Synthesis and Electrochemical Behavior of a New Water Soluble Ca^{2+}-selective Ionophore Based on Calix[4]arene-triacid-monoquinone

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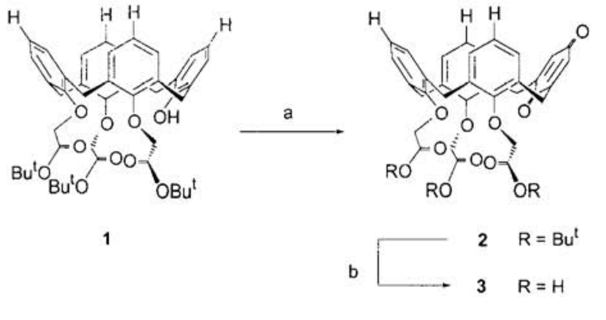
A new water-soluble calix[4]arene-triacid-monoquinone **3** has been synthesized and its ion binding properties in aqueous solution were investigated by means of voltammetry and UV-visible spectrophotometry. The electrochemical behavior of **3** is dependent on the concentration of Ca^{2+} ion rather than that of other alkaline earth metal ions as well as alkali metal cations. The selective response toward Ca^{2+} was achieved even in the presence of a large excess (> 1000-fold) of interfering Na^+ ion.

There have been intense research efforts to develop selective and efficient sensory materials for the biologically important guest species.[1] Especially, the development of selective sensors for Ca^{2+} ion in physiological environment is utterly important.[2] In comparison to conventional potentiometry and optical measurements of Ca^{2+} ion, voltammetric determination is very promising because of its high sensitivity, very small amount of sample needed and applicability for *in situ* monitoring. For voltammetric determination of electrochemically inactive target ions such as Ca^{2+} ion, it is necessary to exploit a redox-active ionophore that can recognize the ion and exhibit characteristic redox behavior. The main difficulty in this end is to prepare water-soluble ionophore that can overcome the presence of Na^+ ion of much higher concentration compared to that of Ca^{2+} ion in the biological fluids.[3]

Recently, a few quinone-derivatized calixarenes[4] have been synthesized and their electrochemical properties have been studied in polar aprotic solvents for integration of molecular recognition and electrochemical signal transduction.[5,6] And, it was reported that the redox behavior of several calix[4]arene derivatives depend sensitively on the size and charge of alkali and alkaline earth metal ions.[6] To the best of our knowledge, however, no water soluble calixarene-based quinone system has been reported yet, which is required to apply to physiological solutions. In this paper, we report a new water soluble calix[4]arene-monoquinone bearing three carboxylic acid groups, which exhibits a remarkably selective response to Ca^{2+} ion in neutral and basic buffer solutions.



a: $Tl(NO_3)_3$, $MeOH/CHCl_3$, b: CF_3CO_2H, CH_2Cl_2

Water-soluble and redox-active triacid-monoquinone **3** was synthesized by the hydrolysis of tri-*tert*-butyl ester derivative **2**,[7] which was obtained by selective trialkylation of calix[4]arene with *tert*-butyl bromoacetate (CaH_2/DMF) utilizing CF_3CO_2H in CH_2Cl_2 (**1**) and then oxidizing the phenol moiety to quinone.[8] Triacid-monoquinone **3** was moderately soluble in 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES) buffer as well as in many common organic solvents, such as acetone, MeCN, MeOH, and $CHCl_3$, etc. The pH of buffer was controlled to be 7.4 by tetraethylammonium hydroxide.

The recognizing capability of **3** is possibly associated with its intrinsic nature of pseudocavity-like structure of lower rim, which is known to be suitable for accommodating Na^+, Ca^{2+},[9] and some of lanthanide ions.[10] In addition, it is expected that three carboxylate terminals in the lower rim and a quinone moiety assist **3** to bind exclusively Ca^{2+} ion among other potentially competing hard metal ions. The formation of complex between **3** and Ca^{2+} ion in aqueous HEPES buffer solution at pH 7.4 is confirmed by UV-visible[11] and ^1H NMR spectroscopy.[12]

As a result of complexation with Ca^{2+} ion, the redox behavior of **3** undergoes a drastic change. Figure 1 shows cyclic voltammograms of 0.5 mM **3** at pH 7.4. The redox behavior of **3** is very similar to that of the simple quinone derivatives such as *p*-benzoquinone(BQ) in the same conditions. The apparently irreversible redox wave of free **3** is due to the proton-coupled electrochemical reduction. When BQ instead of **3** is used, even a large amount of alkali and alkaline earth metal ions have no significant influence on the redox behavior of BQ. Also, the addition of alkali metal ions to free **3** solution leads little change in the electrochemical behavior of **3**.

But the presence of alkaline earth metal ions alters both the reduction/oxidation peak potentials and currents of **3**. The differences between potentials of the reduction peak (ΔE_p) in the absence and presence of metal ions are summarized in Table 1. The results in Table 1 clearly show that **3** gives a selective voltammetric response to Ca^{2+} ion over other alkaline earth metal ions with some degree of interference. With respect to the electrochemical behavior, the irreversible redox wave of free **3** undergoes a dramatic transition to a highly symmetric one upon the addition of Ca^{2+} ion, as shown in Figure 1. The ratio of oxidation (i_a) to reduction current (i_c), i_a/i_c, is about 0.9 and the wave around +0.4 V due to the oxidation of protonated quinone moiety(QH_2) disappears. This result indicates that Ca^{2+} ion effectively blocks the protonation of reduced quinone. Probably, Ca^{2+} ion is positioned so close to the quinone moiety that the reduction of the quinone should be facilitated by strong electrostatic interaction rather than by protonation.

