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Quantitative Estimation of the Bitter Taste Intensity of Oxyphenonium Bromide Reduced by Cyclodextrins from Electromotive Force Measurements

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The bitter taste of oxyphenonium bromide, an antiacetylcholine drug, is suppressed by cyclodextrins. The extent of the suppression can be predicted from the electromotive force measurements with an oxyphenonium bromide-selective electrode. The relationship between the bitter taste intensity and the electromotive force holds true, regardless of the kind and concentration of natural and modified cyclodextrins. This result is explicable on the basis of the observation that both the bitter taste and the electric potential are determined by the concentration of free oxyphenonium bromide. Some implications and limitations of the present approach are discussed.

The developments and various applications of new electrochemical sensors continue to be a rapidly growing area of analytical chemistry. Many researchers are currently working on constructing new drug-sensitive membrane sensors to monitor certain drugs in pure form, complex pharmaceutical formulations, and biological materials. For analytical control of pharmaceuticals, membrane sensor techniques offer several advantages in terms of simplicity, rapidity, specificity, and accuracy over many known methods.[1,2]

Cyclodextrins have homogeneous toroidal structures of different sizes. One side of the torus contains primary hydroxyl groups, whereas the secondary groups are located on the other side. The toroidal structure has a hydrophilic surface resulting from the 2-, 4-, and 6-position hydroxyls, making them water soluble. The cavity is composed of the glucoside oxygens and methylene hydrogens, giving it a hydrophobic character.[3,4] Cyclodextrins are used for separations and quantitative analysis of very similar compounds, including optical isomers, by high-performance liquid chromatography and capillary electrophoresis.[4−6] Cyclodextrins can give beneficial modifications of guest molecules not otherwise achievable: solubility enhancement, stabilization of labile guests, control of volatility and sublimation, and physical isolation of incompatible compounds. Because they are practically nontoxic, they are added into pharmaceuticals and foods for stabilization of labile compounds and long-term protection of color, odor, and flavor.[3] Furthermore, cyclodextrins can mask bitter tastes of drugs, e.g., propantheline bromide.[7] Sensory tests generally depend on individuals. Some instrumental methods, therefore, are desired for such tests.

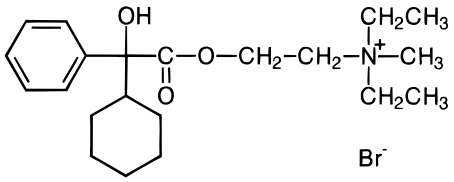

Figure 1. Chemical structure of OB.

In this work, we develop an ion-selective electrode method for the quantitative estimation of the bitter taste intensity of oxyphenonium bromide (Figure 1), an antiacetylcholine drug, in aqueous solutions of cyclodextrins. Electromotive force measurements have been applied to investigate the determination of binding constants of cyclodextrins with guests, such as drugs[8,9] and surfactants.[10,11] It is noted that the electromotive force solely depends on the concentration of free guest, regardless of the concentrations of cyclodextrins and their complexes. Bitter

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Table 1. Degree of Substitution and Binding Constants of Oxyphenonium Bromide for Cyclodextrins at 309.7 K

CD	deg sub	K_1 (M^{-1})	n	s (mV)
α-CD		58	12	0.54
β-CD		8500	11	1.02
γ-CD		96	12	0.38
DMβ-CD	~2	6660	11	2.89
Mβ-CD	0.5−0.7	4290	11	3.08
HPβ-CD	0.6	1460	11	2.84
GLβ-CD	~1	1920	11	5.17
CMβ-CD	?	10010	10	3.80
MLβ-CD	~1	7510	11	4.06

compounds are generally hydrophobic,[12] but their cyclodextrin complexes are rather hydrophilic, because of the hydrophilicity of cyclodextrins.[7] Thus we can expect that the bitter taste of oxyphenonium bromide in a cyclodextrin solution is estimated from the value of electromotive force of an appropriate ion-selective electrode.

