Comparative Study of the Response of Membrane Electrodes Based on Calix[6]arene and Calix[8]arene Derivatives to Organic Ammonium Ions

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p-*tert*-Butylcalix[8]arene-octaacetic acid octaethyl ester and calix[8]arene-octaacetic acid octaethyl ester well recognized 2-phenylethylamine and phenylalanine methyl ester compared with the corresponding calix[6]arene derivatives. Moreover, the calix[8]arene derivatives, especially one having *tert*-butyl groups, gave better selectivity against biologically active amines having a complicated structure, such as norephedrine. We considered the interaction between calixarenes and organic ammonium ions from the viewpoint of molecular symmetries.

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Calixarenes have been successfully used to construct ion-selective electrodes for primary organic ammonium ions as well as alkali metal cations.¹⁻⁴ The hydrophobic cavities generated by the aromatic walls of phenol residues are potentially useful for the inclusion of non-polar moieties of alkylammonium ions.^{1,2,4} Among various calixarene derivatives, calix[6]arene-hexaacetic acid hexaethyl ester (see the structure in Fig. 1) was an excellent ionophore for making a hexylammonium-selective electrode;⁵ this ionophore, named amine ionophore I, soon became commercially available from Fluka.⁶ Later, it was found to induce a strong response to methylammonium among primary ammonium ions.^{7,8} *p*-*tert*-Butylcalix[6]arene-hexaacetic acid hexaethyl ester was also found to be an ionophore for making an ethylammonium-selective electrode.⁹ In general, the hydrophobic cavities of calix[6]arene are potentially useful for the inclusion of linear alkyl groups of small size.^{5,7-9}

Fig. 1 Chemical structures of tested ionophores. Calix[6]arene-hexaacetic acid hexaethyl ester (amine ionophore I) (1), *p*-*tert*-butylcalix[6]arene-hexaacetic acid hexaethyl ester (2), calix[8]arene-octaacetic acid octaethyl ester (3), and *p*-*tert*-butylcalix[8]arene-octaacetic acid octaethyl ester (4).

As a continuation and extension of these studies, we were particularly interested in the use of calix[8]arene derivatives with larger hydrophobic cavities. Shvedene *et al.*¹⁰ reported that an electrode based on one of the calix[8]arene derivatives, *p*-1-adamantylcalix[8]arene-octaacetic acid octaethyl ester, responded strongly to phenylalanine methyl ester as well as 2-phenylethylamine, suggesting that the ability of calix[8]arene derivatives to recognize organic ammonium ions is significantly different from that of calix[6]arene derivatives. In the present study, we used the calixarene derivatives shown in Fig. 1, and systematically compared the response characteristics of calix[8]arene derivatives with those of calix[6]arene derivatives. We tested the organic amines shown in Fig. 2, in addition to various inorganic cations and simple organic ammonium ions tested previously.^{9,11} These calix[8]arene derivatives were also found to well recognize 2-phenylethylamine and phenylalanine methyl ester compared with the corresponding calix[6]arene derivatives. Moreover, the calix[8]arene derivatives, especially 4, gave better selectivity against biologically active amines of a complicated structure, such as norephedrine.

Experimental

Reagents

Calix[8]arene-octaacetic acid octaethyl ester, *p*-*tert*-butylcalix[8]arene-octaacetic acid octaethyl ester, and *p*-*tert*-butylcalix[6]arene-hexaacetic acid hexaethyl ester were synthesized according to similar procedures, as described previously.^{12,13} Other chemicals were obtained from the following sources: amine ionophore I and bis(2-ethylhexyl) sebacate were from Fluka (Buchs, Switzerland); potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB) was from Dojindo Laboratories (Kumamoto, Japan); poly(vinyl chloride) (PVC) (degree of polymerization, 1020) was from Nacalai Tesque (Kyoto, Japan); 2-phenylethylamine hydrochloride and L-phenylalanine methyl ester hydrochloride were from Sigma (St.

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Fig. 2 Chemical structures of organic amines tested in this study. 2-Phenylethylamine (5), phenylalanine methyl ester (6), norephedrine (7), dopamine (8), serotonin (9), and histamine (10).

Louis, MO, USA); and D,L-norephedrine hydrochloride was from Tokyo Kasei (Tokyo, Japan). All other chemicals were of analytical reagent grade.

