Masking Mechanisms of Bitter Taste of Drugs Studied with Ion Selective Electrodes

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The masking mechanisms of the bitter taste of propantheline bromide (PB) and oxyphenonium (OB) bromide by native and modified cyclodextrins, saccharides, surfactants, organic acids, nonionic and anionic polymers, and other compounds were investigated with ion selective electrodes. The intensity of the bitter taste for a mixed solution of cyclodextrin with PB or OB was quantitatively explained from the observed electromotive force with the following assumptions: the complex and the masking agent do not have any tastes and the bitter taste is independent of other tastes. Sodium dodecyl sulfate reduced the bitter taste remarkably, and this reduction was also explicable on the basis of the same mechanism. Sodium taurodeoxycholate enhanced the bitter taste, because of its strong bitterness, although it formed 1 : 1 complexes with PB and OB. The masking mechanism of saccharides was ascribed to overcoming the weak bitterness of the drug by the strong sweetness. λ-Carrageenan suppressed the bitter taste remarkably. This suppression was ascribed to the binding of PB and OB to λ-carrageenan, the effect of the solution viscosity on the bitter taste, and the covering of the bitter taste receptor by λ-carrageenan. It was suggested that the moderate masking by other polymers was attributable to the effect of the solution viscosity or the receptor covering. Native and modified β-cyclodextrins, sodium dodecyl sulfate, λ-carrageenan, Tween 20, and sodium carboxymethyl cellulose are good masking agents for the bitter tastes of PB and OB. The drug ion selective electrode is a useful tool for understanding of the masking mechanism of the bitter taste, screening of masking agents, and estimation of appropriate concentrations of the masking agents.

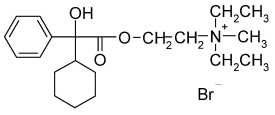
Key words bitter taste; masking mechanism; ion selective electrode; cyclodextrin; polysaccharide

A hydrogen electrode responds specifically to hydrogen ions and is widely used to determine the activity and concentration of hydrogen ions. Metal ion-selective electrodes, such as potassium ions and calcium ions, are also commercially available. Drug ion selective electrodes are prepared and are used to determine the concentrations of drug ions.[1)] Other applications of drug ion electrodes include studies of protein binding of drug ions,[2)] mechanisms of drug action,[3)] and estimation of bitter taste of a drug.[4)] Recently, biosensors of taste and smell are commercially available. For instance, Toko *et al.* have developed a multi-channel taste sensor whose transducer is composed of several kinds of lipid/polymer membranes with different characteristics.[5—7)] This sensor is used to taste foods and medicines objectively, instead of human sensory evaluation.[5—11)]

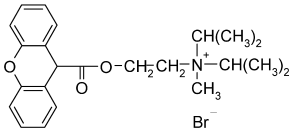
The majority of the orally administrated drugs tastes very bitter. Such drugs are usually administrated as tablets.[12,13)] However, because some of the small children and aged people cannot swallow intact tablets, crushed tablets or liquid formulations are administered to them. In these cases an appropriate masking agent would be added to reduce or eliminate the bitter taste. A number of masking agents of the bitter taste are known.[14)] As well known, a concentrated solution of sucrose masks the bitter taste of drugs by its intense sweetness. Cyclodextrins (CyDs) can reduce the bitter taste of drugs by complex formation[4,14—18)]: The complexed drug is not bitter, whereas the uncomplexed drug is bitter. This masking mechanism was established quantitatively.[4,17,18)] Lipoprotein that is composed of phosphatidic acid, β-lactoglobulin, bovine serum albumin, and other substances is a good masking agent.[19—21)] Some polysaccharides, synthetic polymer, and jellies can mask the bitter taste of drugs.[8,9,22—24)] It is suggested that complex formation of a drug with the lipoprotein and polymers reduces the concentration of the drug in the free state.[9,21)]

Most bitter compounds are hydrophobic and some of them are positively charged.[20,21)] Propantheline bromide (PB) and oxyphenonium bromide (OB), shown in Fig. 1, are bitter anticholinergic drugs. As already reported, suppression of the bitter taste of PB by native CyDs (α-, β-, and γ-CyDs) can be predicted from the observed surface tensions of mixed solutions of PB and one of the CyDs.[17)] Furthermore, masking of the bitter taste of OB by the native CyDs and several modified CyDs has been investigated with the OB ion selective electrode.[4)] The ion selective electrode method will be applicable to more masking agents than the surface tension method, because the latter method is inapplicable to surface active masking agents.

In this work, we aimed to find better masking agents with



Oxyphenonium bromide



Propantheline bromide

Fig. 1. Chemical Structures of Oxyphenonium Bromide and Propantheline Bromide

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ion selective electrodes and to check the applicability of the ion selective electrode method to various masking agents. Candidates for masking agents include native and modified CyDs, saccharides, natural and synthetic surfactants, citric acid, nonionic and anionic polymers, and other compounds. Most of the masking agents are nontoxic compounds or are expected to have high affinities for PB and OB. The masking mechanisms of these agents will be estimated on the basis of the observed electromotive forces and the bitter taste intensities.

