Eur Biophys J (1999) 28: 600–604

BIOPHYSICS LETTER

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A neutral carrier-based liquid membrane microelectrode for divalent putrescine cations

Received: 20 December 1998 / Revised version: 7 May 1999 / Accepted: 27 May 1999

Abstract A new ion-selective liquid membrane microelectrode, based on the neutral carrier 1,1′-bis(2,3-naphtho-18-crown-6), is described that shows the dependence of EMF on the activity of divalent putrescine cations a_{Put}, with the linear slope $s_{\mathrm{Put}} = 26 \pm 3$ mV/decade (mean ± SD, $N = 18$), in the range 10^{-4}–10^{-1} M at 25 ± 1 °C. Values of potentiometric putrescine cation selectivity coefficients of $\log K^{\mathrm{Pot}}_{\mathrm{Put}\,j}$ (mean ± SD, N) are obtained by the separate solution method for the ions K^+ (1.0 ± 0.4, 10), Na^+ (−1.2 ± 0.4, 8), Ca^{2+} (−2.3 ± 0.5, 10) and Mg^{2+} (−2.5 ± 0.5, 7). The microelectrode can be applied for the direct analysis of the activities of free divalent putrescine cations in the range 5×10^{-4} to 10^{-1} M in an extracellular ionic environment. Established analytical methods, e.g. high performance liquid chromatography, determine the total concentration of the derivatives of free and bound putrescine.

Key words Ion-selective microelectrode · Neutral carrier · 1,1′-Bis(2,3-naphtho-18-crown-6) · Putrescine · Polyamines

Introduction

Putrescine (1,4-diaminobutane) is the biosynthetic precursor of the polyamines spermidine and spermine, which are found in all living organisms, extracellularly as well as intracellularly. In recent years, many articles have been published studying the interactions of polyamine cations with ion channels (Drouin and Hermann 1994; Williams 1997); however, no successful attempts have been made up to now to measure the activity of polyamine cations by specific probes or chelating agents. Therefore, in order to measure the activity of divalent putrescine cations (1,4-diammoniumbutane ions), a new cation-selective liquid membrane microelectrode was designed (Ammann 1986) based on the neutral carrier 1,1′-bis(2,3-naphtho-18-crown-6) (Cram 1988). Some results of this work have been published in abstract form (Drouin 1998).

Materials and methods

The electrochemical chain of the whole ion-selective liquid membrane microelectrode assembly consists of the following parts:

$\mathrm{Ag/AgCl/KCl_{sat}/KCl_{sat}}$ – agar bridge//sample solution//
(reference electrode)
liquid membrane/$c = 0.01\,\mathrm{M\ Put \cdot 2HCl/AgCl/Ag}$
(cation-selective microelectrode)

Microelectrodes

After cleaning with bidistilled water and methanol, single-barrelled borosilicate glass capillaries (70 mm long with outer diameter 1.5 mm) were pulled by a vertical puller to obtain two microelectrodes with tip diameters >35 μm; the microelectrodes were then silanized with hexamethyldisilane at 150 °C. The tip of a microelectrode was dipped into the liquid membrane solution and was filled by suction using a water jet pump; then backfilling with 0.01 M Put · 2HCl solution was accomplished by a nonmetallic syringe (World Precision Instruments, MicroFil 28G). The electrical resistances of the microelectrode assembly ranged from 0.2 to 5 GΩ when the glass pipette was filled with liquid membrane solution and 0.01 M Put · 2HCl.

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Liquid membrane

The membrane was composed of 0.7 wt% neutral carrier, 1,1′-bis(2,3-naphtho-18-crown-6); 0.2 wt% additive, sodium tetraphenylborate; 44.4 wt% plasticizer, 2-nitrophenyl octyl ether; 54.7 wt% solvent, cyclohexanone. Chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) and Fluka (Buchs, Switzerland).

