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S,S,S-Tris(2-ethylhexyl) phosphorotrithioate as an effective solvent mediator for a mexiletine-sensitive membrane electrode

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Abstract

S,S,S-Tris(2-ethylhexyl) phosphorotrithioate proved an effective solvent mediator for constructing a mexiletine-sensitive membrane electrode in combination with an ion-exchanger, sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate. Among a series of phosphorus compounds containing phosphoryl (P=O) groups, this solvent mediator showed the highest sensitivity to mexiletine in phosphate-buffered physiological saline containing 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4), giving a detection limit of 2×10^{-6} mol L^{-1} with a slope of 58.8 mV $decade^{-1}$. This is the best detection limit of any mexiletine electrode developed to date. Having high selectivity toward inorganic cations, the electrode was used to determine the level of mexiletine in saliva, the monitoring of which is quite effective for controlling the dose of this drug noninvasively. The mexiletine concentrations determined with the mexiletine electrode compared favorably with those determined by high-performance liquid chromatography.

Keywords Ion-selective electrode • Mexiletine determination • *S,S,S*-Tris(2-ethylhexyl) phosphorotrithioate • Solvent mediator • Drug monitoring

Introduction

There have been few studies devoted to the development of ion-selective electrodes based on the hydrogen-bonding interaction between host and guest molecules [1–8]. Typical examples are the use of hydrogen-bonding ionophores with urea or thiourea units, which were successfully employed to produce anion-selective electrodes, including those specific to nucleotides, Cl^-, SO_4^{2-}, and acetate [2–5]. Hydrogen-bonding solvent mediators have also been used to construct certain specific organic ammonium ion-selective electrodes [6–8]. Notably, phosphate esters, such as tris(2-ethylhexyl) phosphate (TEHP), showed high selectivity toward primary organic ammonium ions through the interaction between NH_3^+ groups of the organic ammonium ions and the negatively polarized oxygen atoms in the P=O groups of phosphate esters [7, 8].

Mexiletine, an antiarrhythmic drug, is one of the primary amines that needs monitoring in terms of its concentration in body fluids [9, 10]. The chemical structure of mexiletine, together with those of other antiarrhythmic drugs tested in this study, is shown in Fig. 1. To develop this electrode, the combination of an ion-exchanger, sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl]borate (NaHFPB), and a solvent mediator, *o*-nitrophenyl octyl ether (NPOE), was effective, because the mexiletine electrode made with such a combination showed good selectivity toward Na^+ present at high levels in body fluids [10]. However, the electrode made using NPOE responded to pharmaceutical substances in terms of their lipophilicity, with more lipophilic ions inducing serious interference with the

electrode's response [10]. This was a general feature of ion-selective electrodes based on the combination of ion-exchangers and solvent mediators [11, 12]. Thus, we were particularly interested in examining the effect of solvent mediators containing a P=O group, such as TEHP, expecting to induce a strong interaction with the NH_3^+ group of mexiletine. We used a series of phosphorus compounds shown in Fig. 2. Among them, *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**) showed the highest sensitivity to mexiletine in phosphate-buffered physiological saline containing 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4), giving a detection limit of 2×10^{-6} mol L^{-1} with a slope of 58.8 mV $decade^{-1}$. Under physiological conditions with a high concentration of NaCl, this is the best detection limit of any mexiletine electrode developed to date [10, 13, 14]. The interference from lipophilic organic ammonium ions was remarkably improved compared to the case of NPOE. We applied the electrode to the determination of mexiletine concentrations in saliva. The results compared favorably with those obtained by high-performance liquid chromatography [15].

Experimental

Reagents

S,S,S-Tris(2-ethylhexyl) phosphorotrithioate (**5**), tris(2-ethylhexyl)phosphine oxide (**4**), and 2-ethylhexyl bis(2-ethylhexyl)phosphinate (**3**) were synthesized by Wako (Osaka, Japan) according to procedures similar to those described previously [16–19]. Other

chemicals were obtained from commercial sources: bis(2-ethylhexyl) 2-ethylhexylphosphonate (**2**) was from Chem Service (West Chester, PA, USA); TEHP (**1**) and NPOE were from Fluka (Buchs, Switzerland); NaHFPB was from Dojindo Laboratories (Kumamoto, Japan); poly(vinyl chloride) (PVC; degree of polymerization, 1020) was from Nacalai Tesque (Kyoto, Japan); mexiletine hydrochloride, *N*-acetylprocainamide hydrochloride, bretylium tosylate, disopyramide phosphate, lidocaine hydrochloride, procainamide hydrochloride, quinidine hydrochloride monohydrate, and sotalol hydrochloride were from Sigma (St. Louis, MO, USA); and tocainide hydrochloride was from USP (Rockville, MD, USA). All other chemicals were of analytical reagent grade.

