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Novel potentiometric sensors based on polysulfone immobilized metallothioneins as metal-ionophores

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ABSTRACT

We show here the use of immobilized metal-binding biomolecules for metal analysis by using novel potentiometric sensors. To this end and as a model, Ag^+-ISEs were developed using polysulfone matrix embedding metallothioneins as ionophores (mouse MT1 (P1) or sea urchin SpMTA (P2)). Polysulfone, a porous polymer that was not used until the present in potentiometric biosensors, has the advantage of being compatible with biological materials. Also, the phase inversion procedure allows protein incorporation into the membrane with minima alterations, since it always remains in the aqueous phase. Construction of these biosensors required small amounts of protein; they can be dry-stored and have long lifetimes. They exhibited linear responses with slopes of *ca.* 61 mV per decade within the 10^{-5} to 10^{-2} M Ag^+ concentration range, detection limits of about 10^{-5} M, and worked in the 2-to-8 pH range. Except for Hg^{2+}, the Pb^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+} cations do not interfere with Ag^+ determination. Significantly, different affinities of Pb^{2+} and Zn^{2+} towards P1- and P2-ISE were found, in good correlation with the higher affinity of these cations towards SpMTA than to MT1. Consequently, the distinct metal-binding features of each MT are conserved and determine the differential properties of their biosensors. These results open a broad range of possibilities for the use of proteins as ionophores in what could be considered a new type of potentiometric biosensor if their response mechanism is taken into account.

1. Introduction

To date, many ion-selective electrodes (ISE) have been reported for metal ions. Most of them are based either on fixed sites (glass, crystalline, and ion-exchange resin membranes) or on mobile sites (liquid membranes with mobile carriers) [1]. Crystalline membranes can be solely constituted by the insoluble salt itself (homogeneous membranes) [2,3] a strategy that has enabled the development of several commercial potentiometric sensors [4]) or with the solid salt dispersed in a polymeric matrix (heterogeneous membranes) [5]. In both cases, the recognition mechanism is based on solubility equilibria between the ions of the solution and the solid salt. In the case of the less common ion-exchange resins dispersed in a matrix [6], this mechanism is only based on electrostatic interactions. Conversely, the recognition mechanism in liquid membranes with mobile carrier is based on liquid–liquid extraction equilibria between an aqueous and an organic phase (mediator solvent). The aqueous phase contains a type of ion (analyte) that is also present in the organic phase bound to the ionophore [7]. Here, the ionophore is a lipophilic species with a high capability for extracting ions from aqueous to organic phases. Normally, the components of the membrane are supported by an inert lipophilic matrix where the mediator solvent behaves as a plasticizer conferring the required final features to the membrane [8]. Among the reported matrixes (polyurethanes, polysiloxanes silicon rubber, etc.) [9–12]) PVC [13] is the most used.

Incorporation of biological material into PVC membranes is not an easy task basically due to the use of organic solvents (normally tetrahydrofuran), into which protein macromolecules dissolve poorly usually altering their subsequent activity [14]. Consequently, the construction of potentiometric biosensors has mainly been achieved by a biomembrane superposition on the ISE. Our research group has recently overcome this drawback by using a matrix of polysulfone (PS), a porous polymer that had not been used until the present in sensor construction but whose stability makes it an attractive material. PS makes it possible to incorporate enzymes [15–17] and antibodies [18–20] for the construction of amperometric biosensor compact membranes, *i.e.* those includ-

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ing all the required elements for the recognition without the need of superposing layers. This has been achieved by incorporating the biological material to the membrane during the phase inversion process, which is when water penetrates into the polysulfone and induces its precipitation by displacement of the dimethylformamide where it was dissolved [15–21]. Among other advantages, this immobilization procedure maintains the protein practically intact throughout the process as it always remains in an aqueous media and at controlled pH. It is also noticeable that by this procedure the amount of required protein is greatly reduced, as it remains mainly bound to the outer membrane layers. Furthermore, PS not only provides a suitable environment to embed hydrophilic components but also hydrophobic ones too, and accordingly we have developed a potentiometric sensor for nitrates [22].