Electrolytes

Unbuffered aqueous solutions of the salts NaCl, KCl, $CaCl_2$, $MgCl_2$ and Put · 2HCl were used in the pH range 6.0–6.4. The ionization constants of the divalent 1,4-diammoniumbutane cations are tabulated as $pK_{a1} = 9.31$ and $pK_{a2} = 10.84$ (Sober 1970) and, therefore, the divalent putrescine cations were present at around pH 6.2.

EMF measurements

Experiments were performed at 25 ± 1 °C using Ag/AgCl pellet half cells connected to a high input impedance differential electrometer (DUO 773 from World Precision Instruments, with an active probe input of $R = 10^{15}\ \Omega$) and a linear ink recorder (Sekonic SS250F) for measurements of the time response. The electrode assembly was located inside a Faraday cage.

Equations

Ion activities

For the analysis of the experiments the concentrations of the cations have to be replaced by the activities of the cations. Therefore, the activity coefficients of the cations for aqueous solutions were calculated according to the Eqs. (1)–(4) of the Debye–Hückel theory and the parameters of Table 1 (Meier 1982; Ammann 1986). An approximation to the mean activity coefficient for an ion and its counterion is proposed

$$\log y_\pm = \frac{-A|z_+z_-|\sqrt{I}}{1 + Ba\sqrt{I}} + CI \qquad (1)$$

Table 1 Debye–Hückel parameters a and C for aqueous electrolytes: for metal chlorides (Meier 1982) and for divalent organic ions, calculated from setting Ba = $2[M^{-1/2}]$ (Kielland 1937)

Electrolyte	a (Å)	C (M^{-1})
KCl	3.65	0.015
NaCl	4.00	0.040
$CaCl_2$	5.00	0.040
$MgCl_2$	5.20	0.060
Put · 2HCl	6.10	

with the ionic strength of the electrolyte solution

$$I = \frac{1}{2}\sum_n c_n z_n^2 \qquad (2)$$

where z_+, z_- are the charge numbers of the cation and anion of the specified electrolyte, z_n is the charge of any ion in the sample solution, c_n is the concentration of any ion in the sample solution (M), and the parameters $A = 0.5108$ (H_2O; 25 °C) ($M^{-1/2}$), $B = 0.328$ (H_2O; 25 °C) ($M^{-1/2}$ Å^{-1}) and a, C = electrolyte specific constants [a (Å); C (M^{-1})].

According to a non-thermodynamic convention proposed by Debye and Hückel, the activity coefficient of a single cation, which per se is immeasurable, is given by

$$\log y_+ = \frac{z_+}{z_-}\log y_\pm \qquad (3)$$

The activity coefficient of a single anion y_- is obtained by replacing the reciprocal of the quotient z_+/z_-. Therefore, the concentration c_n of any ion in the sample solution, multiplied with the single-ion activity coefficient y_n, is substituted for the ion activity a_n according to

$$a_n = c_n y_n \qquad (4)$$

The Nernst and Nicolsky-Eisenman equations

In order to characterize ion-selective electrodes, the Nernst and Nicolsky-Eisenman equations are used (Ammann 1986). A potential difference is generated between the internal filling solution and the sample solution, when the principal ion i is selectively transferred from the sample solution to the membrane phase. Then the EMF of the electrochemical chain is described by the Nernst equation

$$\mathrm{EMF} = E_0 + s\log a_i \qquad (5)$$

where EMF is the electromotive force (mV), E_0 is the reference potential (mV), s is the Nernstian slope ($s = 2.303RT/z_i F = 59.16/z_i$ [(mV); 25 °C]; $s = 29.58$ [(mV); 25 °C] for $z_i = 2$), R is the gas constant (8.314 J K^{-1} mol^{-1}), T is the absolute temperature (K), F is the Faraday equivalent (9.6487×10^4 Coulomb mol^{-1}), z_i is the charge number of principal ion i, and a_i is the activity of principal ion i.

