Magnesium Ion-Selective Optodes Based on a Neutral Ionophore and a Lipophilic Cationic Dye

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Flow-through type magnesium ion-selective optodes based on a neutral ionophore and a lipophilic cationic dye have been developed. The optodes were prepared by coating pellicular octadecylsilica beads with a lipophilic organic liquid containing magnesium ionophores, a lipophilic cationic dye and a lipophilic anionic additive, and packing the beads into a flow-through optical cell. Two neutral magnesium ionophores (K22B5 and K22B1B5) and two novel lipophilic cationic dyes (KDC-1 and KDC-2) were synthesized and tested for preparing the optodes. Among the developed optodes, an optode based on the ionophore K22B1B5 and a cationic dye (KDC-2) produced the best magnesium ion optode with a relatively high Mg^{2+} sensitivity and selectivity. This optode showed a fast and reproducible response to magnesium ion, even in a high concentration of background ions.

Keywords Optode, neutral ionophore, magnesium ion, cationic dye

Following the successful achievement of applying neutral ionophores as a membrane component of an ISE, many novel optodes for cations and anions based on neutral ionophores have been developed in recent years by us¹⁻⁴ and others.⁵⁻¹¹ Unfortunately, there has been no report concerning the development of magnesium ion-selective optodes, which is mainly caused by the lack of a highly selective magnesium ionophore. In practical use, such as in the assay of Mg^{2+} in blood serum^{12,13}, and intracellular liquids¹⁴⁻¹⁶, Ca^{2+} , K^+ , Na^+ and Li^+ cause the most serious interference in the sensing of magnesium ion; therefore, a highly selective magnesium ionophore is crucially needed in order to obtain an excellent magnesium ion-selective optode.

Recently, we have reported on the development of novel magnesium ionophores based on a malonamide subunit attached to an azacrown compound.¹⁷ Among them, a magnesium ionophore (K22B5), which has two bulky adamantyl units in the side chain of the azacrown compound, was found to exhibit the highest magnesium selectivity in potentiometric studies;¹⁷ however the lipophilicity is not sufficient for use in a flow-analysis system. We thus synthesized a new magnesium ionophore (K22B1B5 (one octadecyl chain and one adamantyl unit in the side chain)) as a compromise of high selectivity and high lipophilicity.

Fig. 1 Ion-extraction model for the ion optode response: S, neutral ionophore; A, lipophilic dye; I^{z+} , ion to be sensed.

The optical response principle of the developed Mg^{2+} optode is based on an ion-pair formation reaction, as illustrated in Fig. 1, and expressed as follows:^{18,19}

where I, S and A denote cation, neutral ionophore, and cationic dye, respectively. S_mI^{z+} is the complex species formed by the neutral ionophore and the cation.

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Subscripts o and aq denote the organic and aqueous phases, respectively. A lipophilic anionic additive (R^- (in this investigation, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, TFPB was used)) was added to the organic phase in order to maintain the electroneutrality (see Eq. (2)). Based on this reaction, the cation extraction from the aqueous phase and complex formation with the neutral ionophore organic phase were followed by the release of a proton from the protonated lipophilic cationic dye into the aqueous phase. As a result, a color intensity change in the dye was observed, which could be measured spectrophotometrically in the absorbance mode, which corresponded to the concentration of the AH^+ in the organic phase. In this report we examine the performance of the magnesium optodes based mainly on a newly synthesized ionophore (K22B1B5) and lipophilic cationic dye (KDC-1 or KDC-2).

Experimental

Reagents

Reagents of the highest grade commercially available were used for the synthesis of novel magnesium ionophores, lipophilic cationic dyes and the preparation of aqueous test electrolytes. The distilled and deionized water used had resistivity values of more than $1.5 \times 10^7 \Omega \text{ cm}$ at 25°C . Pellicular ODS beads were purchased from Merck AG (Darmstadt, Germany; Perisorb RP-18, 30–40 μm particle size, ca. 2 μm modified layer thickness). The lipophilic organic liquid NPOE (*o*-nitrophenyl octyl ether) was purchased from Dojindo Laboratories, Kumamoto, Japan, and was used as received.