Electrode system

The electroactive membranes were prepared by incorporating 1 mg of ionophore, 20 mol% of KTpCIPB relative to the ionophore, 60 μ l (55 mg) of bis(2-ethylhexyl) sebacate, and 30 mg of PVC.^{9,11} The materials were dissolved in tetrahydrofuran (about 1 ml) and poured into a flat Petri dish (28 mm in diameter). Then, the solvent was evaporated off at room temperature. The resulting membrane was excised and attached to a PVC tube (4 mm o.d., 3 mm i.d.) with tetrahydrofuran adhesive. Each PVC tube was filled with an internal solution comprising (a) 1 mM 2-phenylethylamine hydrochloride and 10 mM NaCl or (b) 1 mM D,L-norephedrine hydrochloride and 10 mM NaCl. The internal solutions (a) and (b) were, respectively, used when 2-phenylethylamine and norephedrine were examined as primary ions. The sensor membrane was conditioned overnight in the respective internal solution. The electrochemical cell arrangement was Ag,AgCl/internal solution/sensor membrane/sample solution/1 M lithium acetate (salt bridge)/10 mM KCl/Ag,AgCl. Potential measurements were made with a voltmeter produced by a field-effect transistor operational amplifier (LF356; National Semiconductor, Sunnyvale, CA, USA; input resistance $>10^{12} \Omega$) connected to a recorder (R-62; Rikadenki, Tokyo Japan). The volume of the sample solution was 1 ml, because our electrode system was compact, as described previously.^{14–16}

Evaluation of the electrode performance

The detection limit was defined as the intersection of the extrapolated linear regions of the calibration graph.¹⁷ The selectivity coefficients of the electrode, K_{ij}^{Pot} , were determined by a separate solution method¹⁷ using the respective chloride salts. The concentrations were adjusted to 10 mM. In some instances, a matched potential method¹⁸ was also applied to evaluate the selectivity coefficients. In this case, we used a fixed concentration (1.0×10^{-5} M) of 2-phenylethylamine as a background. The selectivity coefficients were calculated from the concentration of the interfering ion that induced the same amount of potential change as that induced by increasing the concentration of 2-phenylethylamine to 2.0×10^{-5} M. This measurement was performed in the presence of 0.1 M MgCl_2 to

keep the ionic strength of the solution constant, and MgCl_2 was chosen because the interference by magnesium was very slight. All measurements were performed at room temperature (about 25°C).

Results and Discussion

We compared the potentiometric ion selectivity coefficients of electrodes based on the calix[6]arene and calix[8]arene derivatives for various organic ammonium ions and inorganic cations, choosing 2-phenylethylamine as the primary ion (Fig. 3). These were determined by a separate solution method.¹⁷ The electrode made from calix[6]arene-hexaacetic acid hexaethyl ester (amine ionophore 1) (1; this number corresponds to that shown in Fig. 3) responded most strongly to 2-phenylethylamine among the organic ammonium ions tested in this study. However, there was a large interference by Tl^+ and Cs^+ , in accordance with the fact that this ionophore also acts as thallium and cesium ionophores.^{11,19} In the case of *p*-*tert*-butylcalix[6]arene-hexaacetic acid hexaethyl ester (2), the response to 2-phenylethylamine decreased remarkably, indicating that the existence of bulky *tert*-butyl groups blocks the inclusion of phenylethylamine in the cavity of calix[6]arene. This ionophore responded strongly to organic ammonium ions of small size, such as ethylammonium, as has been previously reported.⁹ The electrode made from calix[8]arene derivative (3) and *p*-*tert*-butylcalix[8]arene derivative (4) responded most strongly to phenylethylamine among various organic ammonium ions and inorganic cations. Shvedene *et al.*¹⁰ reported that *p*-1-adamantylcalix[8]arene derivative responded to phenylalanine methyl ester in addition to 2-phenylethylamine. This indicates that the cavity of calix[8]arene is large enough to include more bulky molecules than phenylethylamine. In accordance with this fact, both calix[8]arene derivatives with and without *p*-*tert*-butyl groups (3 and 4) responded to L-phenylalanine methyl ester more strongly than calix[6]arene derivatives (1 and 2), though the response was somewhat smaller than in the case of 2-phenylethylamine, as shown in Fig. 3. To cross-check the values of the selectivity coefficients, we measured the selectivity coefficients against L-phenylalanine methyl ester for all of the calixarene derivatives tested using a matched potential method. The logarithmic values were -2.1 for 1, -1.2 for 2, -0.6 for 3, and -0.3 for 4. These values were similar to those obtained by the separate solution method shown in Fig. 3. Such a good correlation between the selectivity coefficients measured by the separate solution method and the matched potential method has generally been observed with electrodes using calixarene derivatives.^{9,11}

