Exploitation of Na^+-Selective Electrodes for Protein Solutions from Crown-Bridged Calix[4]quinones

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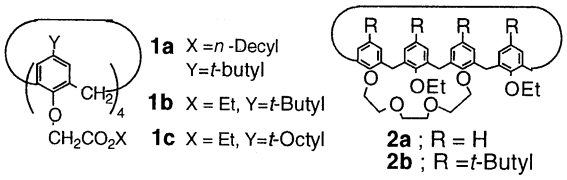
(Received October 2, 1995)

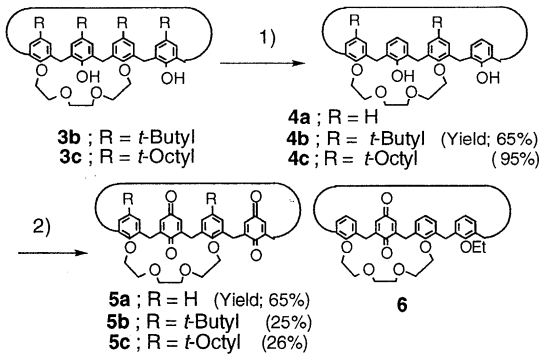
Crown-bridged calix[4]quinones (**5** and **6**) were synthesized from calix[4]crown (**2**). The ion-selective electrodes prepared from **5** or **6** still retained high selectivity for Na^+ against K^+ ($>10^3$). For protein solution samples the electrode prepared from **2** showed a large phase divergence whereas those prepared from **5** or **6** were much more stable in protein solution. The difference is attributed to the less hydrophobic nature of **5** and **6** which strongly influences the protein adsorption onto the membrane surface.

Nowadays it has generally been accepted that calixarene-based ionophores frequently show ion selectivity superior to crown-ether-based ionophores.[1-4] When one applies these calixarene-based ionophores to the design of ion-selective electrodes for protein solutions, one meets a difficult problem. For example, it is known that calix[4]aryl ester derivatives **1** show high Na^+ selectivity[5-7] but the composite membranes prepared from **1**, poly(vinyl chloride) (PVC), and plasticizer cause adsorption and subsequent coagulation of proteins.[7,8] Hence, the electrodes cannot be applied to evaluation of the Na^+ concentration in blood in spite of the high Na^+ selectivity. More recently, we exploited a calix[4]crown **2b** with a short -$(CH_2CH_2O)_2CH_2CH_2$- crown strap on the lower rim, the cavity size of which is adjusted to that between Li^+ and Na^+.[3,9] This calix[4]crown showed record-breaking selectivity for Na^+ against K^+ ($>10^5$). However, the composite membrane again induced protein adsorption onto the membrane surface.[8]



What is the origin of protein adsorption which is observed for calixarene-based membranes but scarcely for crown-ether-based membranes? As a working hypothesis we assumed that the hydrophobic groups present in calixarene-based ionophores are related to such a protein adsorption phenomenon. We thus tried to diminish the hydrophobicity while keeping the basic framework of **2**. New calixarene-based ionophores we designed are crown-bridged calix[4]quinones **5** and **6**. We expected that the quinone unit should be less hydrophobic than the *p-tert*-butylphenyl unit. We have found that although the Na^+ selectivity (against K^+) somewhat drops, protein adsorption is suppressed to a practical level.

Compounds **5**[10] were synthesized from **3**[3] according to Scheme 1 and identified by IR, ^1H NMR and mass spectral evidence and elemental analysis. The synthesis of compound **6**[11] was reported previously.[9] The conformational change



Reagents and conditions: 1) 20 eq. $AlCl_3$, toluene, r.t., 1 h; 2) $TI(NO_3)_3 \cdot 3H_2O$, MeOH, EtOH, $CHCl_3$, 10 min

Scheme 1. Synthetic routes and compounds used in this work.

induced by the Na^+-binding was studied by ^1H NMR (including NOESY and COSY) and ^{13}C NMR. The splitting patterns of the $ArCH_2Ar$ methylene protons for **5a** are illustrated in Figure 1. A pair of doublets with small $\Delta\delta_H$ for the $ArCH_2Ar$ methylene protons and the NOESY correlation between the quinone and the crown protons establish that **5a** adopts "1,3-alternate" in the absence of metal cations. In the presence of Na^+, on the other hand, **5a** adopts "cone" so that quinone oxygens can coordinate to inserted Na^+. The chemical shifts and the peak numbers in ^{13}C NMR also coincide with these conformations. On the other hand, the ^1H NMR spectrum of **6** gave two pairs of doublets with $\Delta\delta_H$ = 1.23 and 1.36 ppm (no metal) or $\Delta\delta_H$ = 0.88 and 0.93 ppm (in the presence of Na^+) for the $ArCH_2Ar$ methylene protons. The results indicate that both **6** and **6**•Na^+ adopt a cone conformation.