Synthesis of ionophores and lipophilic cationic dyes

The preparation of K22B5 (4,13-[bis(*N*-adamantylcarbamoyl)acetyl]-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane) was described in a previous report.¹⁷ K22B1B5, KDC-1 and KDC-2 were synthesized as follows:

K22B1B5 (4-(*N*-octadecylcarbamoyl)acetyl-13-(*N*-adamantylcarbamoyl)acetyl-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane). Monomethyl malonate potassium salt (4.0 g, 25.6 mmol) was suspended in CH_2Cl_2 (20 ml). SOCl_2 was then added to the suspension at 0°C , and the reaction mixture was stirred at room temperature for 2 h. A precipitated white crystal was filtered off, and the filtrate was concentrated to yield monomethylmalonylchloride as a yellowish liquid. Octadecylamine (6.89 g, 25.6 mmol) was dissolved in CH_2Cl_2 (20 ml) and stirred. Monomethylmalonylchloride was slowly added to the mixture at 0°C . The reaction mixture was stirred at room temperature for 1 d and dissolved in chloroform. The mixture was extracted twice by an HCl solution (pH 2) and water. The organic phase was evaporated, and the residue was purified by column chromatography (silica gel, hexane/ethylacetate 1 : 1) to yield 600 mg (5.3%) of (*N*-Octadecylcarbamoyl)methylacetate as a white powder. (*N*-Octa-

decylcarbamoyl)methylacetate (540 mg, 1.92 mmol) was added to a mixture of methanol/water (15 ml/5 ml). LiOH (0.16 g, 3.78 mmol)/ H_2O (5 ml) was added. The reaction mixture was stirred for 15 h, the methanol/water was evaporated, the residue was dissolved in water; the solution was then adjusted to pH 1 with HCl. The solution was extracted 3 times with ethyl acetate. The ethyl acetate was evaporated to yield 486 mg (95%) of (*N*-octadecylcarbamoyl)acetic acid (B1-chain) as a white powder.

A B5-chain ((*N*-adamantylcarbamoyl)acetic acid) was prepared in the same manner as in the preparation of a B1-chain using adamantylamine in place of octadecylamine. (*N*-Octadecylcarbamoyl)acetic acid (300 mg, 0.843 mmol) was dissolved in 18 ml dry dichloromethane on an ice bath; then, triethylamine (0.234 ml, 1.686 mmol) was added to the solution and stirred for 15 min. BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride, 214 mg, 0.843 mmol) was added and stirred for 30 min. Cryptofix K22 (221 mg, 0.843 mmol) was then added; and the reaction was allowed to proceed for 3 h. The reaction mixture was then partitioned 3 times with water (pH 1)-chloroform. The organic layer was collected and dried over anhydrous sodium sulfate. After evaporation, an intermediate product 1 was obtained, and directly used for the next reaction step.

N-Adamantylcarbamoylacetic acid (B5-chain, 200 mg, 0.843 mmol) was dissolved in 18 ml dry dichloromethane in an ice bath; then, triethylamine (0.234 ml, 1.686 mmol) was added to the solution and stirred for 15 min. BOP-Cl (214 mg, 0.843 mmol) was added and stirred for 30 min. Product 1, obtained from the previous step, was then added, and the reaction was allowed to proceed for 24 h. The reaction mixture was then partitioned 3 times with water (pH 1)-chloroform. The organic layer was collected and dried over anhydrous sodium sulfate. The product was separated by HPLC using methanol as the eluent to obtain K22B1B5 as a white solid (121.9 mg, yield 17.6%). Satisfactory analytical data were obtained for K22B1B5 and ^1H NMR (270 MHz, CDCl_3); the spectral data are as follows: δ 0.88 (t, 3H), 1.25 (m, 30H), 1.5–2.1 (m, 15H), 3.21 (m, 4H), 3.45 (m, 2H), 3.5–3.7 (m, 24H), 7.73 (br, 1H).