The presence of interfering ions j in the sample solution generate deviations from the Nernst equation. A successful semi-empirical approach to treating real membrane electrode systems is given by the Nicolsky-Eisenman equation:

$$\mathrm{EMF} = E_0 + s\log\left[a_i + \sum_j K_{ij}^{\mathrm{Pot}}(a_j)^{z_i/z_j}\right] \qquad (6)$$

with K_{ij}^{Pot} the potentiometric selectivity coefficient for ion j with respect to the principal ion i, z_j the charge number of ion j, and a_j the activity of ion j.

Results and discussion

Research on the complexation of organic cations and anions is a well developing field of supramolecular chemistry, and many examples have been given by Cram (1988). 1,1′-Bis(2,3-naphtho-18-crown-6) is a double-ring host, incorporating a 1,1′-dinaphthyl backbone, and is capable of forming a complex with the difunctional 1,4-diammoniumbutane (for the structural formula, see insert of Fig. 1A). When 1,1′-bis(2,3-naphtho-18-crown-6) is dissolved in a solution consisting of the solvents 2-nitrophenyl octyl ether and cyclohexanone and the additive sodium tetraphenylborate, then the cation-selective neutral carrier-based liquid membrane is obtained.

The function EMF versus the activity of divalent putrescine cations, a_{Put}, was measured with different microelectrodes and the linear slope, $s_{\mathrm{Put}} = 26 \pm 3$ mV/decade (mean ± SD, $N = 18$), was found in the range 10^{-4}–10^{-1} M at 25 ± 1 °C; the measured slope was found to be less than the theoretical Nernstian slope for divalent cations, $s_{\mathrm{Nernst}} = 29.58$ mV/decade at 25 °C. The response time was in the range of minutes in unstirred solutions.

The potentiometric selectivity coefficient for ion j with respect to the principal ion i was experimentally obtained by the separate solution method (SSM) (Ammann 1986). The EMF values for the measuring principal ion i and the interfering ion j, both determined in pure single electrolyte solutions, were compared using the transformed Nicolsky-Eisenman equation for $a_i = a_j$:

$$\log K_{ij}^{\mathrm{Pot}} = \frac{\{E_j - E_i\}}{s} + \left(1 - \frac{z_i}{z_j}\right) \log a_i \tag{7}$$

According to measurements as illustrated by Fig. 1A, potentiometric selectivity coefficients of Put^{2+} for K^+ and Na^+ ions were calculated under the reference conditions $a_i = a_j = a_{\mathrm{Put}} = 0.1$ M, $z_i = z_{\mathrm{Put}} = 2$, $z_j = z_{\mathrm{K}} = z_{\mathrm{Na}} = 1$ and $s = s_{\mathrm{Put}}$ (Table 2). Examples of measurements as shown by Fig. 1B were used for the determination of potentiometric selectivity coefficients of Put^{2+} for Ca^{2+} and Mg^{2+} ions under the reference conditions $a_{\mathrm{Put}} = a_j = 0.1$ M, $z_i = z_{\mathrm{Put}} = 2$, $z_j = z_{\mathrm{Ca}} = z_{\mathrm{Mg}} = 2$ and $s = s_{\mathrm{Put}}$ (Table 2).

Table 2 Experimental potentiometric selectivity coefficients for the Put^{2+}-selective neutral carrier, 1,1′-bis(2,3-naphtho-18-crown-6)

Ion j	$\log K^{\mathrm{Pot}}_{\mathrm{Put}\,j}$ (mean ± SD, N)	
K^+	1.0 ± 0.4	10
Na^+	−1.2 ± 0.4	8
Ca^{2+}	−2.3 ± 0.5	10
Mg^{2+}	−2.5 ± 0.5	7

