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Neutral carrier-type Ca^{2+} sensors based on sol-gel-derived membranes incorporating diether-amide derivatives

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We have synthesised Ca^{2+}-selective neutral carriers for sol-gel-derived membranes encapsulating and covalently binding them. These ion-sensing membranes showed excellent EMF responses to Ca^{2+} activity changes with very short response times. Especially, the membranes covalently binding the neutral carriers did not deteriorate for at least 2 months. These sol-gel-derived membranes were selective for Ca^{2+} over Na^+ and K^+. The Ca^{2+}-selective electrode is, therefore, promising for applications to clinical analysis in future.

Keywords: sol-gel-derived membranes; Ca^{2+} sensors; diether-amide derivatives

1. Introduction

Many kinds of ion-selective electrodes (ISE) have been developed in the past 40 years (*1–5*). They are widely used in clinical and environmental analyses because of their high selectivity, easy handling, quick responses and so on. For these advantages, recently, development of ISEs, which can be used for bio-samples, especially *in vivo*, has been desired. However, there are some problems associated with plasticised poly(vinyl chloride) (PVC) membranes, which are generally used for neutral carrier-type ISEs. The ion-sensing membranes require a large quantity of special plasticisers. Due to relatively easy exudation of plasticisers from the PVC membranes to aqueous sample solutions, serious deterioration of ion-sensing membranes could occur, which results in the worst performance of ISEs. When ISEs are used for biological samples, adsorption of proteins or formation of blood clots occur on the surfaces of ion-sensing membranes, because PVC membranes are not biocompatible. Therefore, biocompatible ion sensors are desired for clinical analysis.

Sol-gel glasses are hybrid inorganic–organic materials, which are made from alkoxysilane derivatives such as tetraethoxysilane (TEOS). It is convenient to incorporate organic compounds into inorganic materials because of the low reaction temperature in the sol-gel process. In addition, sol-gel glasses are known as biocompatible materials. Therefore, many biosensors based on sol-gel-derived membranes, in which enzymes are incorporated, have been developed (*6*). We have previously reported on sol-gel-derived membranes encapsulating and covalently binding neutral carriers such as valinomycin (*7*) and bis(crown ether) derivatives (*8*, *9*). They have shown excellent responses to analyte ions and have biocompatible property.

Ca^{2+} plays very important roles in the human body; therefore, development of superior Ca^{2+} ion sensors, which are used especially *in vivo*, are desired in clinical analysis. Up to now, many Ca^{2+}-selective neutral carriers for ISEs have been developed (*10–14*). We have already described above that membranes encapsulating neutral carriers are not very suitable for clinical analysis. Bakker et al. (*15*) have reported on Ca^{2+} potentiometric and optical sensors with an ETH129 derivative grafted covalently onto a methyl methacrylate-*co*-decyl methacrylate polymer matrix.

In this study, we have synthesised new ether-amide derivatives as Ca^{2+}-selective neutral carriers (Figure 1), similar to ETH1001 (*10–12*), and incorporated them into sol-gel-derived membranes in an encapsulating and covalently binding manner. Ion sensor properties have also been examined. To the best of our knowledge, this is the first attempt to apply non-cyclic compounds as neutral carriers to sol-gel-derived membranes.

2. Experimental

2.1 Syntheses

2.1.1 N,N′-Bis[3-(10-undecenyloxycarbonyl)propyl]-N,N′-4,5-tetramethyl-3,6-dioxaoctanediamide (compound 1)

Compound **1** was synthesised by a modification of a published method (*10*). 4,5-Dimethyl-3,6-dioxaoctanedioyl dichloride was synthesised using 2,3-butanediol (mixture of stereoisomers) and oxalyl chloride instead of (*R*,*R*)-(−)-2,3-butanediol and thionyl chloride. 17-Aza-

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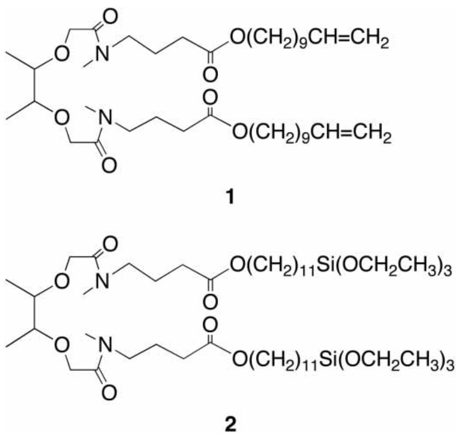

Figure 1. Ca^{2+}-selective neutral carriers used for neutral carrier-encapsulated sol-gel-derived membranes (**1**) and neutral carrier chemically modified sol-gel-derived membranes (**2**).