KDC-1 (4-[(4'-dimethylaminophenyl)azo]-1-octylbenzene). Sodium nitrite (1.11 g, 16.0 mmol) was added to a THF solution of *p*-octylaniline (3.0 g, 14.6 mmol) in an ice bath. Concentrated HCl was then added to the reaction mixture and stirred for 15 min. *N,N*-Dimethylaniline (1.77 g, 14.6 mmol) was dissolved in THF, and added to the reaction mixture and stirred for 2 h. The usual workup and further purification by HPLC yielded KDC-1 (800 mg, 16.1%). Satisfactory analytical data were obtained for KDC-1; the ^1H NMR (270 MHz, CDCl_3) spectral data are as follows: δ 0.85 (t, 3H), 1.28 (m, 10H), 1.65 (m, 2H), 2.65 (t, 2H), 3.15 (s, 6H), 6.75 (d, 2H), 7.28 (d, 2H), 7.78 (d, 2H), 7.88 (d, 2H).

KDC-2 (*N*²-octadecyl-*N*⁸,*N*⁸,3-trimethylphenazine-2,8-diamine). Sodium hydride (0.288 g, 10 mmol) was added to a solution of Neutral Red (3.0 mg, 10 mmol) in

Fig. 2 Chemical structures of the magnesium ionophores and lipophilic cationic dyes.

dry DMF and stirred for 30 min. Then 1-bromooctadecane (3.34 g, 100 mmol) was added to the reaction mixture and stirred at 70°C for 3 h. The usual workup gave KDC-2 as a brown solid (117.4 mg, yield 2.32%). Satisfactory analytical data were obtained for K22B1B5 and ¹H NMR (270 MHz, CDCl₃) spectral data, as follows: δ 0.85 (t, 3H), 1.25 (m, 30H), 2.38 (s, 3H), 3.15 (s, 6H), 3.35 (q, 2H), 4.2 (br, 1H), 6.9 (s, 1H), 7.01 (d, 1H), 7.38 (q, 1H), 7.73 (d, 1H), 7.93 (d, 1H).

The lipophilicity (log *P*_{o/w}; the partition coefficient of oil and water) of the newly synthesized ionophores and dyes were measured according to a previously reported procedure.⁴

Preparation of Mg²⁺-sensing beads

The chemical structures of the ionophores and the lipophilic cationic dyes are shown in Fig. 2. The Mg²⁺ sensing lipophilic organic liquid was prepared by dissolving a neutral ionophore (K22B5 or K22B1B5), a lipophilic cationic dye (KDC-1 or KDC-2) and an anionic additive (sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate; TFPB) in a lipophilic organic liquid (*o*-nitrophenyl octyl ether; NPOE) in a 10-ml glass vessel. The sensing liquid was coated on the surface of the pellicular ODS beads.

Structure of the flow-through type Mg²⁺ optode

The Mg²⁺ optode system is shown schematically in Fig. 3. A 0.05 M Tris-HCl buffer solution, adjusted to pH 7.4, was supplied through a flow-through sensing system at a 1.0 ml/min flow rate using a pulse-free liquid delivery pump (Trirotar 2, JASCO, Tokyo, Japan). Sample solutions were introduced into the flow system through a 2-ml HPLC injector. The absorbance was measured with a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

Preparation of a test solution

All of the sample solutions were prepared from the chloride salts of Li, Na, K, Mg and Ca using a 0.05 M Tris-HCl buffer solution adjusted to pH 7.4.

Fig. 3 Schematic views of a flow-through type optical sensor probe.

Results and Discussion

Performance of the newly synthesized lipophilic cationic dyes

In this investigation, we synthesized two kinds of lipophilic dyes, KDC-1 and KDC-2. In order to examine the performance characteristics of the new dyes, a sensing liquid that contained the dye and only an anionic additive (TFPB) (without the magnesium ionophore) was prepared, and the response to various ions at various concentrations was measured. The typical experimental results are shown in Fig. 4, in which, with the use of KDC-1, a diazo-type dye, the ionic responses were in the order $K^+ \gg Na^+ \approx Ca^{2+} > Li^+ \approx Mg^{2+}$. This experimental response pattern was not advantageous, because KDC-1 appeared to promote the extraction of K^+ , Na^+ and Ca^{2+} , the main source of interference in Mg^{2+} sensing. In contrast to this dye, KDC-2 showed no relative preference for the alkali metal and alkaline-earth metal ions tested. This fact was interpreted to mean that

Fig. 4 Response curves to various ions of the optodes based on dyes (A) KDC-1 and (B) KDC-2. Sensing layer composition: dye:TFPB=2:2 (1 eq.=1.5 μ mol in 40 mg NPOE).

