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Indirect Detection of Alkaline Earth Ions by the Voltammetric Response of Ferricyanide Anion at a Glassy Carbon Electrode Anodized in 1-Octanol

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The suppressed voltammetric response of $Fe(CN)_6^{3-}$ at a glassy carbon (GC) electrode anodized in 1-octanol is restored by addition of alkaline earth ions and this can be applied to the detection of such cations. A good dependence of the cathodic currents of $Fe(CN)_6^{3-}$ at a 1-octanol-modified GC electrode on the concentration of each cation (Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}) is obtained over the range 10 μM—1 mM. The slight increase in enhanced voltammetric response of $Fe(CN)_6^{3-}$ in the presence of Ca^{2+} following added MeOH ruled out an ion-extraction mechanism for the observed phenomena. The effects of Ca^{2+} upon the voltammetric behavior of $Fe(CN)_6^{4-}$ and $IrCl_6^{2-}$ as well as $Fe(CN)_6^{3-}$ at a 1-octanol-modified GC electrode were compared with those previously reported at a carbon microelectrode, suggesting that the suppression of the cathodic reaction of $Fe(CN)_6^{3-}$ and its elimination by the addition of alkaline earth ions are brought about through transformation of a macroelectrode into a pseudo-microelectrode by anodization in 1-octanol. This is strongly supported by the fact that similar phenomena occur at other chemically modified electrodes, providing that modification is controlled to give an effective surface area corresponding to that of a pseudo-microelectrode.

Key words alkaline earth ion; indirect electrochemical detection; modified electrode; ferricyanide anion; cyclic voltammetry

Previously, Umezawa *et al.* reported that the voltammetric response of $Fe(CN)_6^{4-}$ at a glassy carbon (GC) electrode, modified with an LB membrane of didodecyl phosphate, can be used for the indirect detection of Ca^{2+}.[1] As far as the origin of the observed phenomena is concerned, it has been proposed that the LB membrane formed on a GC electrode functions as an ion channel, with switching being controlled by the amount of coexisting Ca^{2+}, resulting in a change in permeability of the marker anion monitored as its voltammetric response. Based on the novel concept of an ion-channel sensor, various types of electrodes modified using molecules with anionic functional groups have been prepared and used for the electrochemical detection of Ca^{2+} and H^+ in the presence of electro-active marker anions.[2-5]

Recently, we have reported that the anodic oxidative treatment of a GC electrode in an alkanol containing a small amount of H_2SO_4 allows the alkanol molecules to be fixed on the surface *via* an ether-linkage (Chart 1).[6] Study of the electrochemical performance of the alkanol-modified electrodes thus obtained has revealed that the electrode reaction of $Fe(CN)_6^{3-}$ at a GC electrode anodized in 1-octanol is totally depressed, but can be restored by the addition of Ca^{2+} even though the modified electrode seems to have no anionic functional groups on its surface.[7] In this paper, we propose cyclic voltammetry (CV) of $Fe(CN)_6^{3-}$ at a GC electrode anodized in 1-octanol as a general tool for the electrochemical detection of alkaline earth ions, together with a plausible explanation of the observed phenomena.

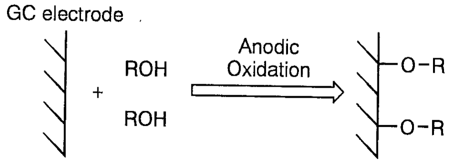

Chart 1

Results and Discussion

Figure 1 shows the results of CV obtained for an aqueous solution of $K_3Fe(CN)_6$ (5 mM) containing KCl (10 mM) as a supporting electrolyte at a GC electrode anodized in 1-octanol (see Chart 1; $R = -(CH_2)_7CH_3$). As previously reported,[7] the electrode response of $Fe(CN)_6^{3-}$ (the marker anion) was totally depressed at the modified electrode, and partly alleviated by addition of $CaCl_2$, the extent of this effect being enhanced by a larger amount of cation. The observed behavior was fully reproducible: the same voltammetric profiles for the marker anion were obtained repeatedly when a solution in the presence or absence of Ca^{2+} was subjected to CV measurements at

