) at 25 °C. After equilibrium was attained (about 4 days), the suspension was centrifuged (1000 × *g*, 5 min), filtered through a membrane filter (Millex-GV13, Millipore, Tokyo, Japan) and analyzed for DY-9760e using high-performance liquid chromatography (HPLC). The conditions were as follows: a Hitachi 655 pump and a 638 UV detector (Tokyo, Japan) at 210 nm; a Hitachi D-2500 Chromato-Integrator (Tokyo, Japan); a GL-Sciences Inertsil ODS-2 column (4.6 mm i.d. × 150 mm, Tokyo, Japan); a mobile phase of 0.05 M phosphate buffer/acetonitrile (1:1 v/v); a flow rate of 1.0 ml/min. The stability constants of higher order complexes ($K_{1:1}$ and $K_{1:2}$) were calculated according to the optimization technique (Higuchi and Christiansen, 1970).

2.4. Adsorption studies

DY-9760e was dissolved in 10 mM PBS (pH 4.0, 6.0 and 7.4) with or without β-CyDs (0.1–2.0 mM) and the aliquot (0.26 ml) was added to PVC tubes (Terumo, 0.288 mm i.d. × 40 mm, 3.26 cm^2, Tokyo, Japan), and left in place at 25 °C for indicated times. The residual amount of DY-9760e was determined by HPLC described above.

2.5. Photo-stability studies

A fluorescent lump (Toshiba FLR40S. W/M.A. 40W, Tokyo, Japan) was used for irradiation. The aqueous solutions containing 4.0 mM DY-9670e in the absence and presence of β-CyDs (8.0 mM) were added to the clear or umber glass ampoule (Fuji Glass, USP Type 1, 10 ml, Tokyo, Japan), and the ampoules were irradiated for 20 days at 25 °C under an aerobic condition. The residual concentration of DY-9760e was determined by HPLC described above.

3. Results and discussion

3.1. CD Spectroscopic studies

The interaction of DY-9760e with SBE-β-CyD and HP-β-CyD in aqueous solutions was investigated by CD spectroscopic method. Fig. 2 shows CD spectra of DY-9760e in the absence and presence of β-CyDs in PBS at pH 4.0, 6.0 and 7.4 where the drug is predominantly in dicationic, monocationic and unionized forms, respectively. DY-9760e alone gave no CD band at any pH studied, because it has no asymmetric carbon in a molecule (Fig. 1). When HP-β-CyD or SBE-β-CyD was added to the DY-9760e solution, a negative Cotton effect was observed at 244 nm. The SBE-β-CyD system gave an additional positive CD band at approximately 290 nm, although its intensity was very small. It is well known that CyDs have no CD band at wavelengths longer than 220 nm and the inclusion of optically inactive compounds within the CyD's cavity generates extrinsic Cotton effects in the wavelength region of guest chromophores. Thus, the CD spectroscopic data indicate that DY-9760e is embedded in the asymmetric locus of the SBE- and HP-β-CyDs cavities. The CD intensity at 244 nm increased with the decrease in pH of the solution,

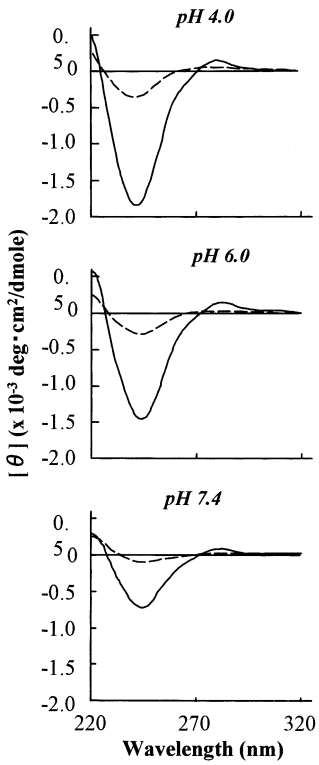

Fig. 2. Effects of β-CyDs on CD spectra of DY-9760e in phosphate buffered saline at 25 °C. The concentrations of DY-9760e and β-CyDs in PBS (pH 4.0, 6.0 and 7.4) were 0.05 and 1.0 mM, respectively. Solid and broken lines represent SBE-β-CyD and HP-β-CyD systems, respectively.