Our next aim was to incorporate biological material into PS for the construction of potentiometric biosensors based on the use of a protein as a metal ion ionophore and to this end we have assayed the use of metallothioneins (MTs). Metallothioneins are low molecular weight metalloproteins (6–10 kDa) present in almost all living organisms. They were discovered nearly 50 years ago and although their physiological function is still a matter of debate, they are well-known for the high metal-binding capability that arises from their high cysteine content (30% of the amino acid sequence). From all known MTs, for this study we have chosen two structurally related MTs: mouse MT1 [23] and sea urchin SpMTA [24] that however show distinct metal-binding features [25]. By this strategy we have explored the possibility of using hydrosoluble proteins, which are immobilized in a membrane that does not inhibit their capability of interaction with metal ions and that gives rise to potentiometric responses.

In recent years, some MTs and other metal-interacting polypeptides have been used for the construction of metal ion biosensors by taking advantage of their coordinating features, but these have been mainly used for capacitance sensors [26–29], which show poor selectivity and require EDTA regeneration after each use. On the contrary, the results we report in this work open a broad range of new possibilities for the use of proteins for potentiometric sensors, as the biosensors we have developed show a better selectivity and neither require incubation nor regeneration processes. As a model, we have chosen a Ag^+-ISE to study the behaviour of the MT1 and SpMTA metallothioneins as ionophores in a PS matrix due to the high affinity of this cation towards these proteins. While the standard Ag_2S-based solid-state electrodes are well known, the design of mobile carrier-based Ag^+-ISEs is gaining ground because of their better selectivity [30–32].

2. Experimental

2.1. Materials

Polysulfone BASF 3010 natur (Frankfurt, Germany) was used as a polymer matrix and N,N-dimethylformamide (DMF) was purchased from Panreac (Barcelona, Spain). All other reagents used were of analytical grade and were obtained from Merck (Darmstadt, Germany). Milli-Q (Bedford, MA, USA) was used throughout. For the preparation of the electrode, graphite powder was obtained from Merck, epoxy resin ARALDIT M from Uneco (Badalona, Spain) and the hardener HY 5162 from VANTICO (Barcelona, Spain).

2.2. Metallothionein biosynthesis

The P1 (mouse MT1) and P2 (sea urchin SpMTA) metallothioneins used in this report as ionophores, were recombinantly synthesized as Zn-complexes in *E. coli* following a GST-fusion strategy that, after a thrombin cleavage step, renders homogeneous preparations of independent MT [33]. MT concentration and Zn-to-protein ratios of the final preparations were assessed by atomic absorption ICP-AES measurements of S and Zn sample content, as previously described [34]. In the current study, the two MTs have been embedded as Zn-complexes of the independent polypeptides, which directly prevented oxidation in contact with air.

2.3. Electrode construction

The electrodes were of all-solid-state type using a solid internal contact commonly used in our laboratories [35–37]. The supporting conductor was prepared by mixing Araldit M and the hardener at a 1:0.4 (w/w) ratio. This epoxy resin was then mixed with graphite powder at a 1:1 (w/w) ratio [38]. The resulting paste was placed in the electrode body and cured at 40 °C for 24 h. After curing of epoxy-graphite, 0.3 mm thickness from the top layer of the surface was removed mechanically so that the electrode was ready for membrane deposition. A solution of 100 mg polysulfone/ml DMF was used to prepare the membrane in accordance with preliminary studies [22]. A drop of this PS solution was deposited on the epoxy-graphite electrode surface using a pipette. Immediately after, it was precipitated by the phase inversion technique [15,18,39], which was achieved by immersing the electrode in the protein solution. This gave raise to the formation of membranes of 100–200 μm thick [40]. Finally, the ISEs were conditioned for 2 h in a 0.1 M Ag^+ solution, this implying a Zn(II)/Ag(I) replacement in all the metal-binding sites of the protein [34].

2.4. Measurements

Potentiometric measurements were performed using a digital potentiometer, Crison pH 2002, with ±0.1 mV resolution (Alella, Spain) and a serial communication line. Up to sixteen electrodes could be calibrated simultaneously through the use of "switch TMI-6017 CONM16". The readings were computer-controlled; using the serial communication line (RS232C) connected to a Pentium 4 computer with specially developed software. Both instrument and software were made by TMI (Alella, Spain). As a reference electrode, a double-junction Ag/AgCl electrode from Orion Thermo (West Palm Beach, Florida, USA) 90-02-00 was used.

Calibration parameters were obtained from the construction of multiple calibration curves in the 1.0×10^{-7} to 2.0×10^{-2} M Ag^+ range and using the constructed P1- and P2-ISE. Calibration experiments were carried out by performing known additions of silver standard solutions to a fixed volume of initial solution in order to reach a working concentration range between 1.0×10^{-7} and 2.0×10^{-2} M. Data was plotted as potential versus logarithm of the Ag^+ activity, calculated by the Debye–Hückel formula. The limit of detection was calculated as the concentration of Ag^+ at the intersection point of the extrapolated linear segments of the calibration plots.