EXPERIMENTAL SECTION

Reagents. Oxyphenonium bromide (OB) was purchased from Sigma Chemical Co. Because this sample was analyzed to be pure by reversed-phase liquid chromatography, it was used without purification. Sodium bromide of analytical grade and α-, β-, and γ-CDs from Nacalai Tesque Co. were used as received. 2,6-*O*-Dimethyl-β-CD (DMβ-CD), 2-hydroxypropyl-β-CD (HPβ-CD), 6-maltosyl-β-CD (MLβ-CD), and 6-glucosyl-β-CD (GLβ-CD) were purchased from Nacalai Tesque Co. These modified CDs were mixtures of CDs different in degrees of substitution as detected by reversed-phase liquid chromatography. The degree of substitution for some cyclodextrins, provided by Nacalai Tesque Co., is shown in Table 1. Carboxymethyl-β-CD (CMβ-CD) and methyl-β-CD (Mβ-CD) were obtained from Cylcolab, Budapest, and Sigma Chemical Co., respectively. Sodium tetraphenylborate, *o*-nitrophenyl octyl ether, and tetrahydrofuran (THF) were obtained from Dojindo Laboratories. Poly(vinyl chloride) (PVC) was from Wako Pure Chemicals Co. The ion-exchanged water was used after double distillation.

Preparation of the PVC Membrane Electrode. The PVC membrane was prepared according to the methods used by Zhang et al.[13] and recommended by Denki Kagaku Keiki Co. (DKK). Sodium tetraphenylborate (5 mg) and 1.9 g of *o*-nitrophenyl octyl ether (plasticizer) were dissolved into 8 cm^3 of THF. Then PVC (0.2 g) was added stepwise into the THF solution under magnetic stirring. Immediately after the DKK membrane filter (6 mm in diameter), previously immersed in THF, was transferred into the THF membrane solution, it was fitted to the tip of an ion-selective electrode body. Further, a drop of the membrane solution was added with a micropipet to the fixed filter, followed by evaporation of the THF in 20 min. This operation was repeated 10 times. The resulting membrane body was soaked in a 10 mM OB solution in 3 h. Then an internal solution containing 1 mM OB and 10 mM NaBr was filled into the body. Finally, an Ag/AgBr electrode was mounted to the body. The electrode was stored in 1 mM OB solution.

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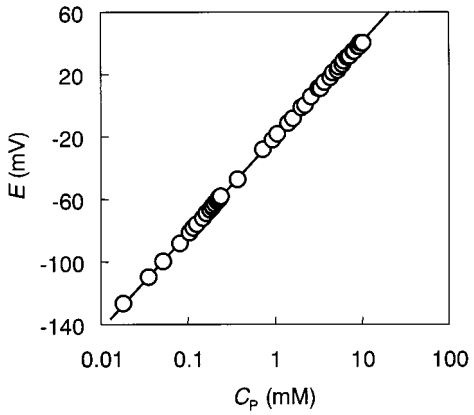

Figure 2. Electromotive force plotted against the logarithm of the OB concentration in the absence of CD. The solid line is calculated from eq 1.

Measurements of Electromotive Forces. Potentiometric measurements were carried out with a DKK model IOL-40 digital pH/mV meter. The electrochemical cell was constructed as follows: Ag/AgCl|KCl solution|PVC membrane|sample solution|1 mM OB, 10 mM NaBr|AgBr/Ag. The Ag/AgBr electrode was kindly supplied by DKK. The electromotive force was referred to a DKK 4083-0.65C double-junction reference electrode. The vessel containing the sample solution was jacketed to maintain a constant temperature of 309.7 ± 0.1 K. The temperature was monitored continuously with a thermometer. The electromotive force of a fresh aqueous solution reached an equilibrium value typically within 2 min. The response was faster as the OB concentration was increased. The calibration curve for OB was determined as follows: 25 cm^3 of a solution containing 0.02 mM OB and 154 mM NaBr was titrated successively by a 20 mM OB solution and the equilibrium potential was measured digitally. The results of three runs are reported herein. The effect of the CD concentration on the potential of a 4 mM OB solution was investigated as follows: 15 cm^3 of a solution containing 4 mM OB, CD, and 154 mM NaBr was titrated stepwise by a solution of 4 mM OB and 154 mM NaBr. For β-CD, a solution of 154 mM NaBr and 4 mM OB was titrated by a solution containing 154 mM NaBr, 4 mM OB, and 12 mM β-CD. The two sets of potentiometric titration data agreed with one another, indicating the reliability of our method.

