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Inclusion Complexation of a Seleno-Organic Antioxidant, Ebselen, with Cyclodextrins in Aqueous Solution

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Abstract

The interaction of ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one, a novel neuroprotective agent, with cyclodextrins (CyDs) in aqueous solution was studied by the solubility method and spectroscopic methods. The ability of sulfobutyl ether β-CyD (SBE7-β-CyD, average degree of substitution= 6.2) to solubilize ebselen was greater, and its stability constant ($> 2000\ M^{-1}$) was significantly higher than those ($< 1000\ M^{-1}$) of other CyD complexes employed. The stability constant of the complexes rose as hydrophobicity of the substituents of CyDs increased, whereas it was negligibly affected by change in ionic strength of the medium, indicating a significant contribution of hydrophobic interaction in the complexation. SBE7-β-CyD gave positive and negative CD bands at around 320 and 350 nm, respectively, indicating that the guest is embedded in the asymmetric locus of the CyD cavity. ^{1}H-NMR spectroscopic studies suggested that the mono-substituted benzene ring of ebselen is preferably included in the cavity of SBE7-β-CyD. The results indicate that SBE7-β-CyD is useful as a solubilizing agent for ebselen.

Introduction

Recently, selenium-containing molecules have received great attention in medicinal chemistry field [1, 2]. For example, glutathione peroxidase is a metalloenzyme containing selenium atom in a molecule, and can block the production of reactive oxygen species such as superoxide radicals, organic peroxides and hydrogen peroxide, protecting biomemebranes from their damage. Therefore, a great deal of effort has been devoted to artificial glutathione peroxidase mimics, including selenium-bearing cyclodextrin derivatives [3, 4], to obtain novel medicines for treatment of oxidative stress related diseases such brain ischemia, reperfusion injury, inflammation and physiological ageing. Ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one (Figure 1), is a small molecular mimics of glutathione peroxidase with a potent antioxidant effect, and the oral formulation of ebselen has been demonstrated to have an evidence of benefit in Phase 3 clinical stroke trials [5, 6]. However, it is desirable for ebselen to develop parenteral preparations, because a rapid delivery of the drug to the brain is necessary. Unfortunately, ebselen exhibits the extremely low solubility (about 3 μg/mL) in aqueous solution, which is about 1/300 of the desirable concentrations (1 mg/mL) needed for parenteral formulations to be used in early clinical studies. Recently,

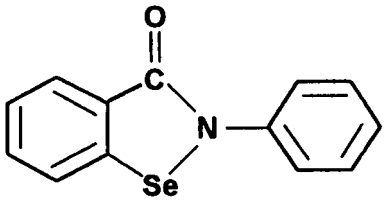

Figure 1. Chemical structure of ebselen.

various hydrophilic cyclodextrins (CyDs) have been applied for the solubilization of poorly water-soluble drugs [7, 8]. Therefore, this study deals with comparison of the ability of various CyDs to solubilize ebselen.

Experimental

Materials

Ebselen was purchased from Sigma (St. Louis, USA). Sulfobutylethers of β-CyD (SBE4-and SBE7-β-CyDs, average degree of substitution (DS) = 4 and 6.2, respectively) were supplied from the CyDex Co. (Overland, KS, USA). α-CyD, β-CyD, γ-CyD, 2-hydroxyethyl-β-CyD (HE-β-CyD, DS = 3), 2-hydroxypropyl-β-CyD (HP-β-CyD, DS = 4.4), 2-hydroxybutyl-β-CyD (HB-β-CyD, DS = 5), and 2,6-di-*O*-methyl-β-CyD (DM-β-CyD) were obtained from Nihon

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Shokuhin Kako Co. (Tokyo, Japan). 6-*O*-Maltosyl-β-CyD (G2-β-CyD) was obtained from the Bio Research Corporation of Yokohama (Yokohama, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Solubility measurements