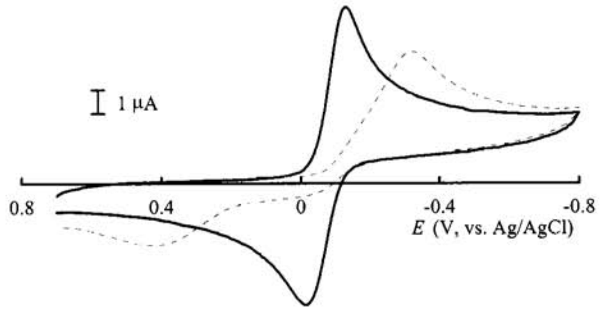


Figure 1. Cyclic voltammograms of 0.5 mM **3** in the absence (dotted line) and presence(solid line) of 0.5 mM $CaCl_2$ at pH 7.4 (HEPES buffer).

Table 1. Voltammetric responses of **3**[a] in the presence of alkali and alkaline earth metal ions

Metal ions	ΔE_p[b] /mV
Li^+, Na^+, K^+, Rb^+, Cs^+	0
Mg^{2+}	32
Ca^{2+}	190
Sr^{2+}	176
Ba^{2+}	132

[a] [**3**] = 0.5 mM (pH 7.4, 0.05 M HEPES buffer). Scan rate: 50 mV/s. [b] ΔE_p: the difference between reduction peak potentials in the absence and presence of 0.5 mM metal ions. When BQ is used instead of **3**, all of the ΔE_p is zero.

This suggestion is supported by the temporal changes of *in situ* UV-visible spectra[13] of **3** in the absence and presence of Ca^{2+} ion during the electrochemical reduction at –0.60 V. The characteristic peaks due to the anion radical form of quinone at 321 nm and 430 nm decay much slowly in the presence of Ca^{2+} ion. This phenomenon demonstrates the unique role of Ca^{2+} ions that stabilize the anion radical form of semiquinone by retarding the progress of subsequent protonation.

To have a further insight into the practical application for the selective recognition of Ca^{2+} ion in physiological fluids, square-wave voltammograms of **3** were obtained in the presence of 0.15 M Na^+ ion while the amount of Ca^{2+} ion varied up to 2.5 mM. Upon the incremental additions of Ca^{2+} ion to **3** solution, the peak height at –0.08 V increases progressively at the expense of the peak of free **3** at –0.27 V, as shown in Figure 2. The electrochemical response toward Ca^{2+} ion is still pronounced in the region of submillimolar concentration range even in the presence of a relatively large background concentration of Na^+ ion, which is around 1000-fold of $[Ca^{2+}]$ and comparable to the blood plasma environment.

In conclusion, we have synthesized a new water soluble calix[4]-quinone-acid which has a remarkable selectivity toward Ca^{2+} ion over Mg^{2+} and Na^+ ion. This implies strongly that the present water soluble triacid-quinone derivative **3** can be utilized to determine Ca^{2+} ion in physiological fluid by voltammetry. Detailed studies on **3** such as exact stoichiometry of the **3**-Ca^{2+} complex and complicated voltammetric behavior are under progress and the results will be reported in separate articles.

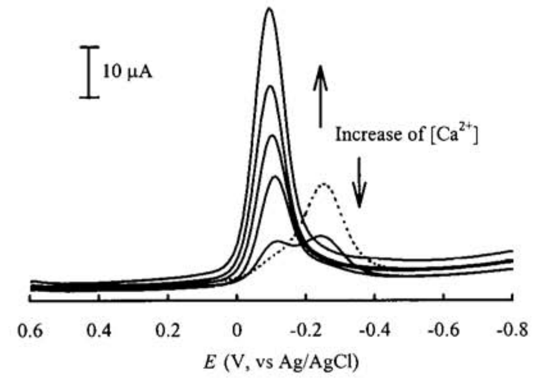


Figure 2. Square-wave voltammograms of **3** in the absence(dotted line) and the presence(solid line) of 0.05, 0.25, 0.50, 1.00, and 2.50 mM Ca^{2+} in pH 7.4 HEPES buffer. [**3**] = 0.5 mM, $[Na^+]$ = 0.15 M, step potential 4 mV, amplitude 25 mV, and frequency 100 Hz.

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8 **3**: Yield: 82%, mp > 281 °C (decomp); ^1H NMR (CD_3CN) δ 7,13 (d, *J* = 7.5 Hz, 2H), 6.92 (t, *J* = 8.1 Hz, 1H), 6.63 (m, 4H), 6.46 (t, *J* = 7.5 Hz, 2H), 6.43 (s, 2H), 4.41 (s, 2H), 4.36 (d, *J* = 15.9 Hz, 2H), 4.33 (d, *J* = 13.5 Hz, 2H), 4.23 (d, *J* = 15.9 Hz, 2H), 3.83 (d, *J* = 13.5 Hz, 2H), 3.21 (d, *J* = 13.5 Hz, 2H), 3.16 (d, *J* = 13.5 Hz, 2H); MS (FAB, *m*-NBA): *m/z* 613 (22.5) $[(M+H)^+]$. Found: C, 65.34; H, 4.75%. Anal. Calcd for $C_{34}H_{28}O_{11}\cdot 0.5\,H_2O$: C, 65.71; H, 4.70%.
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11 Upon titration of **3** with $CaCl_2$ guest solution in HEPES buffer solution at pH 7.4, the electronic transition ($\pi \rightarrow \pi^*$) at 258 nm of the quinone is significantly perturbed, which indicates that **3** forms a strong complex with Ca^{2+}.
12 The chemical shifts of OC**H**$_2$CO at 4.360 ppm and Ar**H**(*p*) at 7.054 ppm, for example, move to the down-field by up to 0.16 ppm and 0.08 ppm, respectively, as the Ca^{2+} ion concentration increases.
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