Fig. 5 Response curves to various ions of the optodes based on KDC-2 and ionophores (A) K22B5 and (B) K22B1B5. Sensing layer composition: Ionophore: KDC-2:TFPB=1:1:0.5 (1 eq.=1.5 μ mol in 40 mg NPOE).

KDC-2 effectively promoted the extraction of Mg^{2+} compared to the optode using KDC-1. Therefore, the use of KDC-2 as a lipophilic cationic dye in a cation optode was recommended. Moreover, KDC-1 was not sufficiently lipophilic ($\log P_{o/w}=8.0\pm0.2$) to provide an optode with a long lifetime.

Performance of the magnesium ionophores

Two kinds of magnesium ionophores, K22B5 and K22B1B5, have been tested as sensing components for a magnesium ion-selective optode. When these ionophores and KDC-2 were incorporated in sensing liquids, all of the optodes were observed to exhibit a high response to Ca^{2+} , rather than to Mg^{2+} . Such a phenomenon is often observed in Mg^{2+} -ISEs based on those ionophores, because the selectivities of those ISEs are strongly affected by the concentration of the anionic additive in the electrode membranes.²⁰ As shown in Fig. 5, the optode based on K22B5 exhibited a

better Mg^{2+}/Ca^{2+} selectivity than the optode based on K22B1B5. However, in consideration for preparation of an Mg^{2+} -optode with a long lifetime, the use of K22B1B5 as the Mg^{2+} -ionophore was advantageous, because this ionophore has a higher lipophilicity than K22B5. Therefore, K22B1B5 was used in further experiments for optimization studies.

Optimization of the sensing liquid composition

As stated in the previous section, the selectivity of the magnesium ionophores used in the experiments was dependent on the concentration of the anionic additive in the sensing membrane. Therefore, optimization of the membrane composition was crucial. Sensing liquids having various compositions, as given in Table 1, were prepared for optimization studies; the responses to various ions tested using the optodes based on those sensing liquids are shown in Fig. 6.

First, in order to experimentally optimize the

Fig. 6 Response curves to various ions of the optodes based on KDC-2, K22B5 and TFPB dissolved in NPOE at various compositions (1 eq.=1.5 mol in 40 mg NPOE).

Table 1 Sensing liquid compositions for optode optimization studies (1 eq.=1.5 μ mol in 40 mg NPOE)

	Composition				
	(i)	(ii)	(iii)	(iv)	(v)
Ionophore (K22B1B5)	1	2	1	1	1
Cationic Dye (KDC-2)	1	1	0.5	2	2
Anionic Additive (TFPB)	0.5	0.5	1	1	2

concentration of the ionophore, two sensing liquids having the mixing molar composition K22B1B5:KDC-2:TFPB of (i) 1:1:0.5 and (ii) 2:1:0.5 were prepared. When the ionophore was two-fold concentrated (composition ii), the response to divalent ions was much greater than that to monovalent ions. This fact indicates that K22B1B5 has an effective binding power to Mg^{2+} . Although the optode response to Ca^{2+} was greater than that to Mg^{2+} , the ionophore capability of binding not only to Mg^{2+} but also to Ca^{2+} is clearly indicated, a fact that was also observed in the ISE studies using K22B1B5 as a magnesium ionophore. In ISE studies, at a lower concentration of anionic sites, the ISEs even exhibited Ca^{2+} -selectivity, rather than Mg^{2+} .²⁰

Secondly, the molar ratio of a cationic dye (KDC-2) to

the ionophore (K22B1B5) was varied, and two kinds of sensing liquids were prepared, *i.e.*, K22B1B5:KDC-2:TFPB of (iii) 1:0.5:1; and (iv) 1:2:1. Optodes with composition (iii) exhibited a similar selectivity pattern to that of an optode based on composition (i). To the contrary, with the molar ratio KDC-2/K22B1B5=2 (composition iv), the optode response to Mg^{2+} and Ca^{2+} ions was significantly increased, although the response to monovalent ions was also increased. This phenomenon was due to the fact that the dye concentration clearly affected the optode sensitivity.