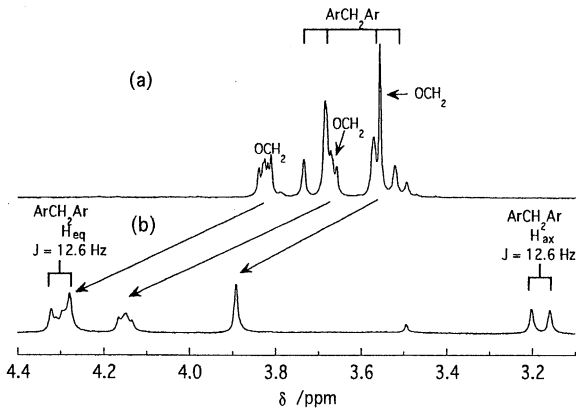


Figure 1. Partial ^1H NMR spectra of **5a** and its Na^+ complex (TMS, $CDCl_3$): (a) in the absence of metal cations, (b) in the presence of an excess amount of sodium picrate.

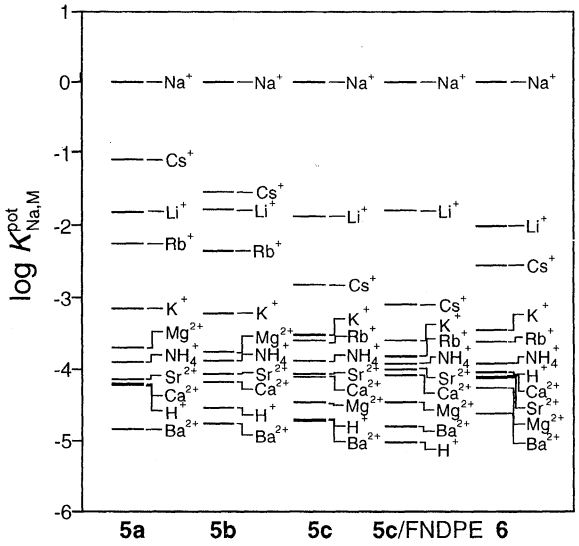


Figure 2. Selectivity coefficients of Na^+-selective electrodes containing NPOE unless otherwise mentioned. The PVC membranes consist of 3.2% (in weight) calix[4]arene derivative, 64.1% NPOE as a plasticizer, 32.1% PVC, and 0.6% potassium tetrakis(*p*-chlorophenyl)borate. The emf measurements were carried out at 25 °C with an electrochemical cell of Ag-AgCl/1 x 10^{-1} mol dm^{-3} NaCl/PVC membrane/sample solution/1 x 10^{-1} mol dm^{-3} NH_4Cl/ sat. KCl/Ag-AgCl.

The preparation of ion-selective electrodes were described previously.[3,6] The electrodes were conditioned in aqueous 0.10 mol dm^{-3} NaCl solution overnight. Both **5** and **6** possessed a near-Nernstian slope (>57 mV/decade) and the lower limit of their working concentration was log[Na^+] = -4.0. This value is slightly inferior to that of **2b** (log[Na^+] > -5.0).[3] The selectivity of Na^+ against other metal cations (M^{n+}) ($\log K^{pot}_{Na,M}$) is summarized in Figure 2.

Examination of Figure 2 reveals that five electrodes prepared from **5** or **6** all show high Na^+ selectivity ($\log K^{pot}_{Na,M}$ < -3), indicating that although Na^+ selectivity itself is somewhat inferior to that of **2**, crown-bridged calix[4]quinones still retain satisfactory Na^+ selectivity for the practical use. The improvement in $\log K^{pot}_{Na,M}$ (= -3.52) for **5c** (compared with -3.16 for **5a** and -3.21 for **5b**) is attributable to high miscibility of **5c** with the plasticizer (NPOE:*o*-nitrophenyl octyl ether).[6] In **6** the presence of one ethoxy group should be effective to construct a rigid ionophoric cavity. As already found for other calixarene-based ion-selective electrodes,[5,6] the use of a polar plasticizer, FNDPE (*o*-nitro-*o'*-fluorodiphenyl ether) further enhanced Na^+ selectivity ($\log K^{pot}_{Na,M}$ = -3.61).