Experimental

Materials Commercial specimen of PB and OB were purchased from Sigma Chemical Co. Because these samples were analyzed to be pure by reversed-phase liquid chromatography, they were used without purification. Methyl β-CyD, sodium taurodeoxycholate, tannic acid, Pluronic F-127, bovine serum albumin, and β-lactoglobulin were also obtained from Sigma Chemical Co. Sodium bromide (analytical grade), sodium cholate, α-, β-, and γ-CyDs, 2,6-*O*-dimethyl β-CyD, 2-hydroxypropyl β-CyD, 6-glucosyl β-CyD, and 6-maltosyl β-CyD were purchased from Nacalai Tesque Co. The modified CyDs were mixtures of CyDs different in degrees of substitution as detected by reversed-phase liquid chromatography.[4] Sodium carboxymethyl β-CyD was obtained from Cylcolab, Budapest. Sodium tetraphenylborate, *o*-nitrophenyl octyl ether, tetrahydrofuran, and calix[6]arene 6-sulfate were obtained from Dojindo Laboratories Kumamoto. Polyethylene glycol 8000 was from INC Biochemicals. Polyvinyl pyrrolidone (K60) and pectin (citrus) were from Tokyo Kasei Kogyo. Polyvinyl chloride, sodium dodecyl sulfate (biochemical grade), sucrose, fructose, glycine, sodium ascorbate, acrylamide, citric acid, arabic gum, dextran, dextrin, corn starch, λ-carrageenan, sodium chondroitinsulfate C, sodium dextran sulfate (molecular weight of 5000), and gelatin (type A) were purchased from Wako Pure Chemicals Co.

Preparation of the Polyvinyl Chloride Membrane Electrode The polyvinyl chloride membrane (PVC membrane) was prepared according to the method 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 ml of tetrahydrofuran. Then, polyvinyl chloride (0.2 g) was added stepwise into the tetrahydrofuran solution under magnetic stirring. Immediately after the DKK membrane filter (6 mm in diameter), previously immersed in tetrahydrofuran, was transferred into the tetrahydrofuran membrane solution, the filter was fitted to the tip of an ion-selective electrode body. Further, a drop of the membrane solution was added with a micropipette to the fixed filter, followed by evaporation of the tetrahydrofuran in 20 min. This operation was repeated 10 times. The resulting membrane was soaked in a 10 mM OB solution in 3 h. Then, an internal solution containing 1 mM OB and 10 mM sodium bromide was filled into the body. Finally, an Ag/AgBr electrode was mounted to the body. The electrode was stored in a 1 mM OB solution. A PB ion selective electrode was prepared in a similar way to the OB electrode. The lifetimes of these membrane electrodes were a few months.

Measurements of Electromotive Forces Potentiometric measurements were carried out with a DKK model IOL-40 digital pH/mV meter *in vitro*. The electrochemical cell was constructed as follows: Ag/AgCl|KCl solution|sample solution|PVC membrane|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 became faster as the OB concentration was increased. The calibration curve for OB was determined as follows: 25 ml of a solution containing 0.02 mM OB and 154 mM sodium bromide was titrated successively by a 20 mM OB solution and the equilibrium potential was measured digitally in a precision of 0.1 mV. The average over three runs is reported herein.

In the investigated range of the OB concentration between 0.02 and 10 mM, the equilibrium electromotive force, E, changed with the drug concentration, C_D, as follow:

$$E = a \log C_D - b \quad (1)$$

Here, a=61.53 mV. This a value remained almost unchanged with elapsed days. Because the b value depended on days within 1 mV, they were determined with 4 mM OB solutions every day. This b value was corrected to a constant of b=65.7 mV. Similarly, Eq. 1 held for PB with values of a=60.66 mV and b=81.8 mV in the PB concentration range between 0.08 and 4 mM. Below 0.08 mM the observed electromotive forces deviated positively from Eq. 1. This deviation will be ascribed to dimerization of PB.[17,25] In this work we neglected the effect of dimerization of PB on the electromotive force, because it was small. Therefore, we can modify Eq. 1 to:

$$E = a \log[\mathrm{D}] - b \quad (2)$$

Here, [D] stands for the concentration of the drug in the free state.

The effects of a masking agent on the electromotive force of a 4 mM OB solution or a 1.5 mM PB solution containing 154 mM sodium bromide were investigated at 309.7±0.1 K. For most cases the highest concentration of masking agents was 10 g/l. The observed change in electromotive force was analyzed to determine the amount of the drug bound to masking agents and used to predict the bitter taste intensity.

Intensity of Bitter Taste Five volunteers were involved in the sensory test. These panelists tasted 35 ml of an aqueous 154 mM sodium bromide solution containing OB or PB alone and a mixture of a masking agent and either 4 mM OB or 1.5 mM PB. The bitter taste intensity of these solutions was rated on 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 bitter taste intensities over the 5 individuals was used for further analysis. The standard deviations of the bitter taste intensities were *ca.* 0.7 in most cases.