3. Results and discussion

Here we report the construction and study of Ag^+ selective electrodes embedding either a mouse (mammalian MT1, P1) or a sea urchin (equinodermata SpMTA, P2) metallothionein (Fig. 1) into the membrane using polysulfone as a polymeric matrix. Although the use of MT polypeptides to construct biosensors has been previously reported [26,29], it has usually been restricted to the bacterial SmtA protein in the GST-fused form. However, in our case we have used the independent proteins.

As a first step, it was considered interesting to study the influence of the concentration of the MT solution to be embedded in

MT1 MDPNCSCSTGGSCTCTSSCACKNCKCTSCKKSCCSCCPVGCSKCAQGCVCKGAADKCTCCA

SpMTA MPDVKCVCCTEGKECACFGQDCCVTGECCKDGTCCGICTNAACKCANGCKCGSGCSCTEGNCAC

Fig. 1. Amino acid sequences of MT1 (P1) and SpMTA (P2). The 20 cysteine residues that each MT uses for heavy metal binding are underlined.

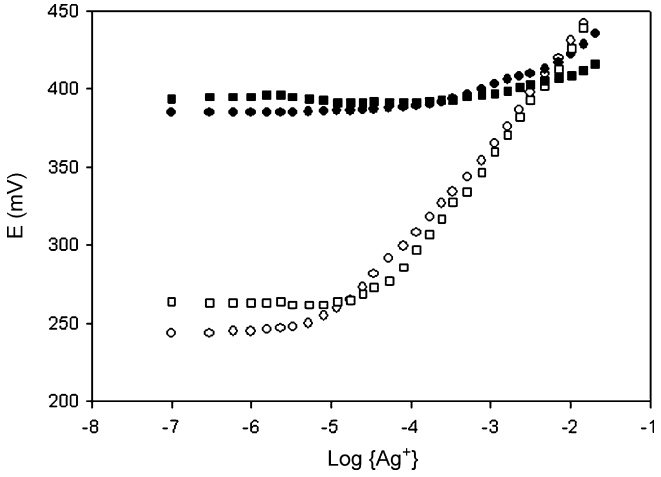

Fig. 2. Potentiometric response of the P1- and P2-ISE stored in different conditions: (○) dry-stored and (●) soaked for P1-ISE, (□) dry-stored and (■) soaked for P2-ISE.

the membrane on the performance of the electrodes. As shown in Table 1, the sensitivity, the linear range and the limit of detection (LOD) values of the P1- and P2-based electrodes remain practically invariant and thus the different MT concentrations assayed are apparently not a key factor in the response of the corresponding electrodes. Consequently, electrodes constructed with MT concentrations of 5.0×10^{-5} M for the sake of protein economy, were used in this work. Furthermore, and due to the costs of the recombinant production of proteins, we also investigated whether there were differences between ISEs that were successively constructed using the same initial MT mother solution. If the DMF released to the aqueous solution during the process of deposition of the membrane on the electrode surface had have altered the MT initial solution, differences in the electrode features could have been observed. To this end, several units were successively constructed, calibrated and their sensibilities and limits of detection compared but no differences between them were observed leading to the conclusion that this variable had no influence on their performance. Under these conditions, 100 μl of the MT solution are enough to prepare at least three ISEs by the phase inversion technique.

Contrarily, some differences in the performance of these ISEs were observed depending on their storage conditions. Although always kept at 4–5 °C, the response of the electrodes stored in dry is much better than that of the electrodes stored soaked in a 0.01 M Ag^+ solution. In fact, soaked electrodes showed a 30, 60 and 100% sensitivity decrease after 4, 6 and 10 days of storage, respectively (data corresponding to 10 days of storage is shown in Fig. 2). This contrasts with the general belief that MTs are oxidized in contact with air and that it is therefore necessary to keep them in an inert atmosphere [26]. Probably, in dry-stored electrodes, PS is able to create adequate conditions inside the membrane that prevent the oxidation of the cysteine MT residues. Additionally, the shorter lifetime of the soaked versus the dry-stored electrodes described in this work could be rationalized by considering that in the hydrated PS the MT backbone would have a higher mobility that could favour the oxidation of cysteines to cystines by formation of S–S bonds. Some authors have proposed that either the water saturation of the membranes or the loss of their permselectivity can be the origin of the observed potential drifts and changes in the response slopes and in the selectivity coefficients [41,42]. In our opinion this two processes, although possibly also exerting some effects, are probably not decisive in the studied electrodes. This last statement is based on previous results found by this group where we reported that soaked-stored nitrate electrodes with PS matrixes showed lifetimes of at least 3 weeks [22], this indicating that chemical rather than physical modifications are responsible of the observed changes. Consequently, all constructed electrodes were stored in dry.