12-oxa-13-oxo-octadeca-1-ene was synthesised from 4-(methylamino)butyric acid hydrochloride and 10-undecen-1-ol, following a general synthetic method of aliphatic ester. Compound **1** was obtained as a brown viscous liquid after purification by column chromatography [alumina (methanol:acetone = 1:1)] and gel permeation chromatography (yield 31%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13–1.21 (6H, m, CH_3CHOCH_2), 1.22–1.43 (24H, m, $-(CH_2)_6-(CH_2)_2-CH=$), 1.53–1.66 (4H, m, $COOCH_2CH_2$), 1.78–1.93 (4H, m, $CH_3NCH_2CH_2$), 2.00–2.10 (4H, m, $CH_2CH=CH_2$), 2.32 (4H, t, $J = 7.5$ Hz, CH_2COO), 2.91 and 3.00 (6H, s, CH_3N), 3.30–3.45 (4H, m, CH_3NCH_2), 3.51–3.66 (2H, m, CH_3CHOCH_2), 4.00–4.09 (4H, m, $COOCH_2$), 4.16–4.33 (4H, m, CH_3-$CHOCH_2$), 4.91–5.02 (4H, m, $-CH=CH_2$), 5.76–5.86 (2H, m, $-CH=CH_2$).

2.1.2 *N,N′-Bis[3-(10-triethoxysilylundecyloxycarbonyl)propyl]-N,N′-4,5-tetramethyl-3,6-dioxaoctanediamide (compound **2**)*

To a dry benzene solution (30 ml) containing trichlorosilane (53.6 mg, 0.396 mmol) and *N,N′*-bis[3-(10-undecenyloxycarbonyl)propyl]-*N,N′*-4,5-tetramethyl-3,6-dioxaoctanediamide (71 mg, 0.100 mmol), a dry 2-propanol solution (1 ml) of $H_2PtCl_6{\cdot}6H_2O$ (3.37 mg, 6.50×10^{-3} mmol) was added. The mixture was refluxed for 45 min while being stirred in an Ar atmosphere. A dry 2-propanol solution (1 ml) of $H_2PtCl_6{\cdot}6H_2O$ (2.88 mg, 5.56×10^{-3} mmol) was added into the mixture, and after further refluxing for 45 min, another dry 2-propanol solution (1 ml) of $H_2PtCl_6{\cdot}6H_2O$ (4.88 mg, 9.42×10^{-3} mmol) was added. The mixture was refluxed for 4 h while being stirred in an Ar atmosphere. After evaporating the benzene and excess trichlorosilane, dry ethanol (40 ml) was added to the residue, and then the mixture was stirred in an ice bath for 12 h. The catalyst was removed by centrifugation (5000 rpm) for half an hour. Evaporation of the ethanol gave a yellow viscous liquid (yield 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (4H, t, $J = 6.7$ Hz, $SiCH_2$), 1.08–1.38 (48H, m, CH_3CH, $-(CH_2)_6-(CH_2)_3-Si$, $SiOCH_2CH_3$), 1.50–1.68 (4H, m, $COOCH_2CH_2$), 1.76–2.12 (12H, m, NCH_2CH_2, $SiCH_2(-CH_2)_2$), 2.31 (4H, t, $J = 7.4$ Hz, CH_2COO), 2.91, 3.00 (6H, s, CH_3N), 3.28–3.45 (4H, m, CH_3NCH_2), 3.51–3.66 (2H, m, CH_3CHOCH_2), 3.76–3.91 (12H, m, $SiOCH_2$), 4.00–4.08 (4H, m, $COOCH_2$), 4.16–4.33 (4H, m, CH_3-$CHOCH_2$).

2.2 *Reagents*

TEOS (Kishida Chemical Co., Ltd, Osaka, Japan) and diethoxydimethylsilane (DEDMS; Tokyo Chemical Industry Co., Ltd, Tokyo, Japan) were used as received. Potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB; Dojindo Laboratories, Kumamoto, Japan) was used without further purification. Alkali and alkaline earth metal chlorides and ammonium chloride were of analytical reagent grade. Water was deionised. All other chemical reagents were of analytical grade and used as received.