Intensity of Bitter Taste. Five volunteers were involved in the sensory test. These panelists tasted aqueous 154 mM NaBr solutions containing OB alone and a mixture of 4 mM OB and CD. The bitter taste intensities of these solutions were evaluated by the following scores: 0, no bitter taste; 1, very slightly bitter taste; 2, slightly bitter taste; 3, appreciably bitter taste; 4, very bitter taste; 5, extremely bitter taste. The average of bitterness scores over the five individuals was used for further analysis.

RESULTS AND DISCUSSION

Electromotive Forces and Bitter Taste Intensities of Aqueous Solutions of Oxyphenonium Bromide. As Figure 2 shows, the equilibrium electromotive force E of an OB solution containing 154 mM NaBr increased with increasing OB concentration. Over the investigated range of the OB concentration C_p (mM) from 0.02 to 10.6 mM, the electromotive force (mV) obeyed

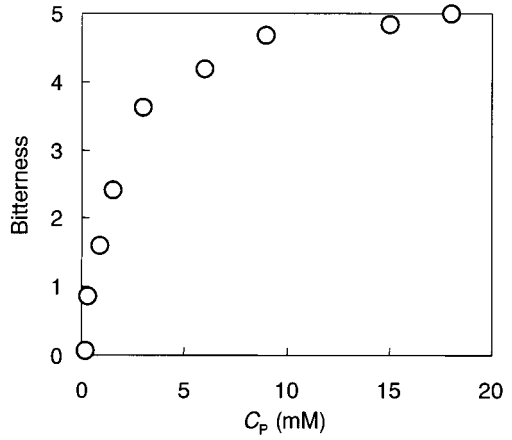

Figure 3. Score of bitter taste of aqueous OB solutions plotted against the OB concentration.

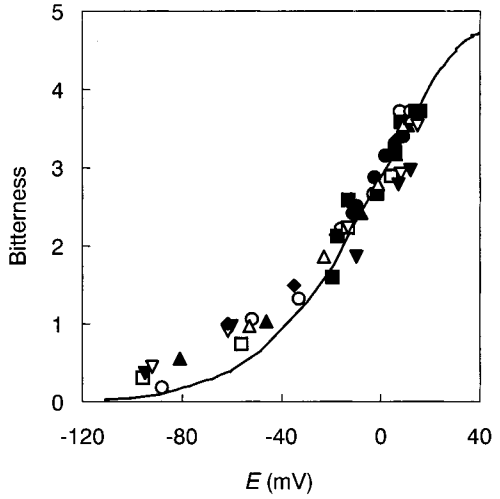

Figure 4. Relationship between the electromotive force and the bitter taste intensity of OB in the absence (solid line) and the presence of α-CD (●), B-CD (○), and γ-CD (■), DMβ-CD (□), HPβ-CD (△), MLβ-CD (▼), GLβ-CD (◆), CMβ-CD (▽), and Mβ-CD (▲).

eq 1. Because OB forms the micelle above a critical micelle

$$E = -22.902 + 61.35 \log C_p \quad (1)$$

concentration (cmc) of 0.108 M,[14] it might form oligomers even at concentrations below this cmc. However, the slope of the electromotive force vs log OB concentration plot does not depend on the concentration in the concentration range investigated. This concentration independence of the slope shows that the self-association of OB is negligible. The slope in eq 1 is very close to a theoretical value of 61.45 mV at 309.7 K. This fact indicates that our electrode responds to OB normally. Furthermore, it is noted that the activity coefficient of OB is almost independent from C_p, because of the presence of excess NaBr.