Solubility studies were carried out according to the method of Higuchi and Connors [9]. The screw-capped vials containing ebselen in excess amounts in aqueous CyD solutions (pH 7.0 phosphate buffer, I = 0.2) at various concentrations were shaken at 25 °C. After equilibrium was attained, the suspension was filtered through a pipette with a cotton-plug, and the filtrate was adequately diluted and analyzed for ebselen using high-performance liquid chromatography under the following conditions: a Jasco PU-1580 pump and a Jasco UV-970 detector (Tokyo, Japan), a column of GL-science inertsil ODS-2 (Tokyo, Japan), a mobile phase of methanol/H_2O (11 : 9 v/v), a flow rate of 1.0 mL/min, and a detection of 230 nm. The 1 : 1 stability constant (K_c) of CyD complexes was calculated from the initial straight line portion of the phase solubility diagrams according to the equation of K_c = slope/{S_0 (1 − slope)} [9], where S_0 is the intrinsic solubility of ebselen. The upward-curve of the Ap-type solubility diagram was analyzed according to the method of Higuchi and Kristiansen [10] to obtain the 1 : 1 and 1 : 2 (guest : host) stability constants.

Spectroscopic studies

Circular dichroism (CD) spectra of ebselen (0.025 mM) in pH 7.0 phosphate buffer (I = 0.2) containing 5% methanol and SBE7-β-CyD (0–2.5 mM) were recorded with a Jasco J-600 polarimeter (Tokyo, Japan). The 1 : 1 stability constant of the ebselen/SBE7-β-CyD complex was determined by analyzing changes in the induced CD intensity of ebselen at 315 nm by the Scott equation [11]. ^{1}H-NMR spectra of ebselen (0.5 mM) in deuterium oxide (D_2O) containing 40% methanol-d_4 and SBE7-β-CyD (0-10.0 mM) were measured with a Jeol JNM-α-500 instrument (500 MHz) at 25 °C.

Results and discussion

Solubility studies

Figure 2 shows the phase solubility diagrams of ebselen with α-, β- and γ-CyDs and various β-CyD derivatives in pH 7.0 phosphate buffer (I = 0.2), where the solubility of the drug increased as a function of CyD concentration. The solubility curve of the α-CyD system deviated positively from a straight line and classified as Ap type [9], indicating a formation of higher order complexes. The 1 : 1 and 1 : 2 (guest : host) complexations were assumed for the α-CyD complex, by considering molecular sizes of the guest and the cavity of α-CyD, and then the 1 : 1 ($K_{1:1}$) and 1 : 2 ($K_{1:2}$) stability constants were determined by analyzing the upward-curvature of the diagram according to the method of

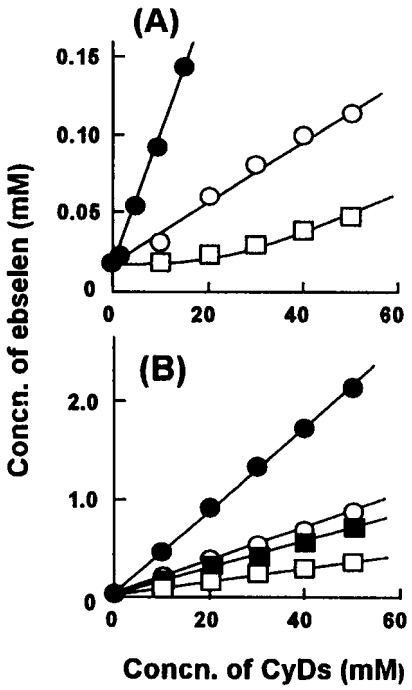

Figure 2. Phase solubility diagrams of ebselen/CyD systems in pH 7.0 phosphate buffer (I = 0.2) at 25 °C. (A) □: α-CyD, ●: β-CyD, ○: γ-CyD. (B) ●: SBE7-β-CyD, ○: DM-β-CyD, ■: HP-β-CyD, □: G_2-β-CyD.