Here, it is of interest to consider the interaction between calixarenes and organic ammonium ions from the viewpoint of their molecular symmetries. Figure 4 shows a schematic illustration of the interaction between calix[6]arene-hexaacetic acid hexaethyl ester and the primary organic ammonium ion. In this figure, we depict the conformation of the calixarene as a cone form. The molecular basis for the recognition of calix[6]arene derivatives by primary organic ammonium ions is that receptors bearing a C_3 -symmetrical proton acceptor site (negatively polarized oxygen atoms of carbonyl groups) of the calix[6]arene derivatives can strongly interact with primary ammonium ions, which act as C_3 -symmetrical proton donors.^{20,21} Furthermore, the hydrophobic cavities generated by the aromatic walls of phenol residues of the derivatives are potentially useful for the inclusion of non-polar moieties of

Fig. 3 Comparison of the selectivity coefficients of electrodes. Each number corresponds to the number of compounds shown in Fig. 1. Bis(2-ethylhexyl) sebacate was used as the solvent mediator. 2-Phenylethylamine was chosen as the primary ion. Abbreviations used: PA, 2-phenylethylamine; Phe-OMe, L-phenylalanine methyl ester.

Fig. 4 Schematic illustration of the complexation geometry between calix[6]arene-hexaacetic acid hexaethyl ester and the primary organic ammonium ion.

organic ammonium ions. Thus, these derivatives were successfully used to construct electrodes responsive to specific organic ammonium ions.^{1,2,4-9,21} However, the calix[6]arene derivatives were effective for rather small molecules, such as methylammonium and ethylammonium.⁷⁻⁹ In the case of bulky organic ammonium ions, the use of calixarenes having a larger cavity, such as calix[8]arene derivatives, would be effective. However, calix[8]arene derivatives, whose proton acceptor site is not C_3 -symmetrical, might restrict the interaction with primary ammonium ions for a difference in symmetry. Nevertheless, we observed that the proton acceptor site of the calix[8]arene derivatives, which deviated from C_3 -symmetry, was still able to interact especially with organic ammonium ions of an “unsymmetrical” structure, such as phenylalanine methyl ester. This implies that calix[8]arene derivatives were rather effective for the recognition of bulky and unsymmetrical organic ammonium ions, using a large cavity and choosing an appropriate position at the proton acceptor sites to interact with these primary ammonium ions.

Thus, we further examined the ability of these calix[8]arene derivatives to discriminate organic ammonium ions having a complicated structure. Because phenylalanine methyl ester

possesses a “2-phenylethylamine skeleton”, we were particularly interested in examining the response characteristics of biologically active amines having this skeleton, *i.e.*, norephedrine, dopamine, serotonin, and histamine. The structures of these amines are shown in Fig. 2, along with 2-phenylethylamine. As shown in Fig. 5, both calix[8]arene derivatives (**3** and **4**) responded most strongly to norephedrine among the biologically active amines, except for 2-phenylethylamine, indicating that the derivatives well recognized certain biologically active amines of unsymmetrical and complex structures. The derivative **4** discriminated norephedrine better than **3**, in accordance with the greater ability of **4** to recognize phenylalanine methyl ester, shown in Fig. 3. The norephedrine and phenylalanine methyl ester had well-separated hydrophilic and hydrophobic parts, which probably fitted the interaction with the derivative **4**.

Then, calibration graphs were obtained for the electrodes by measuring known amounts of D,L-norephedrine hydrochloride added to 0.1 M $MgCl_2$, and plotting the concentrations against the corresponding potential readings. A high concentration of $MgCl_2$ was added to adjust the ionic strength of the solution,⁹ and $MgCl_2$ was chosen because the interference from magnesium was slight ($\log k_{\text{Pot,NER,Mg}^{2+}}^{\text{Pot}} < -4$). As shown in Fig. 6, all of the ionophores tested in this study gave near-Nernstian responses to norephedrine. Table 1 summarizes the response characteristics of the electrodes. Calix[8]arene derivatives (**3** and **4**) showed a higher sensitivity than the corresponding calix[6]arene derivatives (**1** and **2**). The slope and the detection limit for **3** were 57 mV per concentration decade and 2×10^{-6} M, respectively, and those for **4** were 52 mV per concentration decade and 2×10^{-6} M, respectively. The response times (90% final signal) of both electrodes were below 10 s when the concentration of norephedrine hydrochloride was changed from 10 to 100 μM . The lifetimes of the electrodes were more than one month, similar to those of other electrodes using calixarene derivatives reported previously.^{5,10} The pH dependence (pH 2 – 12) of the electrodes using the ionophores **3** and **4** was also examined at 1 mM D,L-norephedrine in a manner similar to that described previously.⁹ The electrode response was independent of the pH over a pH range of 3 – 8.5. The decrease in the potential above pH 8.5 was attributable to an increase in the

Fig. 5 Comparison of the selectivity coefficients of electrodes based on calix[8]arene-octaacetic acid octaethyl ester (3) and *p*-*tert*-butylcalix[8]arene-octaacetic acid octaethyl ester (4). Bis(2-ethylhexyl) sebacate was used as the solvent mediator. D,L-Norephedrine was chosen as the primary ion. Abbreviations used: PA, 2-phenylethylamine; NER, D,L-norephedrine; 5-HT, serotonin; DA, dopamine; Hi, histamine.