Electrode system

A PVC matrix-type ion-selective membrane was prepared using procedures described previously [6, 7, 10]. The components of the sensor membrane were NaHFPB (0.5 mg), solvent mediator (20 mg), and PVC (30 mg). Compared to the traditional ratio for sensor membranes using about 66% (w/w) solvent mediator and about 33% (w/w) PVC [6, 7, 10, 11], the amount of PVC was increased in this study, which improved remarkably the electrode's sensitivity as will be discussed in the results and discussion section. The materials were dissolved in tetrahydrofuran (about 1 mL) and poured into a flat Petri dish (16 mm in diameter) made by Asahi Seisakusho (Ohtake, Hiroshima, Japan). The solvent was then evaporated off at room temperature. The resulting membrane was excised and attached to a PVC tube (4 mm o.d., 3 mm i.d.) with

tetrahydrofuran adhesive. The PVC tube was filled with an internal solution composed of 1×10^{-4} mol L^{-1} mexiletine hydrochloride, 0.01 mol L^{-1} NaCl, and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4), and the sensor membrane was conditioned overnight. The electrochemical cell arrangement was Ag, AgCl/internal solution/sensor membrane/sample solution/1 mol L^{-1} NH_4NO_3 (salt bridge)/0.01 mol L^{-1} KCl/Ag,AgCl. The internal solution was the same as that used to condition the membrane. Potential measurements were made with a voltmeter produced by a field-effect transistor operational amplifier (LF356; National Semiconductor, Sunnyvale, CA, USA; input resistance $>10^{12}$ Ω) connected to a recorder. To examine the pH-dependence of the electrode, a miniature pH glass electrode (1826A-06T; Horiba, Kyoto, Japan), together with test and reference electrodes, was immersed in each sample solution to simultaneously measure the solution pH.

Evaluation of the electrode's performance

The detection limit was defined as the intersection of the extrapolated linear regions of the calibration graph [6, 7, 10, 20]. The selectivity coefficients of the electrode ($k_{i,j}^{\mathrm{Pot}}$) were determined by a separate solution method [6, 7, 10, 20, 21] using respective chloride salts, except for disopyramide and bretylium, for which we used phosphate and tosylate salts, respectively. The concentrations were adjusted to 0.01 mol L^{-1}. In order to obtain the selectivity coefficients under physiological conditions, we adjusted the pH of the solution to 7.4 by adding a small amount of NaOH. The coexistence of a minute amount of NaOH little

affected the estimation of the selectivity coefficient of each ion. The coefficients were calculated from the equation,

$$\log k_{i,j}^{\mathrm{Pot}} = (E_j - E_i)/S + \log c_i - \log c_j^{1/zj},$$

where E_i and E_j represent the e.m.f. readings measured for mexiletine and the interfering ion, respectively, S is the theoretical slope of the electrode for mexiletine (59.2 mV decade^{-1} at 25°C), c_i and c_j are the concentrations of mexiletine and the interfering ion, respectively, and z_j is the charge of the interfering ion. All measurements were performed at room temperature (about 25°C).

Collection of saliva

Saliva secreted in the buccal cavity (defined as mixed saliva or whole saliva) was collected for 5 min by means of continuous mouth and tongue movement [22, 23]. Pre-saliva (i.e. residual saliva in the buccal cavity) was discarded before the periodical collection of saliva. After stimulation, the salivated fluid (i.e. mixed saliva) accumulated in the mouth cavity was expectorated into a beaker, transferred to a plastic tube, and centrifuged at 1200×g for 10 min to remove the mucosal tissue debris. The saliva supernatant obtained was frozen at –20°C until use.