Higuchi and Kristiansen [10]. On the other hand, β-CyD, γ-CyD and β-CyD derivatives showed A_L type-solubility diagrams, where the solubility of the drug increased linearly with CyD concentration, suggesting the 1 : 1 complexation. The 1 : 1 stability constant (Kc) of these complexes was determined by the Higuchi equation (see Experimental). The $K_{1:1}$ and $K_{1:2}$ values of the α-CyD complex were 10 M^{-1} and 50 M^{-1}, respectively. The Kc value of the other complexes increased in the order of SBE7-β-CyD (2700 M^{-1}) > DM-β-CyD (1000 M^{-1}) > HP-β-CyD (800 M^{-1}) > β-CyD (480 M^{-1}) ≈ G_2-β-CyD (430 M^{-1}) > γ-CyD (100 M^{-1}). Among CyDs employed, SBE7-β-CyD had the highest ability to solublize ebselen. For example, the solubility of ebselen increased to 0.6 mg/mL at 50 mM SBE7-β-CyD, suggesting that the concentration of 1 mg/mL ebselen can be obtained at about 80 mM SBE7-β-CyD. The concentration of 17%/w/v (about 80 mM) of SBE7-β-CyD is lower than half as the concentration (40%w/v) of HP-β-CyD used in a commercially available itraconazole preparation [12].

DM-β-CyD is known to have a superior solubilizing ability to poorly water-soluble drugs studied so far [8]. However, the solubilizing ability of SBE7-β-CyD to ebselen was much higher than that of DM-β-CyD, in spite of that SBE7-β-CyD has 6-7 anionic charges as well as the hydrophobic butyl groups in a molecule. These results suggest that hydrophobicity near the cavity plays an important role in the inclusion of ebselen, rather than the ionic character of sulfonate groups extending far from the cavity. Therefore, the interaction of ebselen with 2-hydroxyalkylated β-CyD having different chain lengths was investigated by the solubility method. The solubility of ebselen increased linearly as concentration of HE-β-CyD (2-hydroxyethyl), HP-β-CyD (2-

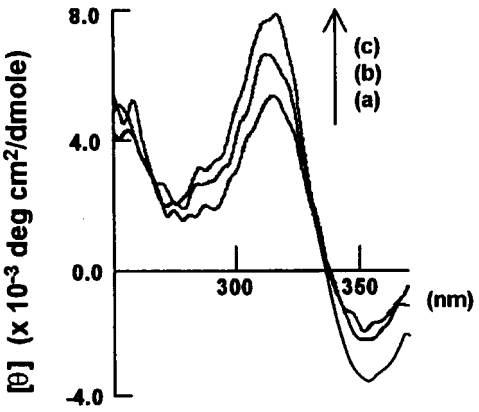

Figure 3. CD spectrum of ebselen (0.025 mM) in the presence of SBE7-β-CyD in pH 7.0 phosphate buffer (I = 0.2) containing 5% methanol at 25 °C. a: with 0.625 mM SBE7-β-CyD, b: with 1.25 mM SBE7-β-CyD, c: with 2.5 mM SBE7-β-CyD.

hydroxypropyl) and HB-β-CyD (2-hydroxybutyl) increased, showing A_L type diagrams (data not shown). The Kc value of the complexes increased in the order of HB-β-CyD (1600 M^{-1}) > HP-β-CyD (800 M^{-1}) > HE-β-CyD (500 M^{-1}). Furthermore, the Kc value (2700 M^{-1}) of the ebselen complex with SBE7-β-CyD (DS 6.2) was higher than that (2000 M^{-1}) of SBE4-β-CyD (DS 4). Effects of ionic strength (I) of media on the Kc value were further investigated by the solubility method to confirm the role of hydrophobic interaction in the complexation of SBE7-β-CyD with ebselen. Only slight change in the Kc value was observed with change in I value, i.e., Kc = 2700 M^{-1} (I = 0.2) and Kc = 2500 M^{-1} (I = 0.02). These results indicate that hydrophobic interaction contributes significantly to the inclusion of hydrophobic ebselen in the SBE7-β-CyD cavities, whereas electrostatic interaction may be of less importance in the complexation.

Spectroscopic studies

Figure 3 shows CD spectra of ebselen in the presence of SBE7-β-CyD in pH 7.0 phosphate buffer (I = 0.2). The optical activity of ebselen was induced at around 320 nm and 350 nm with positive and negative signs, respectively. Since intrinsic Cotton effects of SBE7-β-CyD are observed only below 220 nm and ebselen has no asymmetric carbon in a molecule, these CD bands are attributable to the induced optical activity of ebselen, indicating that the drug molecule is embedded in the asymmetric locus of the SBE7-β-CyD cavity. The induced CD intensities increased as concentration of SBE7-β-CyD increased. These changes in CD intensity at 315 nm were analyzed by the Scott equation [11], and the Kc value of 3600 M^{-1} was obtained for the ebselen/SBE7-β-CyD complex. This value was higher than that obtained by the solubility method (2700 M^{-1}), probably because of different experimental conditions such as concentrations of the guest.