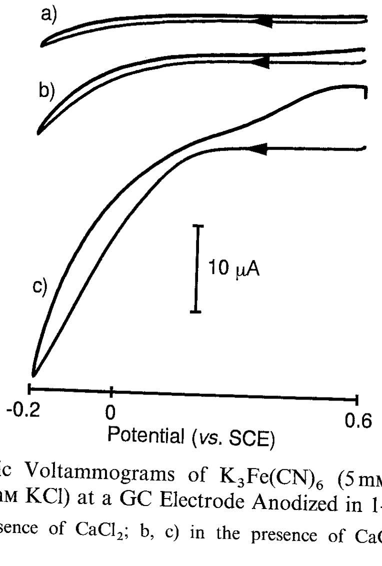

Fig. 1. Cyclic Voltammograms of $K_3Fe(CN)_6$ (5 mM) in Aqueous Solution (10 mM KCl) at a GC Electrode Anodized in 1-Octanol

a) In the absence of $CaCl_2$; b, c) in the presence of $CaCl_2$ (0.1, 1.0 mM, respectively).

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the modified electrode.

Similar voltammetric results were obtained at a GC electrode anodized in $CH_3(CH_2)_nOH$ with $n=0$—6. However, suppression of the electrochemical response of the marker anion at an alkanol-modified GC electrode diminished as n in $CH_3(CH_2)_nOH$ decreased. Thus, we chose a GC electrode anodized in 1-octanol for further study. Although GC electrodes anodized in a 1-alkanol longer than 1-octanol were attractive, they were not investigated. This is because such alkanols are solid and the anodic modification must be performed in some inert organic solvent containing them as a modifier and no satisfactory procedure for this has been developed to date.

In order to establish the conditions for applying the present system as an alkaline earth ion-sensor with a high sensitivity, an investigation was carried out to examine how the identity of constituting cation and concentration of supporting electrolyte affect the Ca^{2+}-induced increment in the voltammetric response of the marker anion. The results are summarized in Table 1, where I_0 and I stand for the cathodic currents observed at -0.2 V *vs.* saturated calomel electrode (SCE) on CV of $K_3Fe(CN)_6$ (5 mM) at an 1-octanol-modified GC electrode in the absence and the presence of $CaCl_2$ (1 mM), respectively (see Fig. 1). The effects on the increment were evaluated from the $I-I_0$ value. When LiCl, NaCl, or KCl was used as a supporting electrolyte (10 mM), addition of Ca^{2+} induced a slightly larger $I-I_0$ in the solution containing KCl than in those containing LiCl or NaCl (runs 1—3). Ammonium salts such as NH_4Cl, Me_4NCl, and Et_4NCl were also used as supporting electrolyte, although each $I-I_0$ was a little smaller than in the presence of KCl (runs 4—6). For solutions of the marker anion containing KCl, the $I-I_0$ value decreased markedly with an increase in the amount of supporting electrolyte (runs 3, 8—10). Of the KCl concentrations examined here, 10 mM proved to be most suitable.

Table 1. Effects of the Constituting Cation and the Concentration of a Supporting Electrolyte on the Voltammetric Response of $K_3Fe(CN)_6$ in the Presence and Absence of $CaCl_2$ at a GC Electrode Anodized in 1-Octanol[a)]

Run	Supporting electrolyte	Concentration (mM)	I_0[b)] (μA)	I[c)] (μA)	$I-I_0$ (μA)
1	LiCl	10	2.6	19.3	16.7
2	NaCl	10	4.0	20.3	16.4
3	KCl	10	5.1	24.2	19.1
4	NH_4Cl	10	5.8	23.6	17.8
5	Me_4NCl	10	7.2	25.0	17.8
6	Et_4NCl	10	14.4	30.1	15.7
7	None	—	1.4	17.0	15.6
8	KCl	40	19.6	32.9	13.3
9	KCl	70	26.2	36.4	10.2
10	KCl	100	31.1	39.9	8.8

a) All values of cathodic current were obtained at -0.2 V *vs.* SCE on CV of an aqueous solution of $K_3Fe(CN)_6$ (5 mM). *b)* Observed in the absence of $CaCl_2$. *c)* Observed in the presence of $CaCl_2$ (1 mM).