Assay procedure

A typical mexiletine assay in saliva proceeded as follows. The electrodes

were placed in 200 µL of saliva and constantly stirred with a bar. This electrode system, including the reference electrode [24], is compact. Therefore, a volume as low as 200 µL can be assayed. Samples containing mexiletine were prepared by adding mexiletine hydrochloride to the saliva. Between measurements, the electrode was soaked in distilled water and wiped. The electrode was stored in a solution of 1×10^{-4} mol L^{-1} mexiletine hydrochloride, 0.01 mol L^{-1} NaCl, and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4), when not in use. All measurements were performed at room temperature (about 25°C).

High-performance liquid chromatography

Mexiletine concentrations in the saliva samples were also determined by means of high-performance liquid chromatography with several modifications of a previous method [15]. The system consisted of a Shimadzu SCL-6B system controller (Kyoto, Japan) equipped with an LC-9A pump, an SPD-6A UV spectrophotometric detector, and a C-R4A chromatopac integrator. The samples (50 µL) were injected into an Inertsil ODS-80A column (150×4.6 mm i.d.; GL Sciences, Tokyo, Japan) maintained at 40°C. The elution was performed isocratically with 0.02 mol L^{-1} KH_2PO_4/acetonitrile (75:25, v/v) at a flow rate of 1.0 mL min^{-1}. The UV wavelength was fixed at 210 nm.

Results and discussion

Response characteristics of the electrodes

We previously used NPOE as a solvent mediator to construct a mexiletine-sensitive membrane electrode [10]. This electrode had markedly suppressed responses to inorganic cations, such as Na^+ and K^+, but suffered marked interference from many lipophilic antiarrhythmic drugs [10]. The interference by lipophilic amines is characteristic of an ion-selective electrode prepared with an ion-exchanger [10–12, 14]. However, we recently found that some solvent mediators with hydrogen bond-forming ability can strongly enhance the response to certain specific organic ammonium ions, even in combination with an ion-exchanger [6–8]. Notably, phosphate esters, such as TEHP (**1**), showed high selectivity toward primary organic ammonium ions through the interaction between NH_3^+ groups of organic ammonium ions and the negatively polarized oxygen atoms in the P=O groups of phosphate esters [7, 8]. Thus, we first paid attention to whether TEHP would act as an effective solvent mediator for mexiletine. Fig. 3 shows a comparison of two solvent mediators, TEHP and NPOE, based on potentiometric ion selectivity coefficients. An electrode made from TEHP showed high selectivity toward mexiletine, with little interference by lipophilic quaternary ammonium ions, such as $(C_3H_7)_4N^+$; however, the ability of TEHP to discriminate inorganic ions, such as Na^+, was much lower than that of NPOE. Thus, it is important to improve the ability to discriminate especially between Na^+ and mexiletine, because we wish to use the electrode to determine levels of mexiletine under physiological conditions with a high concentration of NaCl.

To obtain a highly sensitive mexiletine electrode, we first considered suppressing the transfer of mexiletine in the PVC membrane from the inner electrolyte solution to the outer surface of the PVC membrane, because such a transfer of an analyte ion is now known to be greatly affected by the sensitivity of the electrode [25, 26]. To suppress this transfer, we increased the amount of PVC in the sensor membrane to increase the membrane's thickness and hardness. Fig. 4 shows calibration graphs of three typical electrodes with different amounts of PVC. The graphs were obtained by measuring known amounts of mexiletine hydrochloride added to phosphate-buffered physiological saline containing 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4) and plotting the concentrations against the corresponding potential values. The measurements were performed over a concentration range of 1×10^{-8} to 1×10^{-3} M mexiletine. As was expected, an increase in PVC content in the sensor membrane greatly improved the sensitivity of the mexiletine electrode. Further increases in PVC content above 30 mg were difficult, because such a membrane was very hard and it was difficult to stick the membrane to the PVC tube with THF as adhesive. Thus, we chose 30 mg as the amount of PVC for further studies and the components of the sensor membrane were as described in the experimental section.