Typical calibration plots were obtained for both types (P1- and P2-ISE) of dry-stored electrodes depicting linear ranges from *ca.* 10^{-5} to 10^{-2} M, the latter being the greater working silver concentration assayed. They gave slopes of *ca.* 61 mV per decade of concentration indicating their Nerstian nature and the limits of detection were of *ca.* 10^{-5} M. Consequently, as also shown in Table 1, the nature of the protein does not have a significant influence in these three features of these ISEs.

Both the P1- and P2-ISE, showed good reproducibility and a long lifetime. Reproducibility was determined by successive calibrations over consecutive days ($n = 10$) giving rise to LOD and sensitivity values of respectively 3.1×10^{-5} M (S.D. = 1×10^{-6}) and 60 mV/dec (S.D. = 4) for P1-ISE and of 2.5×10^{-5} M (S.D. = 8×10^{-6}) and 62 mV/dec (S.D. = 3) for P2-ISE. Both types of electrode were used periodically and dry-stored at 4 °C as stated before. After 3 months no significant changes in their performance were observed. These features together with the good repeatability results obtained for a whole day (Fig. 3) enable their applicability as silver sensors after a daily calibration.

To ascertain the functional pH range of the optimized P1- and P2-ISE, we studied their response to the pH of the solution at Ag^+ concentrations of 1.00×10^{-3}, 1.00×10^{-2} and 1.00×10^{-1} M. The pH of the solution was adjusted by appropriate additions of nitric acid or sodium hydroxide. The potentiometric responses were independent of the pH in the 3.0-to-7.5 range (Fig. 4), which may be taken as the functional pH range of these biosensors. It should be noted that the observed potentiometric decrease of over pH 8 is attributable to the formation of silver oxide.

Table 1
Sensitivity, linear range and logarithm of the limit of detection (log LOD) values recorded for dry-stored, P1- and P2-based electrodes as a function of the concentration of the MT solution used. The standard deviations corresponding to three ISEs and three experimental calibration curves each are shown in brackets.

[MT] ($\times 10^{-4}$ M)	Sensitivity (mV/dec)		Linear range (M)		Log LOD	
	P1-ISE	P2-ISE	P1-ISE	P2-ISE	P1-ISE	P2-ISE
0.5	61 (4)	61 (5)	$2 \times 10^{-5} - 0.02$	$5 \times 10^{-5} - 0.02$	−4.8 (0.2)	−4.4 (0.2)
1.0	58 (2)	61 (4)	$5 \times 10^{-5} - 0.02$	$4 \times 10^{-5} - 0.02$	−4.4 (0.3)	−4.5 (0.3)
5.0	62 (5)	–	$5 \times 10^{-5} - 0.02$	–	−4.4 (0.2)	–

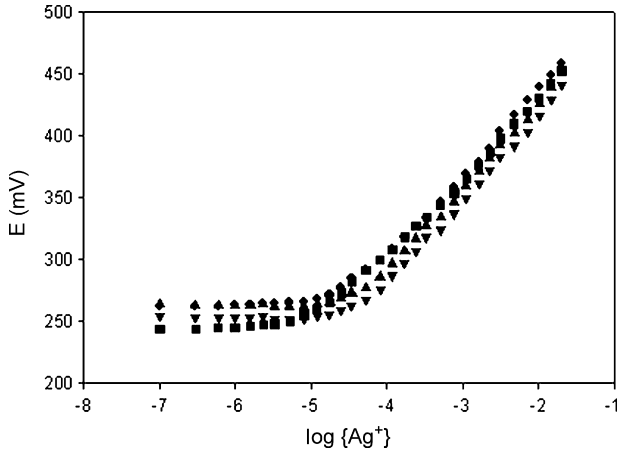

Fig. 3. Successive calibration plots, made in 2 h intervals, to check the repeatability of the P1-based electrode. (■) 1st, (▼) 2nd, (●) 3rd, and (▲) 4th calibration plots.