Results

Complex Formation between Drug and Masking Agent The electromotive force (E) with the drug ion selective electrode increased with increasing free drug concentration, as is expressed by Eq. 2. As shown in Fig. 2, the electromotive force of a 1.5 mM PB solution decreased with addition of sodium taurodeoxycholate, α-CyD, hydroxypropyl β-CyD, and carboxymethyl β-CyD. These decreases were ascribed to decreases of free PB concentration with complex formation and were used to evaluate the 1 : 1 binding constant (K_1) between PB and a masking agent from Eq. 3:

$$[\mathrm{D}] = \{K_1C_D - K_1C_M - 1 + [(K_1C_D - K_1C_M - 1)^2 + 4K_1C_D]^{0.5}\}/2K_1 \quad (3)$$

Although PB forms dimer and micelles, these aggregates are negligible in 1.5 mM PB solutions. One and two β-CyD molecules form 1 : 1 and 2 : 1 complexes with a PB molecule, whereas γ-CyD forms 1 : 1 and 1 : 2 complexes with PB.[17] However, because these 1 : 2 and 2 : 1 binding constants are

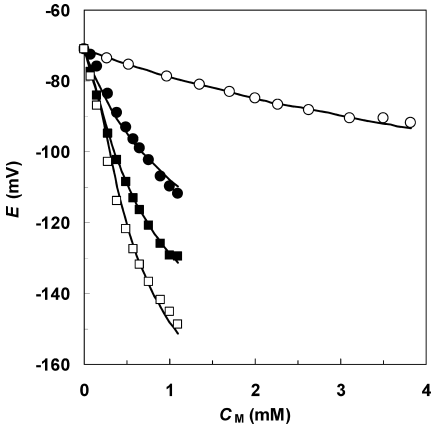

Fig. 2. Electromotive Forces Plotted against the Concentrations of Four Masking Agents in 1.5 mM PB Solutions: Sodium Taurocholate (○), α-CyD (●), 2-Hydroxypropyl β-CyD (□), and Sodium Carboxymethyl β-CyD (■)

The solid lines are calculated from Eq. 3 using the binding constants given in Table 1.

Table 1. Observed and Calculated Bitter Intensities and Electromotive Force of a Mixed Solution of 1.5 mM PB and 10 g/l Masking Agent, the 1 : 1 Binding Constant, and the Slope of Eq. 7

Masking agent	C_M (g/l)	Bitterness		E (mV)	K_1 (M^{-1})	d (mV l/g)
		Obsd	Calcd			
None	0	3.8	3.8	−71		
α-CyD	10	3.8	3.6	−79	40	
β-CyD	10	1.5	1.9	−145	2500	
γ-CyD	10	3.7	3.1	−100	320	
2,6-*O*-Dimethyl β-CyD	10	1.3	1.4	−162	4800	
Methyl β-CyD	10	—[a]	1.9	−144	2300	
2-Hydroxy propyl β-CyD	10	2.8	2.3	−129	1600	
6-Glucosyl β-CyD	10	1.6	1.8	−147	2800	
Sodium carboxymethyl β-CyD	10	1.7	1.9	−145	5700	
6-Maltosyl β-CyD	10	2.7	2.0	−140	2500	
Sodium dodecyl sulfate	10	0.8	0.0	−266	27400	
Sodium cholate	10	2.8	3.1	−97	70	
Sodium taurodeoxycholate	10	5.0	2.8	−110	190	
18-Crown 6-ether	10	—[a]	3.7	−74	3	
Sodium ascorbate	10	3.0	3.6	−78	6	
Citric acid	10	1.8	3.6	−78	7	
Tannic acid	10	1.7	2.9	−107	280	
Polyethylene glycol 8000	10	3.5	3.7	−75		0.3
Dextran	10	1.8	3.8	−71		0.0
Tween 20	10	1.5	3.3	−88		1.6
Pluronic F-127	10	2.7	3.6	−77		0.6
Polyvinyl pyrrolidone	10	2.8	3.7	−75		0.4
λ-Carrageenan	10	1.0	3.2	−95		2.4
Methyl cellulose	10	3.3	3.7	−74		0.4
Sodium carboxymethyl cellulose	10	1.4	3.7	−74		0.3
Sodium chondroitin sulfate C	10	2.1	3.8	−72		0.1
Sodium dextran sulfate	10	3.2	3.7	−74		0.3
Bovine serum albumin	10	4.0	3.8	−72		0.1

a) Not determined.

much smaller than the 1 : 1 binding constants, the 1 : 2 and 2 : 1 complexes were neglected in this work. Sodium taurocholate can form the 1 : 1 ion-pair with PB and micelles at high concentrations. The critical micelle concentration of sodium taurodeoxycholate would be 1.5 mM under the present conditions. The K_1 values are given in Table 1.

In Table 1 the electromotive forces for 10 g/l masking solutions are compiled for comparison of PB binding capacities of masking agents. The electromotive force of 1.5 mM PB solution is −71 mV. Masking agents that decrease significantly the electromotive force below this value have strong binding capacities to PB. Natural and modified β-CyDs have large binding constants. Sodium dodecyl sulfate has a larger binding constant. Sodium cholate and sodium taurodeoxycholate have moderate binding capacities. 18-Crown 6-ether, sodium ascorbate, and citric acid have rather poor binding capacities.