Then, we examined the influence of substituents bound to P=O groups using compounds **1**–**4**, shown in Fig. 2, because the basicity of the oxygen atom in the P=O group is known to be greatly affected by the replacement of an alkoxy group with an alkyl group [6, 27]. Thus, increasing the number of electron-donating alkyl substituents would produce a more negative charge at

the oxygen atom in the P=O group, making a stronger interaction with the NH_3^+ group of organic ammonium ions including mexiletine. The response characteristics of these electrodes are summarized in Table 1, along with those of *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**) which will be discussed later. As expected, the sensitivity of electrodes improved in the order **1** < **2** < **3** < **4**, as the number of alkyl substituents bound to the P=O group increased. This suggested that increases in the number of electron-donating alkyl substituents produced a more negative charge at the oxygen atom in the P=O group, making a stronger interaction with the NH_3^+ group of mexiletine. To obtain more detailed information on the electrode made using **4**, we evaluated the selectivity coefficients for various antiarrhythmic drugs, shown in Fig. 1, as well as organic and inorganic cations examined in Fig. 3. As shown in Fig. 5, the electrode based on **4** exhibited the highest selectivity toward mexiletine among various antiarrhythmic drugs. However, the electrode suffered marked interference from H^+. This indicated that an increase in basicity also promoted the function of **4** as a H^+ receptor.

Then, we were interested in examining *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**). This solvent mediator was expected to suppress the interference from H^+ and to enhance the response to mexiletine, because the presence of a sulfur atom gave weaker basicity than **4** and also increased the response to lipophilic primary organic ammonium ions [28]. It was found that the electrode made using **5** showed the highest sensitivity to mexiletine in phosphate-buffered physiological saline containing 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4) (Table 1). Comparison of the selectivity

coefficients of the electrodes made using **4** and **5** revealed differences in the response characteristics in more detail as shown in Fig. 5. The electrode made from **5**, which showed the highest sensitivity to mexiletine in phosphate-buffered physiological saline, had a more suppressed response to H^+ and alkali metal cations including Na^+ than that made from **4**. However, the electrode made from **5** induced a stronger response to lipophilic quaternary ammonium ions, such as $(C_3H_7)_4N^+$ and bretylium, as well as quinidine than the electrode based on **4**. This result was consistent with the fact that the presence of a sulfur atom generally enhances the response to lipophilic organic ammonium ions [8, 28, 29]. Mexiletine is also lipophilic among primary ammonium ions, and thus the electrode made using **5** had a strengthened response to mexiletine. Although this electrode suffered a large degree of interference from quinidine, it still showed much less interference from various other antiarrhythmic drugs than that made of NPOE reported previously [10]. Hence, we conclude that *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**) produced a sensitive response to mexiletine for the following reasons: (a) the specific interaction of the negatively polarized oxygen atom in the P=O group in **5** with the NH_3^+ group of mexiletine and (b) the presence of the sulfur atom of **5** enhancing the recognition of lipophilic mexiletine.

We examined the pH-dependence to determine the effective pH range for the electrodes based on **4** and **5**. The pH of the solution was adjusted by adding an appropriate amount of dilute hydrochloric acid or sodium hydroxide solution. The measurements were performed in the presence of 0.15 mol L^{-1} NaCl. As shown in Fig. 6, it was clear that the electrode made using **4** suffered a greater

degree of interference with the response to mexiletine in the acidic pH range. Thus, *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**) was a better solvent mediator than **4** in terms of pH insensitivity. Decreases in potential above pH 9, observed for both electrodes, were attributable to an increase in the concentration of unprotonated amine, as the pK_a of mexiletine has been reported to be 9.15 [30]. The response time (90% of the final signal) of the electrode using **5** was below 10 s when the concentration of mexiletine hydrochloride was changed from 5×10^{-6} to 1×10^{-5} mol L^{-1}.

Application of the electrode to clinical analysis

We are particularly interested in the application of the electrode to drug monitoring in body fluids. The clinical range of mexiletine in serum required for antiarrhythmic therapy was 0.7–2 mg L^{-1} (4×10^{-6}–1.1×10^{-5} mol L^{-1}) [9]. However, this concentration was near to the detection limit measured in phosphate-buffered physiological saline and the sensitivity of the present electrode was insufficient to determine mexiletine levels in serum samples.