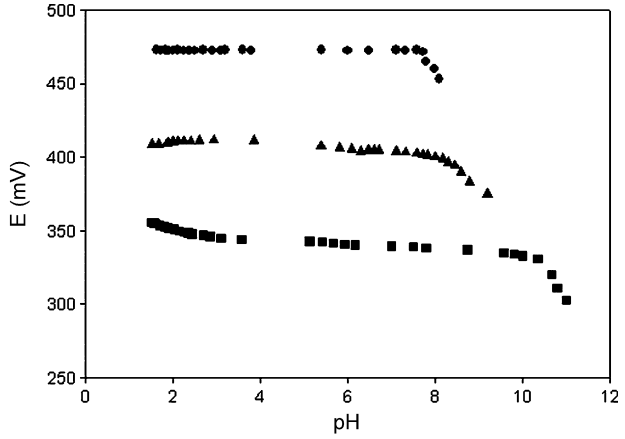

Fig. 4. Effect of the pH on the potentiometric response of the Ag^+-selective electrode based on P1, tested at (■) 1.00×10^{-3}, (▲) 1.00×10^{-2} and (●)1.00×10^{-1} M Ag^+ concentration.

3.1. Selectivity of the electrodes

One of the main features of any ion-selective electrode is its response to the primary ion in presence of other ions. In our case, some interferences between Ag^+ and other metal ions could also be envisaged due to the well-known order of affinity of heavy metal ions for thiolates ($Hg(II) \gg Cu(I) \approx Ag(I) \gg Cd(II) > Pb(II) > Zn(II)$),

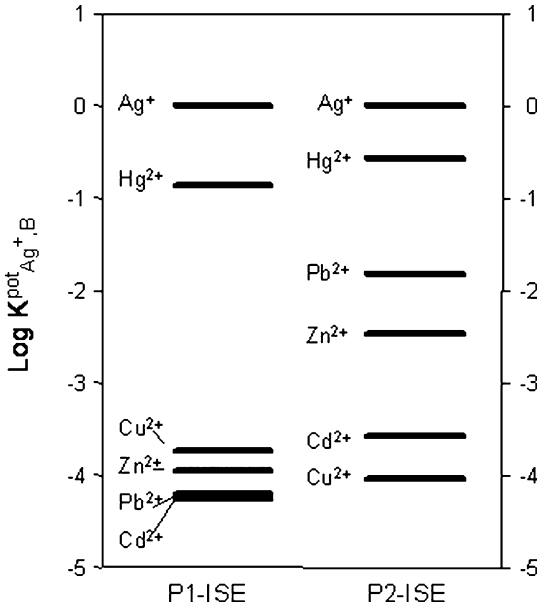

Fig. 5. Potentiometric selectivity coefficients (log $K^{pot}_{Ag^+,B}$) of the proposed silver-selective electrode based on P1 and P2 metalloproteins.

which is very close to that of MTs [43]. The selectivity of ISEs is usually expressed in terms of the potentiometric selectivity coefficient, $K^{pot}_{A,B}$, where A and B respectively are the main and the interfering ions. Here, the selectivity coefficients have been determined by the Fix Interference Method (FIM) [44], a methodology that implies carrying out potentiometric measurements on solutions prepared with a fixed concentration of the interfering ions and by varying the Ag^+ concentrations. Consequently, we conducted the study of the interferences in our P1- and P2-based electrodes with some of the possible competitors of Ag^+ in the following background concentrations: 1×10^{-3} and 1×10^{-2} M for Hg^{2+}; and 1×10^{-3}, 1×10^{-2} and 1×10^{-1} M for Pb^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+}.

From this study, it is concluded that Hg^{2+} is the most interfering ion for both types of electrode (Fig. 5 and Table 2). This is in good correspondence with literature data regarding electrodes of different nature [45–48] and with the higher affinity of this metal ion for the Cys residues if compared with that of the other metal ions studied (Pb^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+}). However, although Hg^{2+} shows a higher affinity than Ag^+ for the binding sites of an MT, both P1- and P2-based electrodes respond in a more selective way to Ag^+ than to Hg^{2+}. Both the sensitivity and the LOD of the P1-based electrode showed only small differences if compared with the results

Table 2
Sensitivities (mV/dec) and log of Limit of Detection (log LOD) obtained with the Ag^+-ISE based on P1 and P2 in different interference medium. Numbers in brackets correspond to the standard deviation of the slope of three experimental calibration curves.