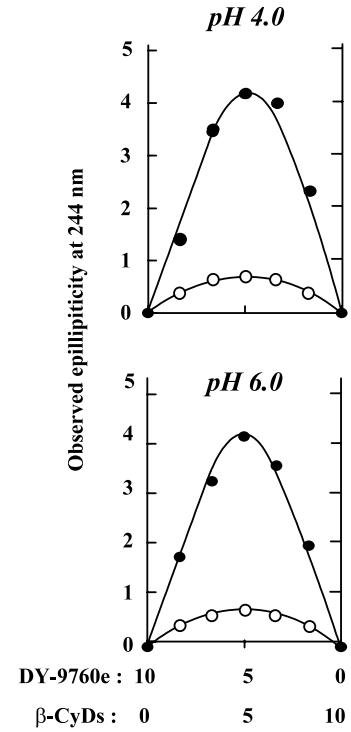

Fig. 3. Continuous variation plots for DY-9760e:β-CyDs systems in phosphate buffered saline at 25 °C. The total concentrations of DY-9760e and β-CyDs in PBS (pH 4.0 and 6.0) were 1.0 mM. Open and closed circles represent HP-β-CyD and SBE-β-CyD systems, respectively.

without band shifts. This intensity change was more significant in the SBE-β-CyD system than in the HP-β-CyD system, suggesting that the magnitude and/or mode of the interaction of DY-9760e with SBE-β-CyD differ from those with HP-β-CyD. To reveal the stoichiometry of the complexes, we performed continuous variation plots for the DY-9760e:β-CyDs system at 244 nm in PBS (pH 4.0 and 6.0 at 25 °C) as shown in Fig. 3. Here we could not make the plot at pH 7.4 because of the small induced CD band. The maximal intensity of the CD band was observed at the molar ratio of 1:1 at both pH, indicating that the guest forms inclusion complexes with SBE-β-CyD and HP-β-CD in a molar ratio of 1:1 under the experimental conditions.

The stability constants (K') of the complexes of DY-9760e with SBE-β-CyD and HP-β-CyD at pH 4.0, 6.0 and 7.4 (37 °C) were determined by analyzing changes in the negative CD band at 244 nm, according to the Scott method (Scott, 1956) assuming the 1:1 stoichiometry, and are summarized in Table 1. The K' values of the SBE-β-CyD complex were higher approximately 15.1, 7.3 and 2.6 times than those of the HP-β-CD complex at

Table 1
Effects of pH on Stability Constants (K') of Complexes of DY-9760e with β-CyDs in Phosphate Buffered Saline at 37 °C

System	K' (M^{-1})		
	pH 4.0	pH 6.0	pH 7.4
HP-β-CyD	630 ± 50	700 ± 70	1190 ± 460
SBE-β-CyD	9550 ± 960	5100 ± 300	3040 ± 430