Recently, increased attention has been paid to the use of saliva samples in place of blood samples for therapeutic drug monitoring in view of the advantage of noninvasive sample collection procedures [22, 23, 31–34]. Mexiletine concentrations were reported to be significantly higher in saliva than in serum (by the factor of 3–8) [22, 23]. Thus, the therapeutic concentration range becomes higher in saliva samples, exceeding 1×10^{-5} mol L^{-1}, and much easier monitoring of this drug by the present electrode was expected. We measured a calibration

graph of mexiletine in saliva and compared it with that in phosphate-buffered physiological saline. As shown in Fig. 7, similar calibration graphs were obtained for saliva and the physiological saline. This is because saliva also contained high concentrations of inorganic ions such as Na^+ and K^+ at levels of 0.01–0.02 mol L^{-1} and a neutral pH of around 6.8–7.2 [22, 32], similar to the situation in the physiological saline. The slope and the detection limit in saliva were 58.1 mV $decade^{-1}$ and 2×10^{-6} mol L^{-1}, respectively. It should be emphasized, however, that the determination of mexiletine concentrations down to 1×10^{-6} mol L^{-1} was still easier with an appropriate calibration as shown in Fig. 7. The sensitivity of the electrode was adequate for measuring therapeutic mexiletine levels in saliva. The response time of the electrode (90% final signal) was below 10 s when the concentration of mexiletine was changed from 5×10^{-6} to 1×10^{-5} mol L^{-1}. We determined the mexiletine concentrations in saliva samples using the calibration graph (Fig. 7, closed circle) and compared the results with those determined by high-performance liquid chromatography. Linear regression analysis of mexiletine concentrations (2×10^{-6}–4×10^{-5} mol L^{-1}) measured by the mexiletine electrode against values obtained by high-performance liquid chromatography showed a good correlation. The slope and the intercept of the line were 1.03 and 0.193, respectively ($r = 0.974$, $n = 38$).

As already mentioned by others, the use of an ion-selective electrode has inherent advantages over various other analytical methods, because it requires no special sample pretreatment, the analysis time is shorter, and the necessary equipment is inexpensive. This method will provide a new means of estimating mexiletine levels in saliva samples.

Conclusions

We demonstrated that *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate is a new solvent mediator, especially useful for constructing a mexiletine-sensitive membrane electrode. This new electrode afforded the best detection limit under physiological conditions of any mexiletine electrode developed to date [10, 13, 14]. Using the electrode, we measured therapeutic mexiletine levels in saliva.

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Table 1 Comparison of the performance of electrodes in response to mexiletine [a]

Solvent mediator	Slope (mV decade^{-1})	Detection limit (mol L^{-1})
Tris(2-ethylhexyl) phosphate (**1**)	54.2	5×10^{-6}
Bis(2-ethylhexyl) 2-ethylhexylphosphonate (**2**)	55.7	4×10^{-6}
2-Ethylhexyl bis(2-ethylhexyl)phosphinate (**3**)	55.5	3×10^{-6}
Tris(2-ethylhexyl)phosphine oxide (**4**)	57.0	3×10^{-6}
S,S,S-Tris(2-ethylhexyl) phosphorotrithioate (**5**)	58.8	2×10^{-6}

[a] All measurements were performed in the concentration range of 1×10^{-8} to 1×10^{-3} mol L^{-1} mexiletine hydrochloride in 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4).

Figure Legends

Fig. 1 Chemical structures of mexiletine and the antiarrhythmic drugs tested.

Fig. 2 Chemical structures of the phosphorus compounds tested.

Fig. 3 Comparison of the selectivity coefficients of electrodes based on TEHP and NPOE.

Fig. 4 Effects of PVC contents on the responses of electrodes to mexiletine in a solution containing 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4). The amounts of PVC were (a) 10 mg, (b) 20 mg, and (c) 30 mg, while the amounts of TEHP (20 mg) and NaHFPB (0.5 mg) were unchanged.

Fig. 5 Comparison of the selectivity coefficients of electrodes made using tris(2-ethylhexyl)phosphine oxide (**4**) and *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**).

Fig. 6 Effects of pH on the response to mexiletine of the electrodes made using tris(2-ethylhexyl)phosphine oxide (**4**) and *S,S,S*-tris(2-ethylhexyl) phosphorotrithioate (**5**) in the presence of 0.15 mol L^{-1} NaCl. The pH of the solution was changed by adding an appropriate amount of dilute hydrochloric acid or sodium hydroxide solution.

Fig. 7 Comparison of the response of electrodes to mexiletine in 0.15 mol L^{-1} NaCl and 0.01 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 7.4) (○) and saliva (●).