Figure 2 shows the cathodic current observed at -0.2 V in the CV of an aqueous solution of $K_3Fe(CN)_6$ (5 mM) and KCl (10 mM) as a function of the added Ca^{2+} concentration. The cathodic response of the marker anion was closely dependent on the concentration of coexisting Ca^{2+} (10 μM—1 mM). Similar concentration–current relationships were found with Mg^{2+} and Ba^{2+} over the same concentration range, which is also shown in Fig. 2. The present method was also used for the indirect detection of Sr^{2+}. The currents obtained for marker anion solution containing various amounts of $SrCl_2$ were close to those induced by Ca^{2+} in the same amounts, although the profile is not shown in the figure for simplicity. Thus, the present method is a potent tool for the electrochemical detection of alkaline earth ions, taking into consideration the feasibility of preparing the modified electrode in a highly reproducible manner (see below).

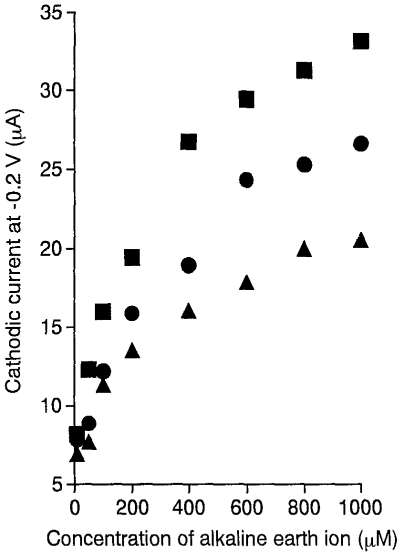

Fig. 2. Relationship between the Concentration of an Alkaline Earth Ion and the Voltammetric Response of $K_3Fe(CN)_6$ (5 mM) in Aqueous Solution (10 mM KCl) at a 1-Octanol-Modified GC Electrode

The response was obtained as cathodic currents at -0.2 V *vs.* SCE; square, $BaCl_2$; circle, $CaCl_2$; triangle, $MgCl_2$.

In obtaining the results shown in Fig. 2, a series of voltammetric measurements for sample solutions containing a particular metal chloride at various concentrations was carried out using an identical modified electrode. Between measurements, the modified GC electrode was simply washed with water. Each series was repeated more than three times at a GC electrode freshly anodized in 1-octanol. The cathodic currents at each modified GC electrode were reproducible within ±10% of the relative standard deviation (RSD) and the average values were used. Although essentially the same procedure was performed in the present work to obtain all cathodic currents on CV at a modified GC electrode, the possibility of using a 1-octanol-modified GC electrode for repeated measurements and the durability of the modified electrode were further investigated. The reproducibility of the cathodic currents for $Fe(CN)_6^{3-}$ at the same modified electrode as measured by the RSD was reasonable: the RSD ($n=20$) was ±13.8% for a sample solution of 100 μM $CaCl_2$ and ±6.7% for 1 mM $CaCl_2$. When the modified GC electrode was kept in deionized and distilled water between daily voltammetric measurements of more than 50 runs, a similar dependence of electrochemical response of the marker anion on the concentration of added Ca^{2+} was observed even after the modified electrode had been subjected to successive measurements for 13 d. However, the cathodic response of the marker anion in the presence and absence of Ca^{2+} at the modified electrode increased with time. After 13 d, the currents became more than twice as large as those observed with a newly modified GC electrode. Although the results indicate that the durability of a 1-octanol-modified GC electrode is fairly good, a daily calibration curve is recommended for the detection of alkaline earth ions using the present method when the modified electrode is in continuous operation.