The effects of Pluronic F-127 and λ-carrageenan on the electromotive force of a 1.5 mM PB solution are shown in Fig. 3. λ-Carrageenan did not form gel in the range of the investigated concentration. Because the molecular weights of these polymeric masking agents are not available, their concentrations, C_M, are expressed on a g/l scale. The mole, ν, of bound PB to 1 g of a polymeric masking agent was calculated from Eq. 4:

$$\nu=(C_D-[D])/C_M \quad (4)$$

The free PB concentration, [D], was calculated from the observed electromotive force using Eq. 2. If a PB molecule is bound to n equivalent sites of a polymer molecule independently, Eq. 5 will hold for this system[2,26]:

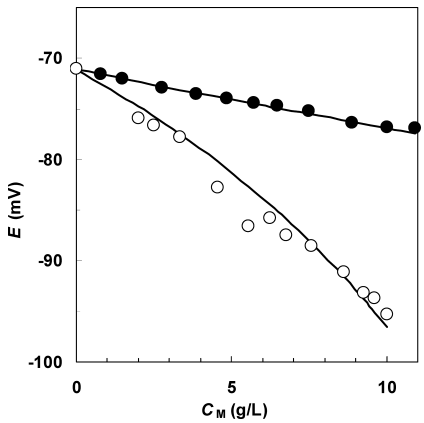

Fig. 3. Electromotive Forces Plotted against the Concentrations of Two Polymeric Masking Agents in 1.5 mM PB Solutions: Pluronic F-127 (○), λ-Carrageenan (●)

The solid line for λ-carrageenan was calculated from Eq. 6 using the optimized values of K=0.124 l/g and n=2.06 mol/g. The solid line for Pluronic F-127 was calculated from Eq. 7 using the values of d=0.6 mV l/g and e=−71 mV.

$$\nu=nK[D]/(1+K[D]) \quad (5)$$

Here K stands for a binding constant. Using Eqs. 4 and 5, we can calculate the free PB concentration, [D]:

$$[D]=\{(KC_D-nKC_M1)+[(KC_D-nKC_M-1)^2+4KC_D]^{0.5}\}/2K \quad (6)$$

This concentration can be calculated for a given set of C_M

and C_D, if appropriate values of n and K are given. Substituting this concentration into Eq. 2, we can obtain a theoretical electromotive force. This theoretical value is fitted to the observed one by nonlinear least squares method. Finally, the optimized values of n and K are determined. λ-Carrageenan decreased the electromotive force of 1.5 mM PB solution rather notably (Fig. 3), and we could determine the optimized values of n and K. The solid line shown in Fig. 3 was calculated with values of $n=2.1$ mol/g and $K=0.12$ l/g. Values of $n=0.27$ mol/g and $K=0.120$ l/g were determined for polyvinyl pyrrolidone, although the electromotive forces are not shown in Fig. 3.

However, because Pluronic F-127 decreased the electromotive force only slightly in the investigated concentration range, we could not determine any converged values of n and K. In this case the slope d of Eq. 7 was used as a measure of the binding capacity:

$$E=-dC_M+e \quad (7)$$

Here the intercept e is -71 mV for 1.5 mM PB solution. For the masking agents that did not give the converged values of n and K for Eq. 6, the slope of Eq. 7 was given in Table 1. Furthermore, the electromotive forces for some nonionic polymers, anionic polymers, and bovine serum albumin at 10 g/l are given in Table 1. The d value and the electromotive force at 10 g/l are measures of binding to PB. Tween 20 and λ-carrageenan have moderate affinities for PB.

The effects of masking agents on the electromotive force of a 4 mM OB solution were also investigated. The values of the electromotive force of a 10 g/l masking agent solution, K_1, and d are summarized in Table 2. All of these values are measures of the binding capacity of masking agents to OB. The general trends for OB are close to those of PB. For instance, native and modified β-CyDs have large binding capacities. Hexasodium calix[6]arenesulfonate remarkably decreased the electromotive force of the 4 mM OB solution. Sucrose, fructose, glucose, and acrylamide at low concentrations only slightly decreased the electromotive force of the 4 mM OB solution. Application of Eq. 3 to these electromotive force data yielded small 1 : 1 binding constants. However, because small changes in electromotive force can result from changes in the activity coefficients of OB, it is uncertain that these non-electrolytes actually form the 1 : 1 complexes with OB. The 850 g/l sucrose solution increased the electromotive force significantly, although it is suspected that the OB electrode worked normally in the highly concentrated

Table 2. Observed and Calculated Bitter Intensities and Electromotive Force of a Mixed Solution of 4 mM OB and 10 g/l (Unless Specified) Masking Agent, the 1 : 1 Binding Constant, and the Slope of Eq. 7