2.3 *Fabrication of sol-gel-derived membranes*

TEOS (73 mg, 3.5×10^{-4} mol), DEDMS (130 mg, 8.8×10^{-4} mol), ethanol (200 μl) and 0.1 M HCl aqueous solution (64 μl) were mixed with compound **1** (5, 6, 8, 10 wt%) and KT*p*ClPB (50 or 70 mol% to the neutral carrier). The mixture was heated at 70–75°C for 1–2 h to afford a viscous sol-gel solution. An aliquot of the solution was placed on the gate surface of a commercially available pH-ISFET tip. Heating at 70–75°C for 17–22 h afforded sol-gel-derived membranes in which a neutral carrier was encapsulated. In the case of sol-gel-derived membranes modified chemically with a neutral carrier, TEOS (20–42 mg, 9.5×10^{-5}–1.9×10^{-4} mol), DEDMS (36–88 mg, 2.4×10^{-4}–5.9×10^{-4} mol), ethanol (200 μl) and 0.1 M HCl aqueous solution (22 μl) were mixed with compound **2** (10 wt%) and KT*p*ClPB (50 or 70 mol% to the neutral carrier). The further procedure was the same as in the case of neutral carrier-encapsulated membranes.

2.4 *EMF measurements*

Potential difference measurements for the resulting ISFETs were made with an ISFET meter (Shindengen Electric, Inc., Tokyo, Japan) at 25°C in a circulating thermostated bath. The external reference electrode was a double-junction type Ag/AgCl electrode (REF4083-0.65C; DKK Corporation, Tokyo, Japan). The electrochemical cell for EMF measurements was Ag|AgCl|3 M

KCl||0.1 M NH_4NO_3||sample solution|membrane|FET. The source–drain voltage (V_{ds}) and current (I_{ds}) for the ISFET measurements were adjusted to 5.0 V and 100 μA, respectively. The measuring metal-ion activities were changed by injection of high-concentration solutions to the sample solutions, while stirring with a magnetic stirring bar. The measurements were made at least three times for the same membranes. The selectivity coefficients for Ca^{2+} with respect to interfering ions (Na^+, K^+) were determined by a matched potential method (*16*). The starting concentration of the primary ion was 1.0×10^{-5} M and its concentration was changed to 3.0×10^{-5} M. On the other hand, an interfering ion was added to the starting primary ion solution until the same EMF values as those on addition of the primary ion was attained. The values are the average of five data and the errors represent the standard deviation.

3. Results and discussion

3.1 EMF responses of sol-gel-derived membranes encapsulating neutral Ca^{2+} carrier (compound 1)

EMF responses of sol-gel-derived membranes encapsulating compound **1** were first investigated. From our preliminary experiment, the optimal ratio of DEDMS to TEOS was 2.5 for the preparation of sol-gel-derived membranes. The content of a neutral carrier was changed from 5 to 10 wt%, and EMF responses to Ca^{2+} were examined (Figure 2). The slopes of EMF responses of the ion-sensing membranes became larger with increasing the content of compound **1**. When the content was 5 or 6 wt%, the EMF responses were near-Nernstian (22.9 and 25.6 mV decade^{-1}, respectively). On the other hand, EMF responses were Nernstian in the case where the content was 8 or 10 wt% (28.7 and 28.2 mV decade^{-1}, respectively). The membrane containing a 10 wt% neutral carrier was more sensitive to Ca^{2+} because it showed the Nernstian slope at a lower concentration (3.2×10^{-5} M). The standard deviations of the slopes were less than 0.8 mV decade^{-1}. The membranes with smaller slopes tended to show a larger standard deviation. The responses to ion activity changing from 3.0×10^{-4} to 1.0×10^{-3} M were very fast in any case and the response times (t_{90}) were less than 10 s. The slope of the EMF response became smaller (19.7 mV decade^{-1}), after immersing the membranes in a 0.10 M $CaCl_2$ aqueous solution for about 2 months. When the membranes, which had just been prepared, were observed by a microscope, small domains of neutral carriers were observed on the sol-gel membrane surface. It seems that neutral carriers were dissolved out of the membranes. But the membranes, which did not show any memory effect, can be used repeatedly before their degradation.