The intensity of bitter taste of an OB solution is shown as a function of OB concentration in Figure 3. A combination of the results of Figures 2 and 3 yields the relationship between the bitter taste intensity and the electromotive force for aqueous solutions of OB alone. As the solid line in Figure 4 shows, the bitter taste intensity of OB solutions increases with elevation in electromotive force.

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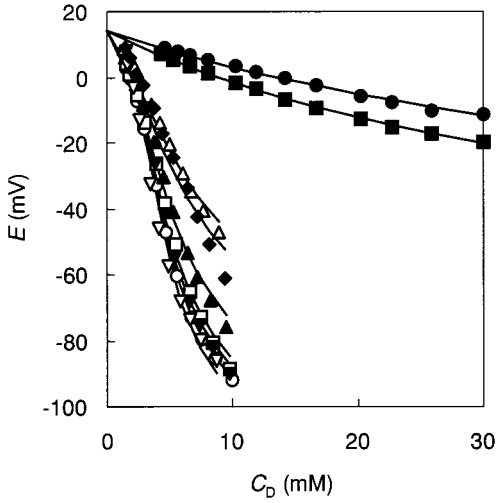

Figure 5. Effects of CDs on the electromotive force of a 4 mM OB solution: α-CD (●), β-CD (○), and γ-CD (■), DMβ-CD (□), HPβ-CD (△), MLβ-CD (▼), GLβ-CD (◆), CMβ-CD (▽), and Mβ-CD (▲). The solid lines are calculated from eq 6 using the equilibrium binding constants shown in Table 1.

Effects of Cyclodextrins on the Electromotive Force of a 4 mM OB Solution. As Figure 5 shows, the electromotive force of a 4 mM OB solution is decreased by the addition of CD. This decrease is ascribed to the reduction of free OB concentration [P], caused by the entrapment of OB into the CD cavity. The extent of decrease is in the following order: CMβ-CD > β-CD > MLβ-CD > DMβ-CD > Mβ-CD > GLβ-CD > HPβ-CD > γ-CD > α-CD.

Binding Constants of Oxyphenonium Bromide for Cyclodextrins. The electromotive force data in Figure 5 were analyzed on the basis of the 1:1 complexation of oxyphenonium ion (P) and CD (D). The equilibrium constant of this complexation is defined as

$$K_1 = [PD]/[P][D] \quad (2)$$

The total concentrations of OB and CD are written as

$$C_P = [P] + [PD] \quad (3)$$

$$C_D = [D] + [PD] \quad (4)$$

From eqs 2−4, we can obtain the concentration of free oxyphenonium ion:

$$[P] = \{K_1C_P - K_1C_D - 1 + [(K_1C_P - K_1C_D - 1)^2 + 4K_1C_P]^{1/2}\}/2K_1 \quad (5)$$

Because the CDs used are hydrophilic, their complexes with the oxyphenonium ion would be also hydrophilic. As the result, these complexes would not form any salt with tetraphenylborate ion and consequently would not interfere with the electromotive force of the present electrode. Under these conditions, the electromotive force of any aqueous solution of OB and CD is determined by the concentration of the uncomplexed oxyphe-

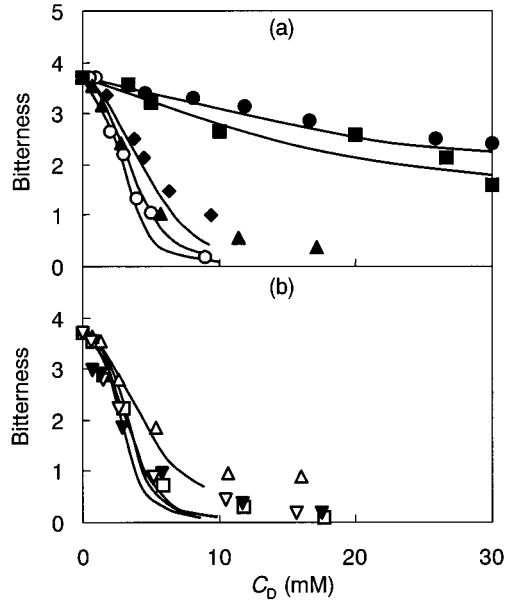

Figure 6. Reduction of the bitter taste intensity of a 4 mM OB solution by the addition of CDs: α-CD (●), β-CD (○), and γ-CD (■), DMβ-CD (□), HPβ-CD (△), MLβ-CD (▼), GLβ-CD (◆), CMβ-CD (▽), and Mβ-CD (▲). The solid lines are calculated from eq 9.