The experimentally observed potentiometric selectivity coefficients of Table 2 lead to the following conclusions. In order to generate the same EMF (mV) as the activity of divalent putrescine cations, $a_{\mathrm{Put}} = 0.1$ M, the following activities of metal ions are required: $a_{\mathrm{K}} = 0.095$ M, $a_{\mathrm{Na}} = 1.3$ M, $a_{\mathrm{Ca}} = 21.9$ M, $a_{\mathrm{Mg}} = 29.7$ M. Calculations of the function EMF versus a_{Put} for typical ion environments (Ammann 1986) [$10^3 a_j$ (M), extracellular: $a_{\mathrm{K}} = 3$, $a_{\mathrm{Na}} = 105$, $a_{\mathrm{Ca}} = 0.37$, $a_{\mathrm{Mg}} = 0.21$; and intracellular: $a_{\mathrm{K}} = 89$, $a_{\mathrm{Na}} = 7.6$, $a_{\mathrm{Ca}} = 1.4 \times 10^{-4}$, $a_{\mathrm{Mg}} = 1.1$] using Eq. (6) yield the dependencies which are presented in Fig. 2.

The response of the sensor to putrescine cations was measured for a typical extracellular ion environment (Ammann 1986) [$10^3 c_j$ (M): $c_{\mathrm{K}} = 4$, $c_{\mathrm{Na}} = 140$, $c_{\mathrm{Ca}} = 1.1$, $c_{\mathrm{Mg}} = 0.6$]. Using Eq. (2) the ionic strength $I = 0.1491$ M was calculated from corresponding concentrations. 1,4-Diaminobutane dihydrochloride was

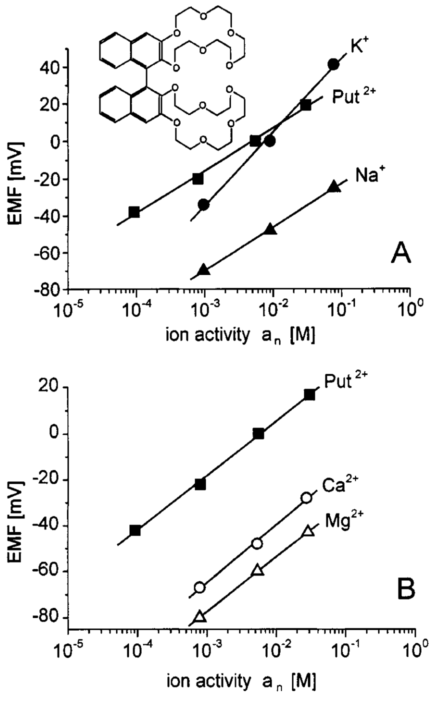

Fig. 1A, B Experimental functions of EMF versus ion activities a_n; the lines are linear fits. **A** Measurements of EMF values versus ion activities a_{Put}, a_{K} and a_{Na}; insert: structural formula of the neutral carrier, 1,1′-bis(2,3-naphtho-18-crown-6). **B** Measurements of EMF values versus ion activities a_{Put}, a_{Ca} and a_{Mg}

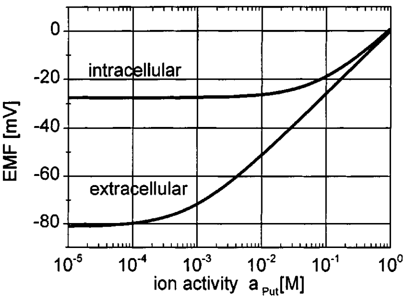

Fig. 2 Calculated functions of EMF versus ion activity a_{Put} for typical ion environments [see text, Eq. (6) and Table 2]

added to this salt solution, to yield four different solutions ($c_{\mathrm{Put}} = 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}$ M); then the corresponding ionic strengths were obtained ($I = 0.1493$, 0.152, 0.179, 0.449 M). The activity coefficients of putrescine cations were calculated by Eqs. (1) and (3) and the parameters of Table 1 as $y_{\mathrm{Put}} = 0.3665$, 0.3647, 0.3481, 0.2675. The activity of putrescine cations were calculated from Eq. (4): $a_{\mathrm{Put}} = 3.67 \times 10^{-5}$, 3.65×10^{-4}, 3.48×10^{-3}, 2.68×10^{-2} M. The range of measured pH values of these solutions was 6.0–6.2 at 24 °C. The experimental results of EMF (mV) versus ion activity a_{Put} (M) are shown in Fig. 3; the linear slope, $s_{\mathrm{Put}} = 20 \pm 3$ mV/decade (mean ± SD, $N = 3$), was in the range 5×10^{-4} to 10^{-1} M at 25 ± 1 °C. This slope appeared to be less than that measured in pure solutions, and also the linear range was narrowed.