MT	Ionic medium	10^{-3}		10^{-2}		10^{-1}	
		Sensitivity (mV/dec)	Log LOD	Sensitivity (mV/dec)	Log LOD	Sensitivity (mV/dec)	Log LOD
P1	$NaNO_3$	63 (4)	−4.3 (0.9)	–	–	61 (3)	−3.7 (0.4)
	$Pb(NO_3)_2$	60 (3)	−4.3 (0.3)	63 (4)	−4.4 (0.3)	65 (5)	−4.7 (0.4)
	$Cd(NO_3)_2$	59 (2)	−4.2 (0.4)	59 (5)	−4.9 (0.5)	60 (5)	−4.7 (0.3)
	$Zn(NO_3)_2$	59 (4)	−4.0 (0.6)	63 (4)	−3.5 (0.4)	61 (6)	−4.4 (0.7)
	$Cu(NO_3)_2$	55 (1.4)	−4.1 (0.2)	58 (1.3)	−4.5 (0.3)	59 (1.6)	−4.2 (0.2)
	$Hg(NO_3)_2$	28 (2)	−2.2 (0.1)	24 (2)	−1.9 (0.1)	–	–
P2	$NaNO_3$	69 (0.8)	−5.1 (0.3)	–	–	66 (0.5)	−3.6 (0.8)
	$Pb(NO_3)_2$	62 (1.6)	−3.4 (0.1)	70 (0.5)	−3.3 (0.6)	79 (5)	−2.3 (0.3)
	$Cd(NO_3)_2$	67 (1.3)	−3.4 (0.1)	60 (0.1)	−3.6 (0.4)	59 (2)	−4.1 (0.2)
	$Zn(NO_3)_2$	64 (0.2)	−3.3 (0.1)	66 (4)	−4.6 (0.5)	66 (0.9)	−3.2 (0.1)
	$Cu(NO_3)_2$	63 (0.2)	−4.7 (0.5)	61 (0.1)	−4.8 (0.3)	65 (0.2)	−4.5 (0.7)
	$Hg(NO_3)_2$	22 (0.2)	−3.7 (0.5)	18 (4)	−1.6 (0.2)	–	–

obtained in a water background. On the other hand, a weak interference of lead and zinc and therefore an increase in LOD were observed in these ionic backgrounds for the P2-based electrodes.

Concerning the P1-based electrode, Fig. 5 presents the low K^{pot} values obtained for the metal ions other than Hg^{2+} here studied, and it can consequently be stated that they would not cause any interference even when present at high concentration. With regards to the P2-based electrode, Fig. 5 also highlights the interferences of Pb^{2+} and Zn^{2+} (weaker), which did not interfered with the P1-based electrode. These differential Pb^{2+} interferences can be rationalized on the basis of the coordinating features of the two MTs employed. MT1 is a positively charged (+5) peptide of 61 amino acid residues with a isoelectric point (IP) of 8.38 and two O-donor amino acids, while SpMTA, which has 64 residues is a neutral peptide with a IP of 6.06 and seven O-donor amino acids (Fig. 1). Considering O-donor ligands are more common in the coordination environments of Pb^{2+} than those of Ag^{+}, a peptide richer in Asp and Glu residues (SpMTA) should show a higher affinity for this cation than other peptides with a lower content of O-donor ligands. On the other hand, it has been described that the inner lower stability of the Zn-S bond makes Zn-binding about 3000 times weaker in mammalian than in equinodermata MTs [25], this explaining the major interfering role of this cation in the P2-based electrodes. Here, it is interesting to note that although embedded in a polymeric matrix the two MT polypeptides used retain their differential metal-binding affinities.

4. Conclusions

In this work we have constructed potentiometric Ag^{+} selective biosensors by using metalloproteins, in particular MTs, as ionophores embedded in a polysulfone matrix. This demonstrates the feasibility of preparing potentiometric biosensors that include polymeric hydrophilic ionophores. The phase inversion procedure used for the construction of the membrane requires small amounts of protein that ensure its low cost in case of non-commercial proteins that have to be recombinantly synthesized or obtained from their native sources. Furthermore, the constructed ISEs have long lifetimes. This is not only related to the physical properties of the membrane and the lack of leaking processes due to the polymeric nature of the ionophore but also to the fact that the use of fully metallated metalloproteins ensures their resistance to air oxidation, the latter probably determining the low durability of other biosensors described (affinity MT sensors [26,29]). These, in comparison with the potentiometric sensors here described, can show lower detection limits, as a function of the incubation time, but contrarily show poor selectivity and require regeneration processes.