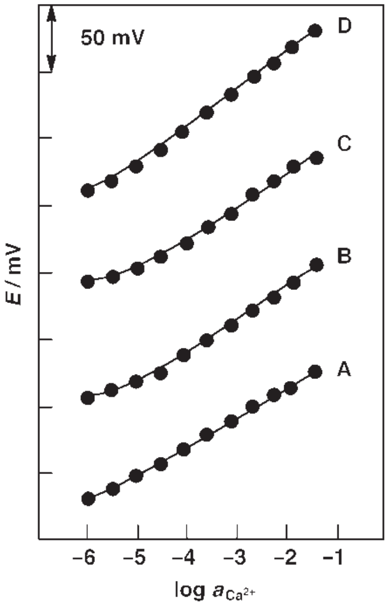

Figure 2. EMF responses of Ca^{2+} ISFETs based on neutral carrier-encapsulated sol-gel-derived membranes with neutral carrier concentrations of (A) 5 wt%, (B) 6 wt%, (C) 8 wt% and (D) 10 wt%.

3.2 EMF responses of sol-gel-derived membranes covalently binding neutral Ca^{2+} carrier (compound 2)

EMF responses of sol-gel-derived membranes covalently binding compound **2** were investigated. When the membranes were observed by a microscope, their surfaces were uniform, differing from the case of the membranes

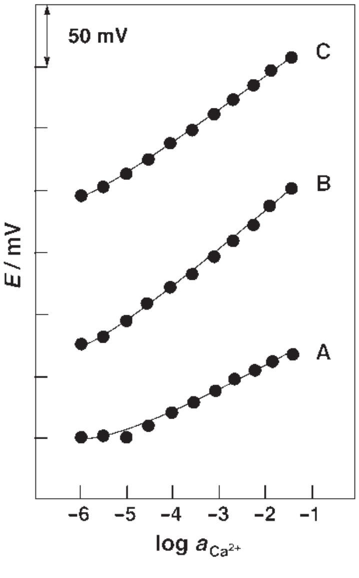

Figure 3. EMF responses of Ca^{2+} ISFETs based on neutral carrier chemically modified sol-gel-derived membranes with initial DEDMS/TEOS ratios of (A) 2.5, (B) 2.7 and (C) 2.9.

Table 1. Selectivity coefficients (log $k^{\mathrm{pot}}_{\mathrm{Ca,M}}$) of Ca^{2+}-selective sol-gel membranes.

Membranes	M = Na^+	M = K^+
Membranes encapsulating compound **1**	− 3.46 ± 0.14	− 3.41 ± 0.20
Membranes covalently binding compound **2**	− 2.43 ± 0.70	− 2.59 ± 0.24
Membranes containing KT*p*ClPB	− 0.187 ± 0.038	0.200 ± 0.129

encapsulating a neutral carrier. In this case, the content of the neutral carrier was fixed to 10 wt%, taking into account optimised conditions in the case of compound **1** (*vide supra*). The membranes were fabricated changing the ratio of DEDMS to TEOS from 2.5 to 2.9. The EMF responses of these membranes are shown in Figure 3. When the ratio of DEDMS to TEOS was 2.7, the membrane showed a very excellent response to Ca^{2+}, and the slope of the EMF response was Nernstian (29.7 mV decade^{-1}), while the slopes were 20.7 and 26.4 mV decade^{-1} when the ratios were 2.5 and 2.9, respectively. The standard deviations for the slopes were less than 0.6 mV decade^{-1} in all the membranes. The membranes did not show any memory effect, as in the case of the membranes encapsulating a neutral carrier. The membranes became softer with increasing the ratio of DEDMS, because DEDMS had only two triethoxysilyl groups that participated in the formation of sol-gel frameworks. Therefore, it is considered that the membrane, whose ratio of DEDMS was 2.5, was too hard to obtain excellent responses, on the other hand, the membrane, whose ratio of DEDMS was 2.9, was too soft. When sol-gel-derived membranes were modified chemically with neutral carriers, the proper ratio of DEDMS to TEOS (2.7) was smaller than that of membranes encapsulating a neutral carrier (2.5). It is because compound **2** for chemically modified membranes bears two triethoxysilyl groups that form a sol-gel framework in itself. The response times were the same as in the case of membranes encapsulating neutral carriers in any case, and the t_{90} were less than 10 s. The membranes covalently binding the neutral carriers showed excellent responses for at least 2 months.