To explain the observed behavior of the marker anion at a GC electrode anodized in 1-octanol, an ion-extraction mechanism[8] was first envisaged, as proposed for the indirect detection of cationic surfactants by the electrochemical response of $Fe(CN)_6^{3-}$ at a gold electrode modified with a long-chain alkanethiol.[9,10] In such a mechanism, the change in the permeability of the marker anion, monitored as an enhanced electrochemical response, should be dictated by its partition coefficient between the aqueous solution and the membrane on the 1-octanol-modified GC electrode. This assumption leads to the prediction that an increase in lipophilicity of the medium will reduce the increment of the voltammetric response of the marker anion induced by the addition of alkaline earth ions. In fact, the response in the detection system of cationic surfactants with the alkanethiol-modified gold electrode decreased as the MeOH content of an aqueous $K_3Fe(CN)_6$ solution increased.[10] Thus, the effects of MeOH on the voltammetric response of $Fe(CN)_6^{3-}$ at a 1-octanol-modified GC electrode in the presence of Ca^{2+} were examined first. CV was performed on aqueous solutions of $K_3Fe(CN)_6$ (5 mM) and KCl (10 mM) containing various amounts of MeOH in the presence and absence of $CaCl_2$ (100 μM). The cathodic currents at -0.2 V were monitored (*cf.* Fig. 1) and the effects of MeOH were estimated from the current ratio, $(I'-I_0')/(I-I_0)$, where I and I_0 denote the currents in media with 0% MeOH, with and without $CaCl_2$, respectively, and I' and I_0' are the corresponding currents in media containing MeOH. Since the voltammetric response of $Fe(CN)_6^{3-}$ at the modified electrode has been found to be enhanced in the presence of dodecyltrimethylammonium bromide in an apparently similar manner as in the presence of Ca^{2+},[7] CV experiments were also performed on solutions containing the surfactant (100 μM) in the place of $CaCl_2$. In this case, well-defined cathodic peaks were observed around 0.1 V, and, hence, peak currents were used for I and I'. The results are illustrated in Fig. 3. The value of $(I'-I_0')/(I-I_0)$ obtained with Ca^{2+} increased slightly as the MeOH content increased. In contrast, the addition of MeOH clearly lowered the $(I'-I_0')/(I-I_0)$ in the case of the surfactant, which reached 0.6 for 30% aqueous MeOH solution. The influence of MeOH upon the voltammetric response of the marker anion in the presence of the surfactant fits well the ion-extraction mechanism. However, the totally different results observed in the presence of Ca^{2+} provide positive proof that the present detection system for alkaline earth ions is not governed by such a mechanism.

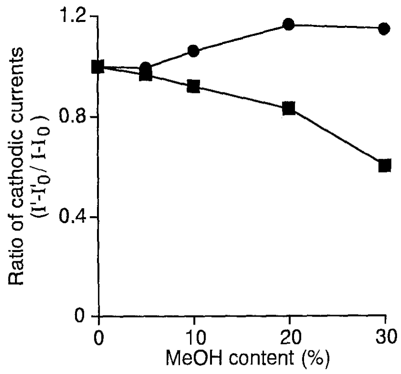

Fig. 3. Effects of MeOH Content on the Voltammetric Response of $K_3Fe(CN)_6$ (5 mM) in Aqueous Solution (10 mM KCl) at a 1-Octanol-Modified GC Electrode

Circle, in the presence of $CaCl_2$ (0.1 mM); square, in the presence of dodecyltrimethylammonium bromide (0.1 mM); for details, see text.

Alternatively, the present behavior of $Fe(CN)_6^{3-}$ at a 1-octanol-modified GC electrode might be ascribed merely to a decrease in the effective surface area of the electrode through anodization in the alkanol, not only because the effect of Ca^{2+} upon the response of the marker anion is negligible at a bare electrode under essentially the same conditions but also because it is likely that the modification results in the formation of a simple hydrophobic membrane without any functionality on the electrode surface. Thus, the observed phenomena in the present study might be induced by transformation of a macro-electrode into a pseudo-microelectrode through anodization in 1-octanol.[11] In fact, Anson *et al.* have recently demonstrated that the voltammetric response of $K_3Fe(CN)_6$ (1 mM) is virtually eliminated at a carbon microelectrode in the absence of supporting electrolyte, and the abolished response is restored by adding a large amount of KCl (0.1 M) or a small amount of $BaCl_2$ (2 mM).[12] Since Ca^{2+} is expected to behave similarly to Ba^{2+}, the effects of K^+ and Ca^{2+} upon the response of $Fe(CN)_6^{3-}$, shown by I_0 and I in runs 7 and 10 of Table 1, are in good agreement with their results, indicating that the 1-octanol-modified electrode exhibits an electrochemical performance similar to that of the microelectrode. The voltammetric behavior of $Fe(CN)_6^{4-}$ and $IrCl_6^{2-}$ was also studied at the microelectrode,[12] and hence CV of the two anions at a 1-octanol-modified GC electrode was carried out in order to compare the performance of both electrodes further.