pH 4.0, 6.0 and 7.4, respectively, indicating that DY-9760e interacts with SBE-β-CyD much more strongly than with HP-β-CyD at any pH studied. Interestingly, the effect of pH on the K' value was the opposite between the SBE- and HP-β-CyD complexes, i.e., the K' value increased with decreasing pH in the former complex whereas it deceased in the latter complex. Since most DY-9760e is in a dicationic form at pH 4.0, SBE-β-CyD with negative charge interacted strongly with DY-9760e at the acidic region, probably through a synergetic effect of both hydrophobic and electrostatic interactions. The contribution of the electrostatic interaction decreased at pH 7.4 where the guest is in a unionized form. On the other hand, HP-β-CyD, a neutral β-CyD derivative, showed a general trend that hydrophobic or lipophilic guests are preferably included in the hydrophobic CyD cavity. The unfavorable interaction of the dicationic DY-9760e with the hydrophobic CyD cavity was observed also for other neutral CyDs such as parent β-CyD and maltosyl-β-CyD which gave the K' values of 430($\pm$50) and 220($\pm$40) M^{-1} (at pH 4.0, 37 °C), respectively, smaller than that of SEB-β-CyD. The involvement of electrostatic interaction in the association of DY-9760e with SBE-β-CyD was confirmed by studying the effect of ionic strength on the K' value. The K' values of the SBE- and HP-β-CyD complexes with DY-9760e at different ionic strengths (I) were determined by the aforementioned Scott method of CD spectroscopy, and were 460($\pm$10) M^{-1} ($I = 0.01$ M) and 630($\pm$50) M^{-1} ($I = 0.16$ M) for the HP-β-CyD complex and 54500($\pm$250) M^{-1} ($I = 0.01$ M) and 9550($\pm$960) M^{-1} ($I = 0.16$ M) for the SBE-β-CyD complex in PBS (pH 4.0) and 37 °C. The K' value of the SBE-β-CyD complex decreased to about 1/6 when the ionic strength changed from 0.01 to 0.16 M, whereas that of the HP-β-CyD complex changed only slightly. This decreased association at high electric environments clearly indicates a significant involvement of the electrostatic attraction, together with hydrophobic interaction, in the complexation of DY-9760e with SBE-β-CyD. The temperature dependence of the K′ value of the SBE-β-CyD complex was studied to obtain thermodynamic parameters. However, the change of the K' value with temperature was too small to be accurately determined (K' values: 9500–8000 M^{-1} for 15–37 °C at pH 4.0 ($I = 0.16$ M)). The small temperature dependence of the K′ value indicates that the interaction between DY–9760e and SBE-β-CyD at pH 4.0 is predominantly an entropy-driven process, which may result from the liberation of water molecules hydrated around the charges of the guest and host molecules through the inclusion complexation.

It is of interest to note that the CD intensity of the HP-β-CyD complex decreased with increase in pH of the solution, in spite of the augmented K′ value at pH 7.4. It is reported that the sign and intensity of CyD-induced CD bands depends on a spatial relationship between the asymmetric center of CyD cavity and the perturbed chromophore of guest molecules, e.g., the electronic transition of an included guest with a transition dipole moment, parallel to the z-axis of the CyD cavity, gives a positive CD, and that with a transition dipole moment perpendicular to the z-axis gives a negative CD (Harata and Uedaira, 1975; Harata, 1981). Therefore, the change of CD intensity at 244 nm with pH suggested that the 3-chloro-2-methyl-benzene ring of DY-9760e, which was assigned to be a preferred inclusion site (as described later), is included within the HP-β-CyD cavity in slightly different orientations between pH 4.0 and 7.4.

3.2. Modes of the inclusion complexes

To gain insight into the inclusion mode of the complexes, ^{1}H- and ^{13}C-NMR spectroscopic studies were carried out for DY-9760e solutions in the absence and presence of SBE- and HP-β-CyDs in D_2O (pD 4.0). The pD 4.0 solution was employed because of the lower solubility of DY-9760e at pD 6.0 and 7.4. As shown in Fig. 4, the proton signals of the benzene ring and the methyl group (number 19–22) of DY-9760e shifted downfield upon the binding to both β-CyDs, suggesting that the benzene ring is involved in the complexation with both β-CyDs. In the ^{13}C-NMR spectra, the signals of the piperazine ring (number 12–15) significantly shifted downfield with concomitant downfield shifts of the benzene ring and the