3.3 *Selectivity of sol-gel-derived membranes incorporating neutral Ca^{2+} carriers (compounds 1 and 2)*

The selectivity coefficients for Ca^{2+} with respect to Na^+ and K^+($k^{\mathrm{pot}}_{\mathrm{Ca,M}}$) were determined for the membranes based on compounds **1** and **2** (Table 1). The $k^{\mathrm{pot}}_{\mathrm{Ca,M}}$ values for a sol-gel-derived membrane containing KT*p*ClPB (3.6 wt%) were also included in the table for comparison. Both the sol-gel membranes based on compounds **1** and **2** were selective for Ca^{2+} over Na^+ and K^+. On the other hand, a sol-gel-derived membrane containing KT*p*ClPB was not selective for specific ions. Therefore, it seems that the selectivity for Ca^{2+} resulted from the incorporation of neutral carriers into the membranes. The $k^{\mathrm{pot}}_{\mathrm{Ca,M}}$ values of membranes based on compound **2** were larger than those of membranes based on compound **1**. The reason can be that the mobility of neutral carriers bonded to the sol-gel framework in the membranes was much lower in the chemically modified membranes. Additionally, since two triethoxysilyl groups were introduced into a non-cyclic neutral carrier, a part of the neutral carriers could not be pre-shaped for suitable complexation with Ca^{2+}. Considering the excellent ion selectivity, it would be possible to apply these membranes to clinical analysis, because the sol-gel-derived membranes are biocompatible (*7*). We believe that this is the first study to apply non-cyclic ligands as neutral carriers to sol-gel-derived membranes for potentiometric ion sensors.

4. Conclusions

We have prepared sol-gel-derived membranes encapsulating and covalently binding Ca^{2+}-selective neutral carriers, which have been synthesised in this study. The membranes encapsulating the Ca^{2+} carrier showed Nernstian responses to Ca^{2+} activity changes and very high Ca^{2+} selectivity over Na^+ and K^+. However, the lifetimes of the membranes were less than 2 months due to the elution of the neutral carrier to the aqueous phase. The membranes covalently binding the Ca^{2+} carrier also showed excellent sensor properties. Moreover, the lifetime was too long, because the neutral carrier was directly bonded to the sol-gel framework, therefore it does not leach out of the membrane. We believe that these membranes will become a clue to Ca^{2+} analysis in bio-samples.

References

(1) Bühlmann, P.; Pretsch, E.; Bakker, E. *Chem. Rev.* **1998**, *98*, 1593–1688.
(2) Buck, R.P.; Linder, E. *Anal. Chem.* **2001**, *73*, 88A–97A.
(3) Bakker, E.; Telting-Diaz, M. *Anal. Chem.* **2002**, *74*, 2781–2800.
(4) Bakker, E. *Anal. Chem.* **2004**, *76*, 3285–3298.
(5) Bakker, E.; Qin, Y. *Anal. Chem.* **2006**, *78*, 3965–3984.
(6) Wang, J. *Anal. Chim. Acta* **1999**, *399*, 21–27.
(7) Kimura, K.; Sunagawa, T.; Yokoyama, M. *Anal. Chem.* **1997**, *69*, 2379–2383.

(8) Kimura, K.; Sunagawa, T.; Yajima, S.; Miyake, S.; Yokoyama, M. *Anal. Chem.* **1998**, *70*, 4309–4313.

(9) Kimura, K.; Yajima, S.; Okamoto, K.; Yokoyama, M. *J. Mater. Chem.* **2000**, *10*, 1819–1824.

(10) Ammann, D.; Bissig, R.; Güggi, M.; Pretsch, E.; Simon, W.; Borowitz, I.J.; Weiss, L. *Helv. Chim. Acta* **1975**, *58*, 1535–1548.

(11) Ammann, D.; Güggi, M.; Pretsch, E.; Simon, W. *Anal. Lett.* **1975**, *8*, 709–720.

(12) Simon, W.; Ammann, D.; Oehme, M.; Molf, W.E. *Ann. New York Acad. Sci.* **1978**, *307*, 52–70.

(13) Schefer, U.; Ammann, D.; Pretsch, E.; Oesch, U.; Simon, W. *Anal. Chem.* **1986**, *58*, 2282–2285.

(14) Suzuki, K.; Watanabe, K.; Matsumoto, Y.; Kobayashi, M.; Sato, S.; Siswanta, D.; Hisamoto, H. *Anal. Chem.* **1995**, *67*, 324–334.

(15) Qin, Y.; Peper, S.; Radu, A.; Ceresa, A.; Bakker, E. *Anal. Chem.* **2003**, *75*, 3038–3045.

(16) Umezawa, Y.; Umezawa, K.; Sato, H. *Pure Appl. Chem.* **1995**, *67*, 507–518.