Masking agent	C_M (g/l)	Bitterness		E (mV)	K_1 (M^{-1})	d (mV l/g)
		Obsd	Calcd			
None	0	3.7	3.7	−29		
α-CyD	10	3.2	3.0	−39	58[a]	
β-CyD	10	0.2	0.2	−124	8500[a]	
γ-CyD	10	3.1	3.0	−41	96[a]	
2,6-*O*-Dimethyl β-CyD	10	0.6	0.3	−116	6660[a]	
Methyl β-CyD	10	0.9	0.4	−104	4290[a]	
2-Hydroxy propyl β-CyD	10	1.7	1.2	−75	1460[a]	
6-Glucosyl β-CyD	10	0.9	0.1	−138	8200	
Sodium carboxymethyl β-CyD	10	1.3	0.6	−93	10010[a]	
6-Maltosyl β-CyD	10	0.8	0.3	−115	7500[a]	
Sodium dodecyl sulfate	10	0.8	0.0	−198	5700	
Sodium taurodeoxycholate	10	4.0	1.5	−67	100	
Hexasodium calix[6]arenesulfonate	5.5	—[b]	2.2	−56	1100	
Sucrose	10	3.8	3.6	−30	1	
Sucrose	850	0.8	5.0	40	—[b]	
Fructose	10	3.9	3.6	−30	1	
Glucose	10	2.4	3.6	−30	1	
Glycine	10	—[b]	3.6	−29	0	
Acrylamide	0.8	—[b]	3.5	−32	10	
Sodium ascorbate	10	2.5	3.3	−35	6	
Citric acid	10	0.8	3.3	−36	4	
Tannic acid	10	1.5	3.0	−40	46	
Polyethylene glycol 8000	10	2.0	3.5	−32	20	0.0
Arabic gum	10	2.3	3.6	−30		0.1
Dextran	10	1.8	3.6	−29		0.0
Pluronic F-127	10	2.0	3.5	−33		0.4
Dextrin	10	2.8	3.6	−29		0.1
Polyvinyl pyrrolidone	10	2.0	3.6	−31		0.2
Starch	10	2.5	3.6	−31		0.2
λ-Carrageenan	10	0.9	3.0	−41		1.3
Sodium chondroitin sulfate C	10	2.5	3.7	−28		−0.1
Pectin	10	2.5	3.7	−28		0.0
Sodium dextran sulfate	10	1.8	3.6	−31		0.3
Bovine serum albumin	10	3.0	3.6	−32		0.4
Gelatin	10	2.5	3.9	−26		0.1
β-Lactoglobulin	10	—[b]	3.6	−30		0.1

a) Taken from ref. 4. *b*) Not determined.

solution. Sodium ascorbate, citric acid, and tannic acid slightly decreased the electromotive force of the 4 mM OB solution. λ-Carrageenan notably decreased the electromotive force. Other nonionic and anionic polymers and proteins exhibited minor changes.

Suppression of Bitter Taste of PB and OB by Masking Agents Five volunteers were involved in the sensory test. The panelists tasted aqueous OB solutions in the presence of 154 mM sodium bromide and evaluated the bitter taste intensities of these solutions. For example, the 20 mM OB solution tasted extremely bitter (a bitter taste intensity of 5). The average standard deviation of the bitter taste intensity over the investigated systems was about 0.7. The bitter taste intensity of the 4.0 mM OB was 3.7. Addition of a 10 g/l masking agent in this solution decreased the bitter taste in most cases, as is given in Table 2. The bitter taste intensities of mixed solutions of 1.5 mM PB and 10 g/l masking agents are given in Table 1. The sensory test of acrylamide, 18-crown 6-ether, and hexasodium calix[6]arenesulfonate was not carried out, because their toxicity is suspected.

The effects of masking agents on the bitter taste intensity of OB are similar to those of PB: the kind and concentration of masking agents are of primary importance. Native and modified β-CyDs decreased remarkably the bitter taste, although α- and γ-CyDs decreased slightly. Sodium ascorbate, citric acid, and tannic acid suppressed the bitter taste, probably because they form ion-pair complexes with OB and PB (Tables 1, 2). As well known, sweet saccharides also masked the bitter taste, although they would not form any complex with OB and PB. Nonionic surfactants and nonionic and anionic polymers, such as Tween 20 and sodium carboxymethyl cellulose, masked the bitter taste remarkably. In particular, λ-carrageenan is the strongest masking agent among these polymers.

Correlation between the Bitter Taste and the Electromotive Force A masking mechanism of the bitter taste of a drug by a masking agent (M) is illustrated in Fig. 4, where several assumptions are made. Any complex between the drug and the masking agent does not taste bitter. The bitter taste intensity is independent of other tastes of masking agents, such as sweetness and sourness. No masking agent tastes bitter: it does not interact with the bitter taste receptor. The amount of the drug adsorbed to all parts of the mouth is negligible, because the sensory test solution has a large volume of 35 ml. If these assumptions are satisfied, the bitter taste intensity of a mixed solution of a drug and a masking agent will be determined by the free OB concentration ([D]) in the solution, irrespective of the kind and concentration of a masking agent:

$$\text{bitter taste intensity} = g([\mathrm{D}]) \tag{8}$$

Here, the g function is determined by sensory experiments and depends on the kind of drug. It cannot be written explicitly.