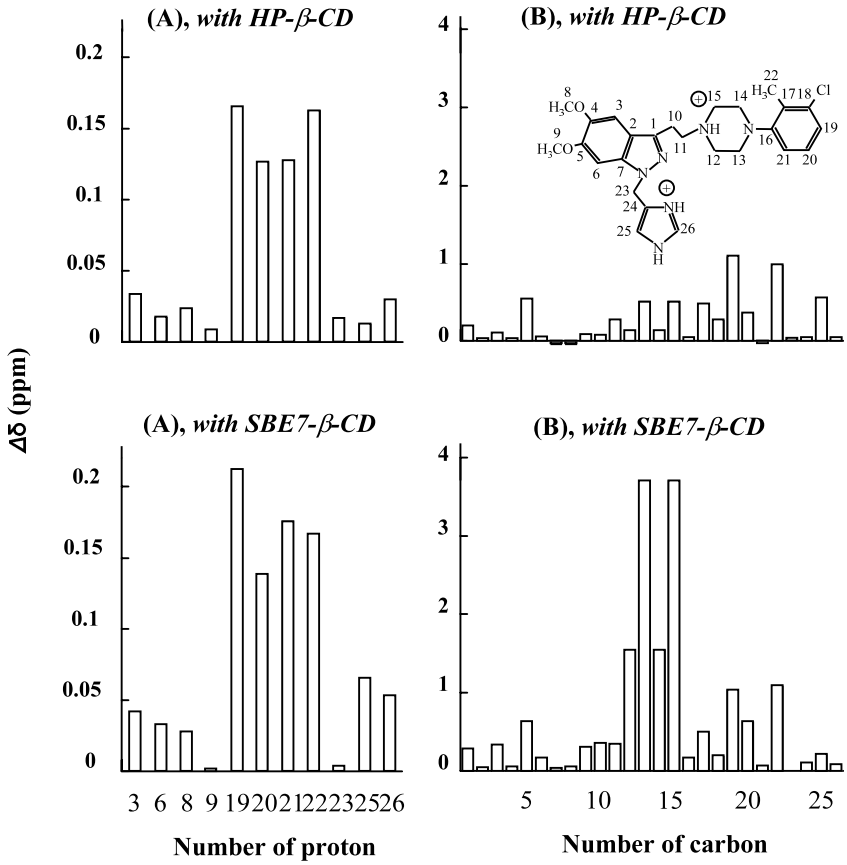

Fig. 4. ^{1}H-(A) and ^{13}C-NMR (B) chemical shift displacements of DT-9760e by binding to β-CyDs in D_2O at 25 °C. The concentrations of DY-9760e and β-CyDs in D_2O (pD 4.0) were 20.0 mM and 20.0 mM, respectively. $\triangle\delta = \delta_{\text{with β-CyDs}} - \delta_{\text{drug alone}}$.

methyl signals (number 19, 20 and 22) by the binding to SBE-β-CyD. HP-β-CyD gave the downfield shift of the benzene and the methyl signals (number 19, 20 and 22) similar to that of SBE-β-CyD, whereas the signals of the piperazine ring shifted negligibly in contrast to the case of SBE-β-CyD. The significant chemical shift of ^{13}C signals corresponding to the piperazine ring may be attributable to the great electrostatic interaction between the positively charged piperazine ring of DY-9760e and negatively charged sulfobutyl group of SBE-β-CyD. The inclusion modes of DY-9760e:β-CyDs are proposed as shown in Fig. 5, on the basis of the results of CD and NMR spectroscopic studies. HP-β-CyD is likely to enclose the benzene ring of DY-9760e due to hydrophobic interaction. Therefore, the stability constant of the HP-β-CD complex augmented with increase in pH of the solution, i.e. with increase in the fraction of unionized DY-9760e species (Table 1). Similarly, SBE-β-CyD encloses the benzene ring of the drug due to hydrophobic interaction, but the resulting complex is synergetically stabilized by the additional electrostatic interaction between sulfobutyl moieties of the host and the piperazine ring of the guest. Therefore, the stability constant of the SBE-β-CyD complex augmented with decrease in pH of the solution, i.e. with increase in the positively charged species of DY-9760e. Unfortunately, the difference in the orientation of the benzene moiety within the CyD cavity between pH 4.0 and 7.4 could not be determined by the NMR spectroscopic studies.