To gain insight into the inclusion structure of the SBE7-β-CyD complex with ebselen, ^{1}H-NMR spectroscopic studies were conducted. Figure 4 shows ^{1}H-NMR spectra of ebselen in SBE7-β-CyD/D_2O solutions (40%v/v methanol/D_2O) at different concentrations. The mixed

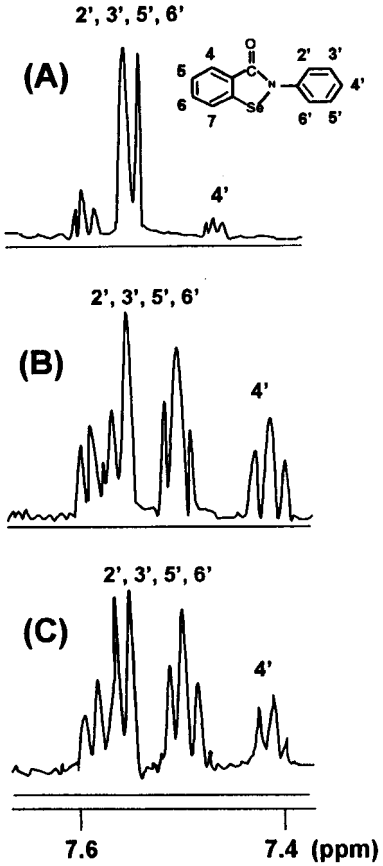

Figure 4. ^{1}H-NMR spectra of ebselen (0.5 mM) in the presence of SBE7-β-CyD in D_2O containing 40% methanol-d4 at 25 °C. (A) with 1.0 mM SBE7-β-CyD, (B) with 5.0 mM SBE7-β-CyD, (C) with 10.0 mM SBE7-β-CyD.

solvent was used to obtain concentrations of ebselen high enough to permit NMR measurements. In the ^{1}H-NMR spectra (Figure 4), large spectral changes of ebselen were observed in the mono-substituted benzene ring (2'∼6' protons) by the addition of SBE7-β-CyD, whereas the benzisoselenazolone ring (4∼7 protons) showed no appreciable spectral changes. These results suggested that SBE7-β-CyD includes preferably the mono-substituted benzene ring of ebselen. Detailed analyses of NMR spectroscopic data are now under investigation, including two-dimensional NMR studies.

Conclusion

The ability of SBE7-β-CyD to include and to solubilize ebselen was much stronger than other CyDs used in this study, indicating that SBE7-β-CyD is useful as a solubilizing agent and enables to develop parenteral formulations of ebselen (1 mg/mL).

References

1. G. Mugesh, W.-W. du Mont, and H. Sies: *Chem. Rev.* **101**, 2125 (2001).
2. M.J. Parnham and E. Graf: *Prog. Drug Res.* **36**, 9 (1991).

3. Y. Liu, B. Li, and H.-Y. Zhang: *Helv. Chim. Act.* **85**, 9 (2002).
4. X. Ren, J. Liu, G. Luo, Y. Zhang, Y. Luo, G. Yan, and J. Shen: *Bioconjugate Chem.* **11**, 682 (2000).
5. M. Parnham and H. Sies: *Exp. Opin. Invest. Drugs* **9**, 1 (2000).
6. T. Yamaguchi, K. Sato, K. Takakura, I. Saito, Y. Shinohara, T. Asano, and H. Yasuhara: *Stroke* **29**, 12 (1998).
7. T. Loftsson and M. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
8. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
9. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instr.* **4**, 117 (1965).
10. T. Higuchi and H. Kristiansen: *J. Pharm. Sci.* **59**, 1601 (1970).
11. R.L. Scott: *Rec. Trav. Chim.* **75**, 787 (1956).
12. K. Miyake, T. Irie, H. Arima, F. Hirayama, K. Uekama, M. Hirano, and Y. Okamoto: *Int. J. Pharm.* **179**, 237 (1999).