The results of CV obtained for $K_4Fe(CN)_6$ (5 mM) in an aqueous solution (10 mM KCl) at a 1-octanol-modified GC electrode in the presence and absence of $CaCl_2$ (1 mM) are compared in Fig. 4 with that at a bare electrode in the absence of $CaCl_2$. Modification of a GC electrode with the alkanol suppressed the anodic response of $Fe(CN)_6^{4-}$, but the reduction was smaller than that observed for $Fe(CN)_6^{3-}$ and a substantial anodic wave was clearly seen (Figs. 4a, 4b). The presence of Ca^{2+} caused a slight change in the peak current of the anodic wave of $Fe(CN)_6^{4-}$ at the modified GC electrode, together with a negative shift of its peak potential (Fig. 4c). As shown in Figs. 5a and 5b, a reversible redox wave was observed for an aqueous solution (10 mM KCl) of K_2IrCl_6 (5 mM) at bare and 1-octanol-modified GC electrodes, respectively, and the electrochemical response was slightly smaller at the modified electrode. Addition of $CaCl_2$ (1 mM) improved

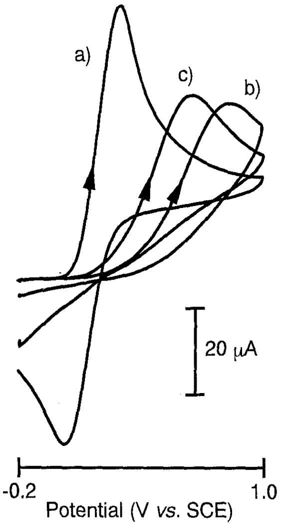

Fig. 4. Cyclic Voltammograms of $K_4Fe(CN)_6$ (5 mM) in Aqueous Solution (10 mM KCl)

a) At a bare GC electrode; b, c) at a GC electrode anodized in 1-octanol; c) in the presence of $CaCl_2$ (1 mM).

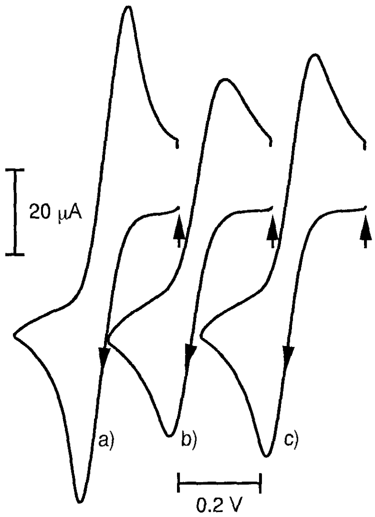

Fig. 5. Cyclic Voltammograms of K_2IrCl_6 (5 mM) in Aqueous Solution (10 mM KCl)

Vertical arrows denote 1.0 V vs. SCE; a) at a bare GC electrode; b, c) at a GC electrode anodized in 1-octanol; c) in the presence of $CaCl_2$ (1 mM).

the shape of the voltammogram at the modified electrode and a small increment in redox current was noted. The observed voltammetric results again resemble those for the marker anions at a microelectrode in the presence and absence of a large amount of K^+, which is considered to be equivalent to a small amount of Ca^{2+} based on the reported results mentioned above.[12] Thus, it is strongly suggested that a 1-octanol-modified GC electrode behaves as a pseudo-microelectrode, and thus the present detection system will be useful for the detection of alkaline earth ions.

According to speculations about the nature of a 1-octanol-modified GC electrode, it seems plausible that the packing environment of a 1-octanol-modified GC electrode may play an important role in the detection of alkaline earth ions by the present system. Thus, the effects of membrane packing on an 1-octanol-modified GC electrode upon the voltammetric response of marker anion were examined. The surface condition of the modified electrode was adjusted by changing the amount of electric current during the anodic treatment in 1-octanol. In the CV of $K_3Fe(CN)_6$ (5 mM) in aqueous KCl (10 mM) solution at the GC electrodes anodized by passing a different electricity, the cathodic currents in the absence and the presence of $CaCl_2$ (1 mM) (I_0 and I, respectively) were measured. The values of $I-I_0$ at the modified electrode prepared using 10 and 20 mC were 3.6 and 0.9 μA, respectively, which were substantially smaller than that at the GC electrode anodized by passing 5 mC of electricity (see run 3 in Table 1). Since a 1-octanol-modified GC electrode seems to have a more pinhole-free membrane as the electricity during anodic treatment is increased,[6] the pinhole defects in the membrane on the modified electrode must be essential for the present system to detect alkaline earth ions.