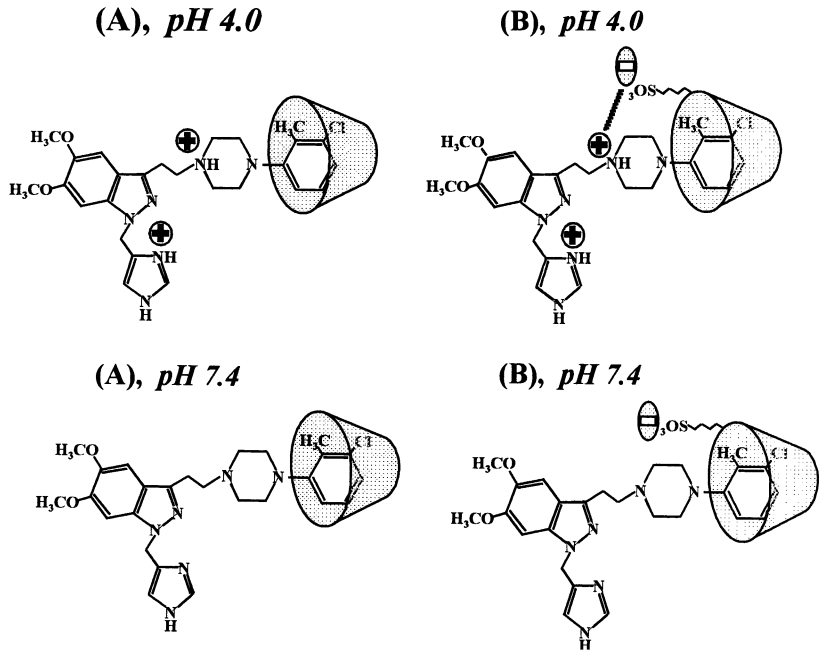

Fig. 5. Proposed modes for the inclusion complexes of DY-9760e with HP-β-CyD (A) and SBE-β-CyD (B) at pH 4.0 (upper) and 7.4 (lower).

3.3. Solubility studies

Fig. 6 shows the effects of β-CyDs on solubility of DY-9760e in PBS (pH 4.0, 6.0 and 7.4) at 25 °C. The intrinsic solubility of DY-9760e decreased with increasing pH due to the increase in the fraction of the unionized DY-9760e form. Ten mM of SBE-β-CyD and HP-β-CyD increased the solubility of DY-9760e, where the solubilizing effect of SBE-β-CyD was higher than that of HP-β-CyD at any pH studied. Fig. 7 shows phase solubility diagrams of DY-9760e with HP^{v} and SBE-β-CyDs in PBS (pH 7.4) at 25 °C. The extremely low solubility of DY-9760e (5.7 μM in pH 7.4 phosphate buffer at 25 °C) increased with β-CyD concentrations, showing a positive deviation from linearity. Thus, the solubility diagrams can be classified as the A_p type, defined by Higuchi and Connors (1965), suggesting the formation of higher-order complexes. Therefore, the upward curvatures were quantitatively analyzed according to the optimization technique to obtain the $K_{1:1}$ and $K_{1:2}$ values of 1:1 and 1:2 (guest:host) complexes (Higuchi and Christiansen 1970), and were $K_{1:1} = 830(\pm 100)\ M^{-1}$ and $K_{1:2} = 30(\pm 10)\ M^{-1}$ for the HP-β-CyD complex and $K_{1:1} = 2400(\pm 710)\ M^{-1}$ and $K_{1:2} = 110(\pm 20)\ M^{-1}$ for

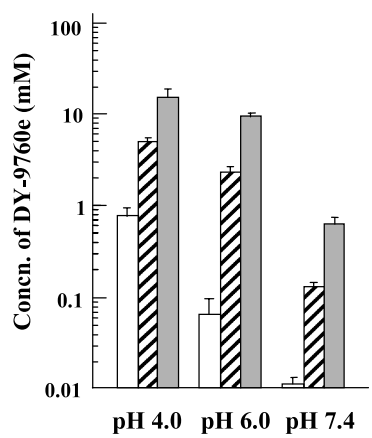

Fig. 6. Solubilities of DY-9760e in the absence and presence of β-CyDs in phosphate buffered saline at 25 °C. The concentrations of β-CyDs in PBS (pH 4.0, 6.0 and 7.4) were 10.0 mM. The open, hatched and shaded columns represent DY-9760e alone and HP-β-CyD and SBE-β-CyD systems, respectively.