The electromotive force is also a function of [D] alone; namely, Eq. 2. Therefore, we can expect that the bitter taste intensity of a mixed solution of OB and the masking agent is determined from the observed E value:

$$\text{bitter taste intensity} = g(10^{E/a+b/a}) \tag{9}$$

The bitter taste intensity and the electromotive force of an OB solution were measured and the relation between them was plotted in Fig. 5. This relation was determined for OB solutions at a number of concentrations in the absence of any masking agent. The solid line was drawn through these plots and shows the relation of Eq. 9 for OB. Next, the bitter taste intensities and the electromotive forces of 4 mM OB solutions containing α-CyD at different concentrations were measured. Some of the circles in Fig. 5 showed the relation between them. The same experiments were carried out for the other masking agents given in Table 1. All data of the CyDs (circles) are very close to the solid line, regardless of the kind of CyD: Eq. 9 held true for the CyDs. The same agreement was obtained for PB, as shown by the circles in Fig. 6.

Equation 9 was applied to other masking agents at a concentration of 10 g/l. The bitter taste intensity calculated from Eq. 9 using the observed electromotive force is given in Tables 1 and 2. For clarity some typical data only are shown in Figs. 5 and 6. Prior to detailed consideration, we must keep in mind that because bitter taste intensities and electromotive forces for very dilute solutions of masking agents are close to those of the solutions of PB or OB alone, the relation between them obeys Eq. 9. In general, as the concentration of a masking agent is increased, the agreement between experiment and theory becomes worse.

Common features for PB and OB are observed in Tables 1 and 2 and Figs. 5 and 6. All the CyDs obey Eq. 9. Sodium taurodeoxycholate tastes bitter. The solutions of 10 g/l

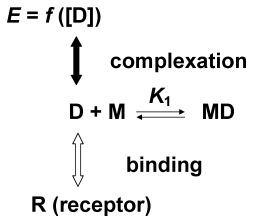

Fig. 4. Schematic Relationship between the Equilibria of Masking Agent (M) Complexation and Receptor Binding of a Bitter Drug (D), Monitored with a Drug Ion Selective Electrode

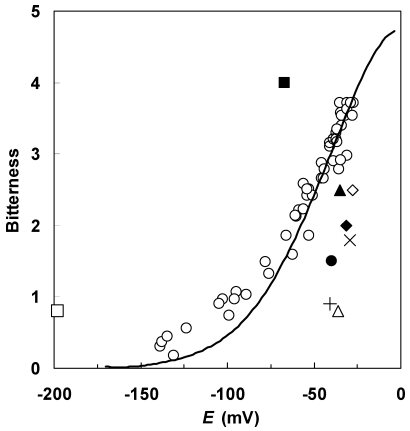

Fig. 5. Bitter Taste Intensities Plotted against the Observed Electromotive Forces for Mixed Solutions of OB and Masking Agents

The solid line is drawn for OB solutions in the absence of any masking agent. The concentration of OB was fixed at 4 mM in the presence of masking agents. The concentrations of native and modified CyDs (○) were changed, whereas for the other masking agents their concentrations were 10 g/l. Some of the data given in Table 2 are shown: sodium taurocholate (■), sodium dodecyl sulfate (□), citric acid (△), λ-carrageenan (+), sodium chondroitin sulfate C (◇), dextran (×), tannic acid (●), sodium ascorbate (▲), and polyethylene glycol 8000 (◆).

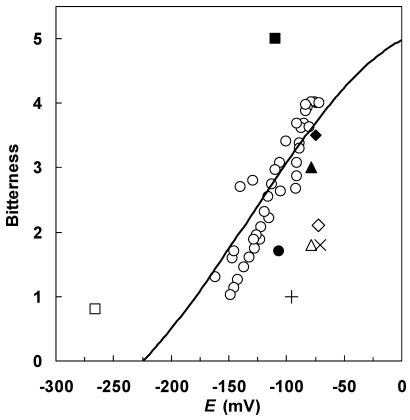

Fig. 6. Bitter Taste Intensities Plotted against the Observed Electromotive Forces for Mixed Solutions of PB and Masking Agents

The solid line is drawn for PB in the absence of any masking agent. The concentration of PB was fixed at 1.5 mM in the presence of masking agents. The concentrations of native and modified CyDs (○) were changed, whereas the concentrations of the other masking agents were 10 g/l. Some of the data given in Table 1 are shown: sodium taurocholate (■), sodium dodecyl sulfate (□), citric acid (△), λ-carrageenan (+), sodium chondroitin sulfate C (◇), dextran (×), tannic acid (●), sodium ascorbate (▲), and polyethylene glycol 8000 (◆).

sodium taurodeoxycholate mixed with 1.5 mM PB and 4 mM OB tasted much bitterer than predicted by Eq. 9. Sodium dodecyl sulfate, which tastes slightly bitter, exhibited small positive deviations from the predicted ones. Saccharides, sodium ascorbate, citric acid, tannic acid, and nonionic and anionic polymers at 10 g/l all suppressed the bitter taste more strongly than predicted by Eq. 9. Bovine serum albumin did not affect it.