Then, the next question is whether the observed behavior of $Fe(CN)_6^{3-}$ at a 1-octanol-modified electrode is common to other chemically modified electrodes, provided that the modification can produce an electrode surface area corresponding to that of a microelectrode, regardless of the identity of the modifier and modification method. In order to answer this, a GC electrode anodized in triethyleneglycol monomethyl ether, a GC electrode covered with a poly(phenyleneoxide) film[13,14] and a gold electrode with a self-assembled monolayer of 1-octanethiol were prepared, and their electrochemical performances studied.

Anodization of a GC electrode in triethyleneglycol monomethyl ether seemed to cover the electrode surface with a more hydrophilic membrane than that in 1-octanol, since the contact angles of water on the GC electrodes anodized in triethyleneglycol monomethyl ether and 1-octanol were 48° and 82°, respectively. The effects of alkaline earth ions on the voltammetric response of $Fe(CN)_6^{3-}$ at a GC electrode anodized in the former alcohol were investigated under essentially the same conditions used in Fig. 2. As shown in Fig. 6, a similar relationship between the cathodic current and the concentration of each alkaline earth ion was observed.

When a GC electrode was anodically treated in a solution of phenol and Me_4NOH in CH_3CN by applying a constant electric current consuming 10 mC of electricity, similar Ca^{2+} effects were observed in CV of an aqueous $K_3Fe(CN)_6$ solution at this polymer-coated electrode, as shown in Fig. 7A. However, the consumption of 50 mC electricity during the electrochemical deposition of the polymer film resulted in a negligible increment in the cathodic current of the marker anion at the modified electrode in the presence of Ca^{2+}. Similar phenomena induced by Ca^{2+} were noted at a gold electrode covered with 1-octanethiol when the modification was performed by dipping a gold electrode for 1 h—not in a 1 mM solution, which is generally used to produce a self-assembled monolayer of various thiols[15]—but in a 0.5 mM EtOH solution of the thiol (Fig. 7B). This proves that suppression of the cathodic reaction of $Fe(CN)_6^{3-}$ and its removal by addition of alkaline earth ions is generally observed at an electrode covered partially with an insulating molecule,

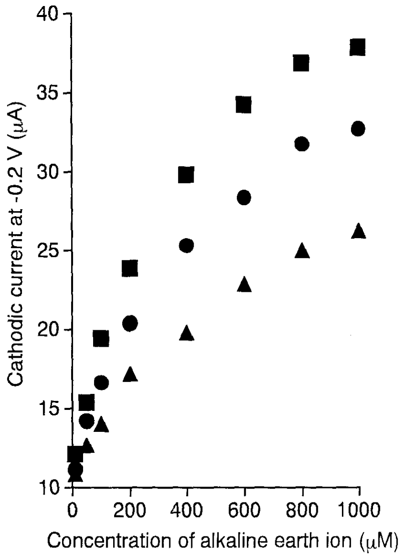

Fig. 6. Relationship between the Concentration of an Alkaline Earth Ion and the Voltammetric Response of $K_3Fe(CN)_6$ (5 mM) in Aqueous Solution (10 mM KCl) at a GC Electrode Anodized in Triethyleneglycol Monomethyl Ether

The response was obtained as the cathodic current at -0.2 V *vs.* SCE; square, $BaCl_2$; circle, $CaCl_2$; triangle, $MgCl_2$.