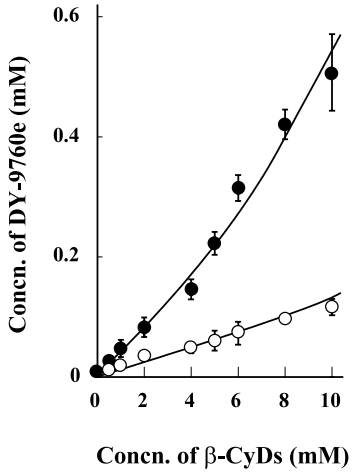

Fig. 7. Phase solubility diagrams for DY-9760e/β-CyDs systems in phosphate buffered saline (pH 7.4) 25 °C. Open and closed circles represent HP-β-CyD and SBE-β-CyD systems, respectively. Each point represents the mean ± S.E.M. of 2–4 experiments.

the SBE-β-CyD complex. The $K_{1:1}$ and $K_{1:2}$ values of the SBE-β-CyD complex were higher than those of the HP-β-CyD complex. The stoichiometry of the complexes was different from that estimated by the continuous variation method of CD spectroscopy. This may be due to the different experimental conditions, i.e. higher concentrations of β-CyDs used in the solubility method relative to those used in the CD method. The second β-CyD molecule may include the dimethoxybenzene moiety of DY-9760e, together with an additional interaction of the imidazole moiety with the sulphobutyl and hydroxyl groups of β-CyDs outside the cavity, because the small, but apparent downfield shifts were observed for both dimethoxybenzene and imidazole moieties. The solubilizing effect of SBE-β-CyD (D.S. = 6.2) on DY-9760e was much greater than that of SBE-β-CyD with a lower D.S. of 3.9) (data not shown), indicating the pivotal role of the sulfobutyl group in the solubilizing effect of SBE-β-CyD on the drug in PBS.

3.4. Adsorption of DY-9760e to PVC tubes

The adsorption of drugs to clinical tubes and containers should be reduced to prevent the decrease in drug contents in the solutions. To test whether DY-9760e adsorbs to clinical tubes for infusion, we studied the effect of the initial concentration of DY-9760e, incubation time and pH in PBS on the adsorption of DY-9760e to PVC tubes. As shown in Fig. 8A, the residual amounts of DY-9760e in PBS 60 min after the incubation increased with an increase in the initial concentration of DY-9760e, showing the adsorption isotherm curve of Freundlich's type, i.e. the monomolecular adsorption of DY-9760e to the tube. Fig. 8B shows the time-course of DY-9760e adsorption to the tubes. The residual amounts of DY-9760e in PBS rapidly decreased in the initial phase (< 10 min) and then gradually decreased, showing a biphasic pattern of the adsorption. In addition, the adsorption of DY-9760e to the tube increased with a rise in pH of PBS (Fig. 8C). This adsorption pattern of DY-9760e suggested that the adsorption of DY-9760e results from the hydrophobic adsorption mechanism, rather than from the ionic adsorption mechanism. Fig. 9 shows the effect of β-CyDs on the adsorption of DY-9760e to the tubes. SBE-β-CyD reduced the adsorption of DY-9760e much more significantly than HP-β-CyD, reflecting the higher stability

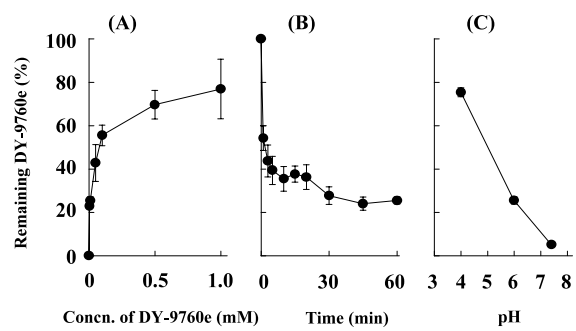

Fig. 8. Adsorption behaviors of DY-9760e to polyvinyl chloride tubes in phosphate buffered saline. (A) Effect of initial concentrations of DY-9760e on residual percentage of DY-9760e in PBS 60 min after incubation. (B) Time-course of adsorption of DY-9760e to the PVC tubes. (C) Effect of pH of PBS on residual percentage of DY-9760e in PBS 60 min after incubation. In the (B) and (C) studies, the initial concentration of DY-9760e in PBS (pH 4.0, 6.0 and 7.4) was 10 μM. The residual concentration of DY-9760e in PBS at various times after incubation was measured by HPLC. Each value represents the mean ± S.E.M. of 2–4 experiments.