Discussion

Estimation of Binding between Drug and Masking Agent In this work, we determined electromotive forces in the presence of 154 mM sodium bromide. Under this condition the activity coefficients of PB and OB did not change with increasing drug concentration: the slopes of Eq. 1 are close to the theoretical value (61.52 mV). Under this condition we can estimate accurate concentrations of PB and OB from the observed electromotive forces. The electromotive forces for very dilute PB solutions deviated positively from Eq. 1. This deviation allowed us to estimate a dimerization constant of 17 M^{-1}. This value is similar to 13, 20, 30, and 30 M^{-1} determined by gel filtration chromatography,[29] NMR,[25] surface tension,[17] and UV spectra,[17] respectively. The 1 : 1 binding constants of PB with α-, β-, and γ-CyDs (Table 1) are close to those already determined by measurements of the NMR chemical shift,[25] the surface tension, and the UV absorbance.[17]

The 850 g/l sucrose solution, which is named "tan siroppu (simple syrup)" in Japanese Pharmacopoeia, increased the electromotive force of the 4 mM OB solution to 40 mV (Table 2). If we assume that the OB ion selective electrode worked normally, the activity coefficient of 4 mM OB in the 850 g/l sucrose solution is estimated to be 13. It is not certain whether this large value is the true activity coefficient of OB.

We have taken into consideration two binding models. Either of these models will be applicable to mixed solutions of the drugs with masking agents. The 1 : 1 model, Eq. 3, is applicable to native and modified CyDs. Sodium dodecyl sulfate, sodium taurocholate, and sodium cholate can form micelles above the critical micelle concentration (cmc). Below the cmc the 1 : 1 model will be applicable, whereas it would be inapplicable above the cmc. For non-electrolytes the 1 : 1 model will not hold, although it has been applied (Tables 1, 2). Citric acid and tannic acid are weak electrolytes. Their anions could form 1 : 1 complexes with OB and PB, whereas their acidic (protonated) forms will not form them. The 1 : 1 binding constants (K_1) will be invalid for these acids, although they can be used to predict rough electromotive forces at arbitrary concentrations of the acids and the drugs. The second binding model, Eq. 6, is based on the independent binding of the drug to the n equivalent sites of polymer.[26] This model has been applied to binding of PB to λ-carrageenan and polyvinyl pyrrolidone to determine the values of n and K. The model will be applicable to all of the polymers listed in Tables 1 and 2, although it was not actually applied, because of the deficiency of the electromotive force data in concentrated solutions of these polymers.

Mechanisms and Quantitative Prediction of Masking of Bitter Taste As already reported,[4] the bitter taste intensities of mixed solutions of OB and CyDs can be predicted quantitatively from the observed electromotive forces with the OB ion selective electrode (Fig. 5, Table 2). The same result was also obtained for mixed solutions of PB and CyDs (Fig. 6, Table 1). This prediction is based on the scheme shown in Fig. 4, where non-taste of the complex and the masking agent and independence of the bitter taste from other tastes are assumed. The solution structures of complexes of OB and PB with native CyDs have been determined, and these structures suggest that the complexes do not taste bitter, because of their expected hydrophilicity.[25,27,28] According to the mechanism illustrated in Fig. 4, the concentration of a drug in the free state determines the bitter taste intensity of a mixed solution of the drug and a masking agent. The free drug concentration can be determined by other methods; for instance, UV absorbance data had been used for this purpose.[30] Dimethyl β-CyD and β-CyD are effective masking agents for PB and OB.

The other masking agents listed in Tables 1 and 2 do not completely follow the above mechanism. Although sodium dodecyl sulfate tastes slightly bitter, it suppressed the bitter tastes of OB and PB remarkably, because of large binding constants. Other anionic surfactants would be useful as masking agents, and the ion selective electrode would be used to screen such surfactants. Sodium taurodeoxycholate is a very bitter bio-surfactant. This bile salt increased bitter tastes of OB and PB solutions because of its strong bitterness, although it can form ion-pairs with OB and PB.

Since sucrose tastes very sweet, it is added to mask the bitter taste of a medicine in syrups. When humans taste a sample having several tastes, they seem to sense the strongest taste. As well known, this is the masking mechanism of the bitter taste by sucrose and other sweet saccharides. Sodium ascorbate, citric acid, and tannic acid taste sour or astringent. These intense tastes could mask the bitter taste of a drug. Ion selective electrodes are not suitable to predict the bitter tastes of samples having other intense tastes. Multi-channel taste sensors developed by Toko and his coworkers can be used for such multi-taste samples.[5—7] The electromotive force of a channel sensor responses the intensities of multi-tastes differently. Multi-component analysis of the electromotive

forces of multi-channel sensors can be correlated with the intensities of different tastes obtained by human sensory tests. Therefore, these sensors can predict the kind and intensity of taste.[5—7] However, the sensors cannot be used to determine the binding constant between a drug and a masking agent.

Anionic and nonionic polymers can mask the bitter tastes of OB and PB. As a masking mechanism of the bitter tastes of drugs by lipoprotein, binding between them has been suggested.[21] Bovine serum albumin and β-lactoglobulin bind PB and OB very weakly. λ-Carrageenan masks the bitter taste remarkably and binds PB and OB rather strongly. As already suggested,[8,9] the strong binding capacity of λ-carrageenan is one of the masking mechanisms. However, this is not all. For λ-carrageenan the observed bitter taste intensities are significantly smaller than those calculated from Eq. 9 (Tables 1, 2). Similar differences are found for other nonionic and anionic polymers. The viscous solution may decrease the bitter taste. Saccharides are generally biocompatible and are widely used as preservatives of biological tissues and proteins. Polysaccharides may cover the receptor of the bitter taste to inhibit binding of bitter molecules. Multi-channel taste sensors may be used to evaluate the bitter taste intensities of such polymeric systems.[8,9,24] The masking mechanisms of sodium carboxymethyl cellulose may be based on the solution viscosity and/or coverage of bitter taste receptor. Tween 20 binds to PB significantly, is rather biocompatible, and increases the viscosity of water. These properties of Tween 20 are favorable for masking the bitter taste of PB.