More interestingly, from the point of view of biosensors classification, the Ag-ISE described here probably provides a new kind of sensor as it cannot be considered to be based either on crystalline membranes with fixed sites, or on membranes with mobile sites. Therefore, their response mechanism could be closer to that described for ion-exchange resin membranes but differing in the fact that the interaction between the ionophore and the analyte responds to a complexation process, this latter feature conferring a higher selectivity to these biosensors.

Here, two types of electrodes embedding two structurally similar metallothioneins (*i.e.*, mouse MT1 and sea urchin SpMTA) have been developed. Both types of ISEs show high sensitivity, wide linear and working pH ranges, and good selectivity towards silver ion. Interference studies were carried out for several metal ions, and, except for Hg^{2+}, which has a strong interfering effect on both types of electrodes, Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+} do not cause important interferences with Ag^{+}. However, a different affinity of Pb^{2+} and Zn^{2+} towards P1- and P2-ISE was found that interestingly correlates well with the higher affinity of SpMTA (P2-ISE) than MT1 (P1-ISE) towards these metal ions. Thus, the use of different MT isoforms has allowed us to make a comparative study whose results reveal that the distinct metal-binding features of each MT are reflected in the different properties of the biosensors constructed with them. To our understanding this provides a large number of possibilities regarding the incorporation of other water-soluble metalloproteins as ionophores for the construction of potentiometric sensors.