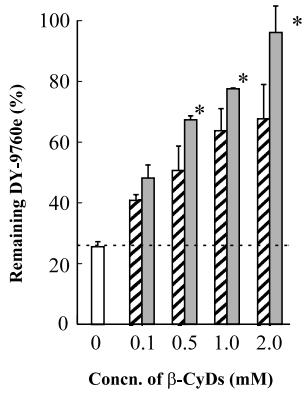

Fig. 9. Effects of β-CyDs on the adsorption of DY-9760e to polyvinyl chloride tube in phosphate buffered saline (pH 6.0) 25 °C. The residual concentration of DY-9760e in PBS 60 min after incubation was measured by HPLC. The open, hatched and shaded columns represent DY-9760e alone and HP-β-CyD and SBE-β-CyD systems, respectively. Each value represents the mean ± S.E.M. of 3 experiments. $*P < 0.05$ versus HP-β-CyD system.

constants. Thus, the inhibitory effect of SBE-β-CyD on the adsorption of DY-9760e can be ascribed to the masking effect of SBE-β-CyD against the hydrophobic interaction between the surface of PVC tubes and DY-9760e molecules.

3.5. Photo-stability of DY-9760e

The effect of SBE-β-CyD on the photo-stability of DY-9760e was investigated, because its solubilizing and adsorption-inhibiting abilities were much greater than those of HP-β-CyD. Fig. 10 shows the residual percentage of DY-9760e after irradiation (2500 lux) for 20 days at 25 °C in the absence and presence of SBE-β-CyD. Here we used clear and umber ampoules to perform the photo-stability tests. When the clear ampoules were used, approximately 10% of DY-9760e degraded for 20 days and the solution's color changed from white to pale yellow. Adding SBE-β-CyD to the solution, the degradation of DY-9760e and coloration of the solution were markedly reduced. When the umber ampoules were used, DY-9760e scarcely degraded with only slight coloration in the absence of SBE-β-CyD. On the other hand, no degradation or coloration of DY-9760e was observed in the presence of SBE-β-CyD. These results suggest that SBE-β-CyD can improve the photo-stability of DY-9760e in aqueous solution.

In conclusion, the present data indicate that DY-9760e forms a more stable inclusion complex with SBE-β-CyD than HP-β-CyD, resulting in significant improvements of the aqueous solubility and the photo-stability of DY-9760e and in the reduction of the adsorption of the drug to PVC tubes. Moreover, our preliminary study has revealed that SBE-β-CyD reduced the local irritation of DY-9760e more significantly against erythrocytes and vascular endothelial cells, and the alleviating effect of SBE-β-CyD was greater than that of HP-β-CyD (data not shown). These results suggest that SBE-β-CyD is a useful carrier for parenteral solutions of DY-9760e.