In conclusion, ion selective electrodes are useful to predict the bitter taste intensities of mixed solutions of a drug and a masking agent and to clarify the masking mechanisms of bitter tastes. Five masking mechanisms (reduction of free drug concentration, formation of a non-bitter complex, overcoming by a stronger taste, the effect of solution viscosity, and covering of the bitter taste receptor) were taken into consideration and were used to explain the masking by CyDs, synthetic surfactants, bile salts, saccharides, organic acids, nonionic and anionic polymers, and proteins. Native and modified β-CyDs, sodium dodecyl sulfate, Tween 20, sodium carboxymethyl cellulose, and λ-carrageenan are good masking agents for the bitter tastes of PB and OB. Anionic surfactants may be used as masking agents of bitter drugs in the future. The drug ion selective electrode is a good tool for understanding of the masking mechanism of the bitter taste, screening of masking agents, and estimation of appropriate concentrations of the masking agents.

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References

1) Cosofret V. V., Buck R. P., "Pharmaceutical Applications of Membrane Sensors," CRC Press, Boca Raton, 1992.
2) Angelakou A. T., Sideris E. E., Valsami G. N., Koupparis M. A., Macheras P. E., *J. Pharm. Sci.*, **83**, 1150—1154 (1994).
3) Katsu T., *Farumashia*, **37**, 129—132 (2001).
4) Funasaki N., Kawaguchi R., Ishikawa S., Hada S., Neya S., Katsu T., *Anal. Chem.*, **71**, 1733—1736 (1999).
5) Takagi S., Toko K., Wada K., Yamada H., Toyoshima K., *J. Pharm. Sci.*, **87**, 552—555 (1998).
6) Takagi S., Toko K., Wada K., Ohki T., *J. Pharm. Sci.*, **90**, 2042—2048 (2001).
7) Toko K., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 7—10.
8) Harada T., Kato T., Owaki T., Iwamoto K., *Pharm. Tech. Jpn.*, **17**, 1367—1373 (2001).
9) Harada T., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 17—21.
10) Nakamura T., Tanigake Y., Miyanaga T., Ogawa T., Uchida T., *Chem. Pharm. Bull.*, **50**, 1589—1593 (2002).
11) Uchida T., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 11—16.
12) Nakamura Y., *Pharm. Tech. Jpn.*, **21**, 817—823 (2005).
13) Nakamura Y., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 22—29.
14) The Pharmaceutical Society of Japan (ed.) "Iyakuhin Tenkabutu (Additives for Pharmaceutical Formulations)," Farumashia Rev., 22, Chap. 5—7 and Appendix, Nanzando, 1987.
15) Szejtli J., Szente L., *Eur. J. Pharm. Biopharm.*, **61**, 115—125 (2005).
16) Szejtli J., "Cyclodextrin Technology," Chap. 3 and 4, Kluwer Academic Publishers, Dordrecht, 1988.
17) Funasaki N., Uemura Y., Hada S., Neya S., *J. Phys. Chem.*, **100**, 16298—16301 (1996).
18) Funasaki N., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 36—41.
19) Katsuragi Y., Sugiura Y., Lee C., Otsuji K., Kurihara K., *Pharm. Res.*, **12**, 658—662 (1995).
20) Kurihara K., "Mikaku·Shukaku (Taste and Smell)," Chap. 6, Kagaku Dojin, Kyoto, 1990.
21) Kurihara K., Proceedings of the 22nd Meeting of the Society for Physicochemical Research of Physical Properties, Japan, 2004, pp. 1—6.
22) Ukai K., Harada T., Suzuki Y., *PCT Int. Appl.*, **1998**, 15 (1998).
23) Ukai K., Harada T., *PCT Int. Appl.*, **2000**, 20 (2000).
24) Tsuji E., Uchida T., Fukui A., Fujii R., Sunada H., *Chem. Pharm. Bull.*, **54**, 310—314 (2006).
25) Hada S., Ishikawa S., Neya S., Funasaki N., *J. Phys. Chem.*, *B*, **103**, 2579—2584 (1999).
26) Tanford C., "Physical Chemistry of Macromolecules," Chap. 8, John Wiley and Sons, New York, 1961.
27) Funasaki N., Yamaguchi H., Ishikawa S., Neya S., *Bull. Chem. Soc. Jpn.*, **76**, 903—911 (2003).
28) Funasaki N., Sumiyoshi T., Ishikawa S., Neya S., *Mol. Pharmceut.*, **1**, 166—172 (2004).
29) Funasaki N., Uemura Y., Hada S., Neya S., *Langmuir*, **12**, 2214—2219 (1996).
30) Funasaki N., Kawaguchi R., Ishikawa S., Hada S., Neya S., *J. Pharm. Sci.*, **88**, 759—762 (1999).