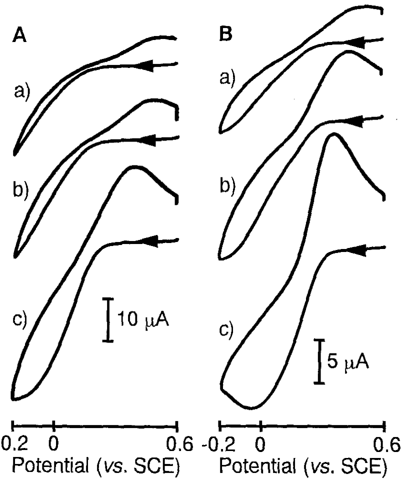

Fig. 7. Cyclic Voltammograms for an Aqueous Solution (10 mM KCl) of $K_3Fe(CN)_6$ (5 mM) (A) at a GC Electrode Covered with a Poly(phenyleneoxide) Film and (B) at a Gold Electrode Modified with a Self-Assembled Monolayer of 1-Octanethiol

a) In the absence of $CaCl_2$; b, c) in the presence of $CaCl_2$ (0.1, 1.0 mM, respectively).

irrespective of the structure of the modifier or modification method. However, it should be mentioned here that we have experienced difficulty in reproducing electrodes covered with less-packed membranes of poly(phenyleneoxide) and 1-octanethiol, in comparison with alkanol-modified GC electrodes.

Anson and Lee explained the voltammetric results at a carbon microelectrode as follows[12]: 1) migration of $Fe(CN)_6^{3-}$ will be inhibited by electrostatic repulsion between the multiply-charged marker anion and the electrode, which is negatively charged to function as a cathode, that is, by a so-called dynamic diffuse layer effect[16]; 2) electron transfer to the marker anion might be suppressed in the absence of any supporting electrolyte; 3) cathodic response of the marker anion will be abolished due to 1) and 2); 4) a small amount of Ba^{2+} or a large amount of K^+ can release the effects in 1) and 2), resulting in restored response of the marker anion.[12] Although the voltammetric results at a 1-octanol-modified GC electrode described so far might be explained by a mechanism similar to that proposed by Anson and Lee, further study is needed to elucidate why such effects can occur in the voltammetry, depending on the surface area of the electrode. In any case, however, the present findings should be taken into consideration for other ion-sensing systems based on the electrochemical response of $Fe(CN)_6^{3-}$ at an electrode modified by molecules with anionic functionality, since such functional groups might induce a rather coarse surface coverage by the modifiers due to electrostatic repulsion of the negative charge, resulting in the formation of a pseudo-microelectrode.

As far as practical application is concerned, selectivity between alkaline earth ions must be achieved by the present methodology. This requirement would be satisfied in two ways: 1) anodic modification with an alkanol substituted with an ionophore will produce a selective detection of an alkaline earth ion interacting with the ionophore on the electrode surface; 2) addition of an ionophore to a test solution may allow the electrochemical response at a 1-octanol-modified GC electrode to correspond to an alkaline earth ion that cannot form a complex with the additive. Although this study did not investigate how a coexisting compound would affect the voltammetric detection of alkaline earth ions at a 1-octanol-modified GC electrode, such effects must have important practical implications as well. Further studies of these factors are underway.

Experimental

Reagents Deionized water, MeOH, and CH_3CN were distilled and used throughout the present study. All other reagents were of reagent grade and used without further purification.

Apparatus A GC electrode was polished using a Maruto polishing system, Model ML-150P. Electrochemical modification of a GC electrode with 1-octanol, triethyleneglycol monomethyl ether, or phenol was performed using a Hokuto Denko potentiostat/galvanostat, Model HA 301, connected to a Hokuto Denko coulomb/amperehour meter, Model HF 201. Cyclic voltammograms were recorded with a Huso potentiostat, Model 315 A, equipped with a Riken Denshi X-Y recorder, Model F-5C. A three-electrode configuration was employed: glassy carbon disk ($7.07\ mm^2$) or gold disk ($3.14\ mm^2$) electrodes, with or without modification as the working electrode, a SCE as the reference electrode, and a platinum wire as the counter electrode. GC disks (GC 30, 3 mm i.d.) were obtained from Tokai Carbon. The preparation of GC and gold disk electrodes has been described previously.[17] Contact angles were determined at room temperature with a Kyowa Interface Science contact angle meter, Model CA-D.