nonium ion. Thus, C_P in eq 1 is replaced by [P] for aqueous solutions containing both OB and CD:

$$E = -22.902 + 61.35 \log\{K_1C_P - K_1C_D - 1 + [(K_1C_P - K_1C_D - 1)^2 - 4K_1C_P]^{1/2}\}/2K_1 \quad (6)$$

This equation was applied to the observed electromotive force data shown in Figure 5. Thus, we determined the best-fit binding constants by a nonlinear least-squares method.[14] These values are shown in Table 1, together with the number of data, *n*, and the standard deviation, *s* (mV):

$$s = \{(\sum^{n}(E_{obsd} - E_{calcd})^2/(n-1)\}^{1/2} \quad (7)$$

The fitting procedure has already been reported in detail elsewhere.[15] The standard deviations for three native CDs are very small, so that we need not take into consideration the other stoichiometries. The standard deviations for the six modified CDs are beyond experimental errors. This will be explicable by taking into consideration that these modified CDs contain many homologues different in binding constant. The binding constants for most of the modified β-cyclodextrins are smaller than that for β-CD. The reason for this decrease with a modification would be the steric hindrance of the substituted group. The only exception is CMβ-CD. Because this CD has a negative charge, its binding ability with a positive OB ion will be enhanced by electrostatic attraction.

Effects of Cyclodextrins on the Bitter Taste Intensity of OB. As Figure 6 shows, the bitter taste intensity of a 4 mM OB solution is reduced by the addition of CD. The order of this reduction by the kind of CD is almost consistent with the result of Figure 5 and that of the magnitude of the equimolar binding constant.

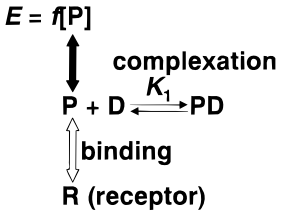

Figure 7. Schematic relationship between the equilibria of CD complexation and receptor binding of OB.

All the data of bitter taste intensities shown in Figures 3 and 6 and electromotive forces shown in Figures 2 and 5 are included in Figure 4. These data on solutions containing both OB and CD are close to the data on solutions containing OB alone. The electromotive force and the bitter taste intensity of an aqueous solution of OB and CD will be determined by the concentration ([P]) of uncomplexed OB in the solution, irrespective of the kind and concentration of CD. That is,

$$E = f([P]) \quad (8)$$

$$\text{bitter taste intensity} = g\{[P]\} = g\{f^{-1}(E)\} \quad (9)$$

These equilibria are illustrated in Figure 7. The receptor of bitter taste binds hydrophobic compounds. Although OB is a hydrophobic compound, its complex with CD is not hydrophobic. Therefore, CDs and their complex do not taste bitter. Thus, eq 9 holds true for all solutions containing OB and CDs. This important result enables us to predict the intensity of bitter taste of an OB and CD solution from the observed electromotive force of their mixed solution and the relation between the bitter taste intensity and the electromotive force of an aqueous OB solution.

Implications and Limitations of the Present Work. The present approach will apply to other ionic bitter compounds but does not apply to nonionic compounds and other tastes, such as sweetness, acidity, and saltiness. Many drugs self-associate in aqueous media by hydrophobic interactions.[16] The present approach will apply to such drugs, though OB does not self-associate in the concentration range investigated. The binding of OB and CD is explicable in term of the 1:1 stoichiometry, though other stoichiometries are observed for CD inclusion systems.[15,17] The present approach will apply to such multiple-complexing systems. Because the relation of eq 9 depends on each compound, it does not apply to other bitter compounds. It is desired to develop a universal electrode that determines the absolute intensity of bitter taste for all compounds.

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