The potential responses of ion-selective electrodes containing **2a**, **5a**, or **6** to a control serum solution (Wako I) are shown in Figure 3. It is seen from Figure 3. that a calix[4]crown-type **2a**-based electrode induces a large phase divergence whereas it can be relatively suppressed in calix[4]quinone-type **5a**- and **6**-based electrodes. The similar tendency was also observed for a 0.15 mol dm^{-3} NaCl containing 7wt% BSA (bovine serum albumin) solution.

In conclusion, the present study established that less hydrophobic crown-bridged calix[4]quinones are very useful to design Na^+-selective electrodes with high Na^+ selectivity and preferable performance for protein solutions.

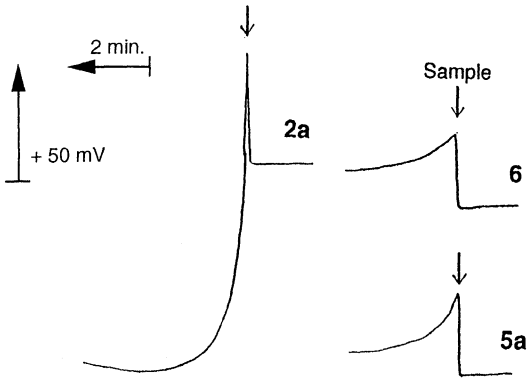


Figure 3. Potential response to a control serum solution (Wako I). A 1.0 ml of sample solution was injected into a 1.0 x 10^{-3} mol dm^{-3} NaCl solution (25 ml) adjusted to pH 7.4 with 1.0 x 10^{-1} mol dm^{-3} Tris-HCl buffer.

References and Notes

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10 **5a**; yellow crystal, mp >300 °C; MS (SIMS(+)), 589 ([M+Na]$^+$), IR (KBr) $\nu_{C=O}$ 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05-6.92 (m, 6H, ArH), 6.60 (s, 4H, quinone), 3.84-3.80 (m, 4H, OCH_2), 3.75-3.52 (m, 16H, OCH_2, $ArCH_2Ar$); Anal. Found: C, 69.94; H, 5.30%. Calcd for $C_{34}H_{30}O_8$+H_2O: C, 69.85; H, 5.52%. **5b**; yellow crystal, mp >300 °C; MS (SIMS(+)), 701 ([M+Na]$^+$), IR (KBr) $\nu_{C=O}$ 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.04 (s, 4H, ArH), 6.50 (s, 4H, quinone), 3.90-3.76 (m, 12H, OCH_2, $ArCH_2Ar$), 3.64 (s, 4H, OCH_2), 3.41 (d, J= 14.7 Hz, $ArCH_2Ar$), 1.25 (s, 18H, t-C_4H_9); Anal. Found: C, 73.88; H, 6.40%. Calcd for $C_{42}H_{46}O_8$: C, 74.30; H, 6.84%. Mp' 210-214 °C for **5c**.
11 **6**; yellow crystal, mp 217-220 °C; MS (EI), 580 (M^+), IR (KBr) $\nu_{C=O}$ 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (dd, J= 1.8, 7.2 Hz, 2H, ArH), 7.04 (dd, J= 1.8, 7.2, 2H, ArH), 6.98 (t, J= 7.2 Hz, 2H, ArH), 6.27-6.16 (m, 3H, ArH), 5.61 (br. s, 2H, quinone), 4.52-4.42 (m, 4H, OCH_2, $ArCH_2Ar$), 4.26 (d, J= 14.4 Hz, 2H, $ArCH_2Ar$), 4.03-3.65 (m, 12H, OCH_2), 3.31 (d, J= 13.8 Hz, 2H, $ArCH_2Ar$), 2.88 (d, J= 14.4 Hz, 2H, $ArCH_2Ar$), 1.50 (t, J= 7.0 Hz, 3H, OCH_2CH_3); Anal. Found: C, 74.25; H, 6.15%. Calcd for $C_{36}H_{36}O_7$: C, 74.46; H, 6.25%.