Electrode Modification and Measurements A GC electrode modified with 1-octanol or triethyleneglycol monomethyl ether was prepared as follows: a GC electrode was polished mechanically using the Maruto system with a polishing paper (#1200) followed by alumina powder (0.05 μm) on a polishing cloth, sonicated in deionized water for 5 min, washed with water and MeOH, dried under a stream of nitrogen, and subjected to anodic treatment at $+2.0$ V *vs.* Ag wire in either of the alkanols (5 ml) containing 0.1 M H_2SO_4, where the electricity consumption was 5 mC in 1-octanol and 20 mC in the other solvent unless otherwise specified. After treatment, the modified electrode was washed with MeOH and water and treated by CV in 0.1 M aqueous KCl with five repetitive sweeps between 0 and -0.5 V *vs.* SCE, which guaranteed stable background currents at the modified electrodes. A poly(phenyleneoxide) coated GC electrode was obtained by a modification of the reported procedure,[13,14] *i.e.*, anodic treatment of a similarly polished GC electrode by passing a constant electric current (7 mA, 10 mC) through a CH_3CN solution containing phenol (50 mM) and $Me_4NOH \cdot 5H_2O$ (50 mM), followed by washing with MeOH and water. The procedure for the modification of a gold electrode with 1-octanethiol has been reported.[7] All voltammetric measurements were carried out at room temperature with a sweep rate of $100\,mVs^{-1}$. The voltammetric data at an alkanol-modified electrode are the average values of more than three measurements obtained using freshly prepared electrodes.

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References and Notes

1) Sugawara M., Kojima K., Sazawa H., Umezawa Y., *Anal. Chem.*, **59**, 2842—2846 (1987).
2) Maeda M., Tsuzaki Y., Nakano K., Takagi M., *J. Chem. Soc., Chem. Commun.*, **1990**, 1529—1531.
3) Nakashima N., Taguchi T., Takada Y., Fujio K., Kunitake M., Manabe O., *J. Chem. Soc., Chem. Commun.*, **1991**, 232—233.
4) Takehara K., Ide Y., Aihara M., *Bioelectrochem. Bioenerg.*, **29**, 113—120 (1991).
5) Takehara K., Ide Y., Aihara M., Obuchi E., *Bioelectrochem. Bioenerg.*, **29**, 103—111 (1991).
6) Maeda H., Yamauchi Y., Hosoe M., Li T.-X., Yamaguchi E., Kasamatsu M., Ohmori H., *Chem. Pharm. Bull.*, **42**, 1870—1873 (1994).
7) Maeda H., Li T.-X., Hosoe M., Itami M., Yamauchi Y., Ohmori H., *Anal. Sci.*, **10**, 963—965 (1994).
8) The mechanism refers to the situation that hydrophilic $Fe(CN)_6^{3-}$ will be extracted into the hydrophobic membrane on the electrode surface by a cation more lipophilic than its original counter cation K^+, such as a cationic surfactant, allowing the marker anion to show the electrochemical response.
9) Kawaguchi T., Yamauchi Y., Maeda H., Ohmori H., *Anal. Sci.*, **8**, 691—692 (1992).
10) Kawaguchi T., Yamauchi Y., Maeda H., Ohmori H., *Chem. Pharm. Bull.*, **41**, 1601—1603 (1993).
11) As far as we are aware, the exact definitions for macroelectrode and microelectrode are not given. However, it is likely that the terms are routinely utilized for electrodes with dimensions in the milimeter and micrometer scales, respectively.
12) Lee C., Anson F. C., *J. Electroanal. Chem.*, **323**, 381—399 (1992).
13) McCarley R. L., Thomas R. E., Irene E. A., Murray R. W., *J. Electroanal. Chem.*, **290**, 79—92 (1990).
14) McCarley R. L., Irene E. A., Murray R. W., *J. Phys. Chem.*, **95**, 2492—2498 (1991).
15) For examples: *a*) Dubois L. H., Zegarski B. R., Nuzzo R. G., *J. Am. Chem. Soc.*, **112**, 570—579 (1990); *b*) Walczak M. M., Chung C., Stole S. M., Widrig C. A., Porter M. D., *ibid.*, **113**, 2370—2378 (1991).
16) Levich B., *Dokl. Akad. Nauk SSSR*, **67**, 309—312 (1949) [*Chem. Abstr.*, **44**, 461 (1950)].
17) Ohmori H., Matsumoto A., Masui M., *J. Chem. Soc.*, *Perkin Trans. 2*, **1980**, 347—357.