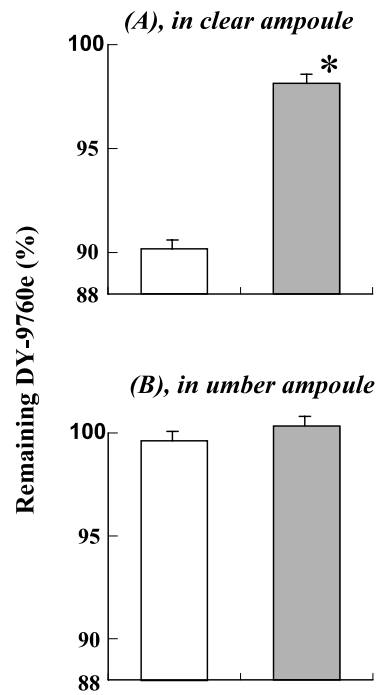

Fig. 10. Effects of SBE-β-CyD on photodecomposition of DY-9760e in water for injection. The solutions (pH 4.0) in clear ampoule (A) or umber ampoule (B) were irradiated to 2500 lux for 20 days at 25 °C. The open and shaded columns represent DY-9760e alone and SBE-β-CyD systems, respectively. The initial concentrations of DY-9760e and SBE-β-CyD were 4.0 and 8.0 mM, respectively. Each value represents the mean ± S.E.M. of 3 experiments. $*P < 0.01$ versus DY-9760e alone.

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References

Albers, E., Müller, B.W., 1995. Cyclodextrin derivatives in pharmaceutics. CRC Crit. Rev. Ther. Drug Carrier Syst. 12, 311–337.

Frijlink, H.W., Visser, J., Hefting, N.R., Oosting, R., Meijer, D.K.F., Lerk, C.F., 1990. The Pharmacokinetics of β-cyclodextrin and hydroxypropyl β-cyclodextrin in the rats. Pharm. Res. 7, 1248–1253.

Frömming, K.H., 1994. In: Szejtli, J. (Ed.), Cyclodextrins in Pharmacy. Kluwer, Dordrecht.

Fukunaga, K., Ohmitsu, M., Miyamoto, E., Sato, T., Sugimura, M., Uchida, T., Shirasaki, Y., 2000. Inhibition of neuronal nitric oxide synthase activity by 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperazinyl]ethyl]-5, 6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydrochloride 3.5 hydrate (DY-9760e), a novel neuroprotective agent. in vitro and in cultured neuroblastoma cells in situ. Biochem. Pharmacol. 60, 693–699.

Harata, K., 1981. Induced circular dichroism of cycloamylose complexes with meta- and para-disubstituted benzenes. Bioorg. Chem. 10, 255–265.

Harata, K., Uedaira, H., 1975. The circular dichroism spectra of the β-cyclodextrin complex with naphthalene derivatives. Bull. Chem. Soc. Jpn. 48, 375–378.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instr. 7, 117–212.

Higuchi, T., Christiansen, H., 1970. Binding specificity between small organic solutes in aqueous solution: Classification of some solutes into two groups according to binding tendencies. J. Pharm. Sci. 59, 1601–1608.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Pitha, J., 1993. Pharmacological role of hydroxypropyl-β-cyclodextrins. In: Duchêne, D. (Ed.), New Trends in Cyclodextrins and Derivatives. Edition de Santé, Paris, pp. 351–367.

Saenger, W., 1980. Cyclodextrin inclusion compounds in research and industry. Angrew. Chem. Int. Ed. Engl. 19, 344–362.

Sato, T., Morishita, Y., Sugimura, M., Uchida, T., Shirasaki, Y., 1999. DY-9760e, a novel calmodulin antagonist, reduces brain damage induced by transient focal cerebral ischemia. Eur. J. Pharmacol. 370, 117–123.

Scott, R.L., 1956. Some comments on Benesi-Hildebrand equation. Rec. Trav. Chim. 75, 787–789.

Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: Their future in drug formulation and delivery. Pharm. Res. 14, 556–567.

Stevens, D.A., 1999. Itraconazole in cyclodextrin solution. Pharmacotherapy 19, 603–611.

Sugimura, M., Sato, T., Nakayama, W., Morishita, Y., Fukunaga, K., Omitsu, M., Miyamoto, E., Shirasaki, Y., 1997. DY-9760e, a novel calmodulin antagonist with cytoprotective action. Eur. J. Pharmacol. 336, 99–106.

Szente, L., Szejtli, J., 1999. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv. Drug Deliv. Rev. 36, 17–28.

Thompson, D.O., 1997. Cyclodextrin-enabling excipients: their present and future use in pharmaceuticals. CRC Crit. Rev. Ther. Drug Carrier Syst. 14, 1–104.

Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier system. Chem. Rev. 98, 2045–2076.