Fig. 6 Comparison of the response of electrodes to D,L-norephedrine in 0.1 M MgCl₂. Calix[6]arene-hexaacetic acid hexaethyl ester (amine ionophore I) (1), *p*-*tert*-butylcalix[6]arene-hexaacetic acid hexaethyl ester (2), calix[8]arene-octaacetic acid octaethyl ester (3), and *p*-*tert*-butylcalix[8]arene-octaacetic acid octaethyl ester (4).

concentration of unprotonated amine, since the pK_a of norephedrine has been reported to be 9.55.²² The decrease in the potential below pH 3 may be due to the protonation of calix[8]arene derivatives in the membrane phase, resulting in a loss of the ability to complex with norephedrine. A similar observation was made with an ethylammonium electrode using ionophore 2 and a thallium electrode using ionophore 1.^{9,11}

These results demonstrated that calix[8]arene derivatives, especially 4, can discriminate biologically active amines of a complicated structure, such as norephedrine.

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Table 1 Comparison of the electrode performance in response to D,L-norephedrine^a

Ionophore	Slope/mV per decade	Detection limit/ μ M
1	50	30
2	52	30
3	57	2
4	52	2

a. Bis(2-ethylhexyl) sebacate was used as the solvent mediator. All measurements were performed in 0.1 M MgCl₂.

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References

1. P. Bühlmann, E. Pretsch, and E. Bakker, *Chem. Rev.*, **1998**, 98, 1593.
2. R. Ludwig, *Fresenius J. Anal. Chem.*, **2000**, 367, 103.
3. Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, and S. Amemiya, *Pure Appl. Chem.*, **2000**, 72, 1851.
4. Y. Umezawa, P. Bühlmann, K. Umezawa, and N. Hamada, *Pure Appl. Chem.*, **2002**, 74, 995.
5. W. H. Chan, K. K. Shiu, and X. H. Gu, *Analyst*, **1993**, 118, 863.
6. Fluka Chemika Selectophore®, **1996**, Fluka, Buchs, Switzerland.
7. T. Katsu, D. Xu, K. Tsuji, and T. Nagamatsu, *Anal. Chim. Acta*, **1997**, 354, 301.
8. T. Katsu, M. Akagi, T. Hiramatsu, and T. Tsuchiya, *Analyst*, **1998**, 123, 1369.
9. T. Katsu and K. Ido, *Anal. Sci.*, **2002**, 18, 473.
10. N. V. Shvedene, M. Yu. Nemilova, V. V. Kovalev, E. A. Shokova, A. K. Rozov, and I. V. Pletnev, *Sens. Actuators B*, **1995**, 26–27, 372.
11. T. Katsu, K. Ido, K. Takaishi, and H. Yokosu, *Sens. Actuators B*, **2002**, 87, 331.
12. M. A. McKervey, E. M. Seward, G. Ferguson, B. Ruhl, and S. J. Harris, *J. Chem. Soc., Chem. Commun.*, **1985**, 388.
13. F. Arnaud-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill, and E. M. Seward, *J. Am. Chem. Soc.*, **1989**, 111, 8681.
14. T. Katsu, H. Kobayashi, and Y. Fujita, *Biochim. Biophys. Acta*, **1986**, 860, 608.
15. T. Katsu, K. Ido, S. Sagara, K. Tsubaki, and K. Fujii, *Electroanalysis*, **2003**, 15, 287.
16. K. Yasuda, C. Ohmizo, and T. Katsu, *J. Microbiol. Methods*, **2003**, 54, 111.
17. R. P. Buck and E. Lindner, *Pure Appl. Chem.*, **1994**, 66, 2527.
18. Y. Umezawa, K. Umezawa, and H. Sato, *Pure Appl. Chem.*, **1995**, 67, 507.
19. A. Cadogan, D. Diamond, M. R. Smyth, G. Svehla, M. A. McKervey, E. M. Seward, and S. J. Harris, *Analyst*, **1990**, 115, 1207.
20. S.-K. Chang, M. J. Jang, S. Y. Han, J. H. Lee, M. H. Kang, and K. T. No, *Chem. Lett.*, **1992**, 1937.
21. K. Odashima, K. Yagi, K. Tohda, and Y. Umezawa, *Anal. Chem.*, **1993**, 65, 1074.
22. T. B. Vree, A. Th. J. M. Muskens, and J. M. van Rossum, *J. Pharm. Pharmacol.*, **1969**, 21, 774.