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References

[1] G.J. Moody, J. Biomed. Eng. 7 (1985) 183.
[2] E. Santos, M. Conceiçao, B.S.M. Montenegro, C. Couto, A.N. Araújo, M.F. Pimentel, V. Lins Da Silva, Talanta 63 (2004) 721.
[3] Y.E. Ermolenko, V.V. Kolodnikov, S. Al-Marok, Y.G. Vlasov, Sens. Actuators B 26–27 (1995) 369.
[4] Handbook of Electrode Technology, Orion Research. Inc., Cambridge, MA, 1982.
[5] S. Alegret, J.L.F.C. Lima, A.A.S.C. Machado, E. Martínez-Fàbregas, J.M. Paulís, Química Analítica 6 (1987) 176.
[6] A. Jyo, T. Imato, H. Kohno, N. Ishibashi, Bull. Chem. Soc. Jpn. 56 (1983) 3177.
[7] E. Bakker, M. Willer, M. Lerchi, K. Seller, E. Pretsch, Anal. Chem. 66 (1994) 516.
[8] E. Bakker, P. Bühlmann, E. Pretsch, Chem. Rev. 97 (1997) 3083.
[9] S.Y. Yun, Y.K. Hong, B.K. Oh, G.S. Cha, H. Nam, S.B. Lee, J.I. Jin, Anal. Chem. 69 (1997) 868.
[10] D.N. Reinhoud, J.F.J. Engebersen, Z. Brzozka, H.H. Van Der Vlekkert, G.W.N. Honing, H.A.J. Holterman, U.H. Verkerk, Anal. Chem. 66 (1994) 3618.
[11] M.E. Poplawski, R.B. Brown, K.L. Rho, S.Y. Yun, H.J. Lee, G.S. Cha, K.J. Phaeng, Anal. Chim. Acta 355 (1997) 249.
[12] E. Pungor, K. Toth, M.K. Papay, L. Polos, H. Malissa, M. Grasserbauer, E. Hoke, M.F. Ebel, K. Persy, Anal. Chim. Acta 109 (1979) 279.
[13] G.J. Moody, R. Oke, J.D.R. Thomas, Analyst 95 (1970) 910.
[14] S.G. Cha, D. Liu, M.E. Meyerhoff, A.C. Cantor, A.R. Mdgley, H.D. Goldberg, R.B. Brow, Anal. Chem. 63 (1991) 1666.
[15] B. Prieto-Simon, E. Fàbregas, Biosens. Bioelectron. 22 (2006) 131.
[16] B. Prieto-Simon, E. Fàbregas, A. Hart, Biosens. Bioelectron. 22 (2007) 2663.
[17] B. Prieto-Simón, J. Macanás, M. Muñoz, E. Fàbregas, Talanta 71 (2007) 2102.
[18] S. Sanchez, E. Fàbregas, Biosens. Bioelectron. 22 (2007) 965.
[19] S. Sanchez, M. Pumera, E. Cabruja, E. Fàbregas, Analyst 132 (2007) 142.
[20] S. Sanchez, M. Pumera, E. Fàbregas, Biosens. Bioelectron. 23 (2007) 332.
[21] S.P. Nunes, K.V. Peinemann (Eds.), Membrane Technology in the Chemical Industry, Wiley–VCH Verlag GmbH, Weinheim, Germany, 2001, pp. 12–33.
[22] A. González-Bellavista, J. Macanás, M. Muñoz, E. Fàbregas, Sens. Actuators B 115 (2006) 691.
[23] A.H. Robbins, D.E. McRee, M. Williamson, S.A. Collett, N.H. Xuong, W.F. Furey, B.C. Wang, C.D. Stout, J. Mol. Biol. 221 (1991) 1269.
[24] R. Riek, B. Prêcheur, Y. Wang, E.A. Mackay, G. Wider, P. Güntert, A. Liu, J.H.R. Kägi, K. Wüthrich, J. Mol. Biol. 291 (1999) 417.
[25] Y. Wang, E.A. Mackay, M. Kurasaki, J.H.R. Kägi, Eur. J. Biochem. 225 (1994) 449.
[26] I. Bontidean, C. Berggren, G. Johansson, E. Csoregi, B. Mattiasson, J.R. Lloyd, K.J. Kenneth, J. Jakeman, N.L. Brown, Anal. Chem. 70 (1998) 4162.
[27] I. Bontidean, J. Ahlqvist, A. Mulchandani, W. Chen, W. Bae, R.K. Mehra, A. Mortari, E. Csöregi, Biosens. Bioelectron. 18 (2003) 547.
[28] P. Corbisier, C. Van der Lelie, B. Borremans, A. Provoost, V. De Lorenzo, N.L. Brown, J.R. Lloyd, J.L. Hobman, E. Csoregi, G. Johansson, B. Mattiasson, Anal. Chim. Acta 387 (1999) 235.
[29] I. Bontidean, J.R. Lloyd, J.L. Hobman, J.R. Wilson, E. Csöregi, B. Mattiasson, N.L.J. Brown, Inorg. Biochem. 79 (2000) 225.
[30] R.K. Mahajan, O. Parkash, Talanta 52 (2000) 691.
[31] M.K. Amini, M. Ghaedi, A. Rafi, I. Mohamadpoor-Baltork, K. Niknam, Sens. Actuators B 96 (2003) 669.
[32] K. Kimura, S. Yajima, K. Tatsumi, M. Yokohama, M. Oue, Anal. Chem. 72 (2000) 5290.
[33] N. Cols, N. Romero-Isart, M. Capdevila, B. Oliva, P. Gonzàlez-Duarte, R. Gonzàlez-Duarte, S. Atrian, J. Inorg. Biochem. 68 (1997) 157.
[34] O. Palacios, K. Polec-Pawlak, R. Lobinski, M. Capdevila, P. González-Duarte, J. Biol. Inorg. Chem. 8 (2003) 831.
[35] E. Martínez-Fabregas, S. Alegret, J. Chem. Educ. 71 (1994) A67.
[36] J.L.F.F. Lima, A.A.F.C. Machado, Analyst 111 (1986) 799.
[37] F. Céspedes, E. Martínez-Fàbregas, S. Alegret, Trends Anal. Chem. 15 (1996) 296.
[38] S. Alegret, F. Florido, Analyst 116 (1991) 473.

[39] M. Mulder, Basic Principles of Membrane Technology, Kuwer Academic Publishers, Dordrecht, 1992, p. 59.
[40] S. Sánchez, M. Roldán, S. Pérez, E. Fàbregas, Anal. Chem. 80 (2008) 6508.
[41] E. Pretsch, Trends Anal. Chem. 26 (2007) 46.
[42] R. De Marco, J.-P. Veder, G. Clarke, A. Nelson, K. Prince, E. Pretsch, E. Bakker, Phys. Chem. Chem. Phys. 10 (2008) 73.
[43] M. Vasak, Methods Enzymol. 205 (1991) 452.
[44] R.P. Buck, E. Lindner, Pure Appl. Chem. 66 (1994) 2527.
[45] D. Siswanta, K. Nagatsuka, H. Yamada, K. Kumakura, H. Hisamoto, Y. Shichi, K. Toshima, K. Suzuki, Anal. Chem. 68 (1996) 4166.
[46] W. Wróblewski, Z. Brzozka, Sens. Actuators B 24-25 (1995) 183.
[47] D. Xu, T. Katsu, Anal. Chim. Acta 43 (2001) 235.
[48] L. Chen, X. He, B. Zhao, Y. Liu, Anal. Chim. Acta 417 (2000) 51.