According to the results shown by Figs. 2 and 3, the cation-selective microelectrode can be applied for the direct analysis of activities of free divalent putrescine cations in the range 5×10^{-4} to 10^{-1} M in an extracellular ion environment, but not for intracellular measurements because of interference with potassium ions. Extracellular measurements are reliable within the normal range of a_{K} (2.6–3.7 mM; Ammann 1986).

Established analytical methods, e.g. high performance liquid chromatography (HPLC), are used to determine the total concentration of derivatives of free and bound putrescine in biological systems (e.g. urine, blood, serum, Liquor cerebrospinalis). The new ion-selective liquid membrane microelectrode opens the possibility to measure the activity of free putrescine cations. Both the established and the new methods provide better analytical insight into the action of putrescine cations. Concentrations of the order of 10^{-6}–10^{-2} M of putrescine have been determined by chromatographic methods in relevant biological systems (Tabor et al. 1973; Seiler et al. 1998), but no activities of putrescine cations have been measured so far. Therefore, optimizing the selectivity characteristics of microelectrodes for putrescine cations needs further research. Large improvements of the lower detection limits of ion-selective electrodes have been reported, when the principal ions can be buffered to low activity in the inner electrolyte to prevent leaching into the sample (Sokalski et al. 1997). Rather large openings of the tips of the microelectrodes were used for testing the function of the neutral carrier, in order to lower the electrical resistance and to minimize interference from static electricity.

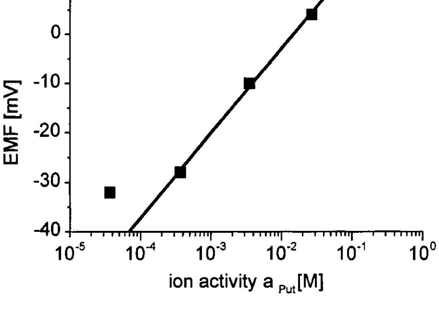

Fig. 3 Experimental function of EMF versus ion activity a_{Put} for a typical extracellular ion environment (see text)

Small tips generate very high electrical resistances and extensive shielding is then usually necessary, which can greatly restrict access to the preparation (Thomas 1978). Keeping the composition of the liquid membrane phase as simple as possible, no attempt has been made to stabilize the liquid membrane by poly(vinyl chloride); a substantial increase in specific membrane resistance with addition of poly(vinyl chloride) has been reported (Ammann 1986). Lipophilic salts were incorporated into the membrane phase with the aim to decrease the membrane resistance and to overcome problems of shielding (Ammann 1986). More recently it has been reported that the addition of lipophilic inert electrolytes to the membrane renders the ion-selective electrode more selective for divalent over monovalent ions (Nägele et al. 1998). New carriers can be tested: basket-shaped hosts are promising candidates (Smeets et al. 1989, 1990). It is well known that putrescine is intimately involved in growth-related processes, and there is increasing evidence that excessive intracellular accumulation (up to the millimolar range) of putrescine favors the malignant transformation of cells (Seiler et al. 1998). Monitoring the levels of activity of putrescine ions (in the micromolar range) in extracellular body solutions is one prominent goal of future research.

Acknowledgements The author expresses his gratitude to Professor E. Bamberg for financial support of this research, and thanks Professor W. Schwarz for the use of his facilities, and particularly appreciates Dr. E. Grell's hospitality and many helpful discussions of this investigation. This research was made possible by Drs. I. and G. Hayenga's kind support in providing the rare chemical 1,1′-bis(2,3-naphtho-18-crown-6) from Fluka Chemie AG and Sigma-Aldrich Chemie GmbH.

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