To optimize the amount of the anionic additive, a sensing liquid with the composition K22B1B5:KDC-2:TFPB of 1:2:2 (composition v) was prepared, which was doubly concentrated in anionic sites (TFPB) compared to composition (iv). An optode based on composition (v) showed a decrease in the optode response to Ca^{2+} . Coinciding with the ISE optimization studies, the selectivity of the optodes for Mg^{2+} relative to Ca^{2+} and monovalent cations was increased with the increasing molar ratio of the anionic additive (TFPB). This fact indicates that TFPB is effective to increase the extractability of magnesium ion from the aqueous phase into the organic ion-sensing phase. Composition (v) was considered to be the best composition in view of the optode selectivity and sensitivity.

Fig. 7 Calibration curve for Mg^{2+} with and without background ions obtained with the optodes based on K22B1B5 and KDC-2. Sensing layer composition: K22B1B5:KDC-2:TFPB=1:2:2 (1 eq.=1.5 μmol in 40 mg NPOE). The background ions were 3.0 mM LiCl, 150 mM NaCl, 5.0 mM KCl and 1.2 mM CaCl_2 . (●) Mg^{2+} without interfering ion; (○) Mg^{2+} containing ions.

Magnesium ion response curve

The response curve of the Mg^{2+} -optode of the optimized composition was measured for both test solutions not containing the background ions and those containing the background ions. The background ions were 3.0 mM LiCl, 150 mM NaCl, 5.0 mM KCl and 1.2 mM CaCl_2 , which represent a typical artificial serum sample.²¹ The response curves for Mg^{2+} are shown in Fig. 7. Although the background ion concentration was relatively high, the optode did not show severe interference in the concentration range of 5×10^{-3} – 10^{-1} M MgCl_2 . Below this concentration range, the optode response showed serious interference due to the background ions.

Response profile of the optode

The response profile of the magnesium ion selective optodes based on K22B1B5 and KDC-2 with the optimized composition is shown in Fig. 8. The response time of the optode was ca. 1 min, and the analysis time per sample was ca. 4 min. This study provided a novel magnesium ion selective optode with a relatively fast response time. In a test solution of 10^{-1} M MgCl_2 at pH 7.4 containing 3.0 mM LiCl, 150 mM NaCl, 5.0 mM KCl and 1.2 mM CaCl_2 , a highly reproducible response was observed (see Fig. 8). Ionophore K22B1B5 and a color-changeable dye (KDC-2) are highly lipophilic ($\log P_{o/w}$ of K22B1B5 and KDC-2 are 9.6 ± 0.3 and 13.4 ± 0.8 , respectively) and are suitable for the sensing components of the optode.

Fig. 8 Typical response profiles of the Mg^{2+} -selective optode for the test solution containing 3.0 mM LiCl, 150 mM NaCl, 5.0 mM KCl and 1.2 mM CaCl_2 . Sensing layer composition: K22B1B5:KDC-2:TFPB=1:2:2 (1 eq.=1.5 μmol in 40 mg NPOE).

In summary, a magnesium-selective optode was successfully developed based on a novel neutral magnesium ionophore (K22B1B5) and a lipophilic cationic dye (KDC-2), as the first optode for magnesium ion. The optode exhibited high selectivity and sensitivity to Mg^{2+} over Ca^{2+} , Li^+ , Na^+ and K^+ . However, the optode prepared in this work was still inapplicable for measurement of magnesium ion in the human serum (ca. 10^{-4} M Mg^{2+}); thus further development of a highly selective Mg^{2+} -optode is required.

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