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Cytosine-substituted metalloporphyrins: receptors for recognition of nucleotides in ion-selective electrodes

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Received (in Pittsburgh, PA, USA) 19th January 2004, Accepted 26th February 2004
First published as an Advance Article on the web 18th March 2004

A new series of cytosine-substituted metalloporphyrin conjugates (containing Co(II) and Zn(II) as the coordinated metals) were designed and investigated as nucleotide receptors in PVC-membrane-based ion-selective electrodes under neutral conditions. The potentiometric results indicate that these systems, in particular the Co(II)-containing complex, may be potentially useful sensors for complementary nucleotide substrates in the presence of 10 mol% tridodecylmethylammonium chloride ($K^{Pot.}_{5'\text{-GMP}/5'\text{-AMP}} = 0.045$).

Introduction

A key step in rendering practical many of the inspirational results arising from the current explosion in the area of supramolecular chemistry involves showing that the selectivity in recognition demonstrated in solution can be reproduced to good effect on surfaces, membranes, electrodes and other supports through which an interface to conventional instrumentation may be readily established. One approach we and others have been pursuing in this regard is focused on the development of selective, molecular recognition-based ion-selective electrodes. Here, one of the goals has been and remains the generation of systems that recognize and sense mononucleotides. Such species play a critical role in biology and medicine and thus, not surprisingly, the development of receptors, carriers, and sensors for nucleotides has attracted the attention of many researchers working in the supramolecular field.[1–19] From this body of work, it is now well-appreciated that various combinations of complementary base pairing, directed multisite hydrogen bonding interactions, specific pi–pi (π–π) stacking effects, and generalized electrostatic interactions can be used to design synthetic receptors, just as these various binding motifs, individually or in concert, are thought to contribute to the exquisitely sensitive nucleotide recognition observed in biological systems. Incorporating one or more of these features into appropriately designed synthetic receptors could culminate in the production of highly specific potentiometric sensors (in particular, ion-selective electrodes, ISEs), which by virtue of their interfacial nature are potentially well-suited for use in various "real world" bioanalytical applications. Whether this goal will be fully realized remains to be seen. However, its pursuit has stimulated the evolutionary development of several elegant nucleotide-targeting ISEs in recent years.[1,3,6,9,11,13] Here, in addition to the ultimate goal of generating a field-usable working nucleotide sensing system, an important ancillary motivation for making and studying various potential carrier-based ISEs, is that their analysis can provide a means for testing quickly whether a given supramolecular receptor system displays selectivity for the targeted analyte(s) under what can be considered model "real world" conditions.[7,13] In particular, the potentiometric methods involved in the testing of potential ISEs can be used to derive insights into recognition events that take place at an aqueous–organic interface.

In recent years, we have devoted considerable effort to the design of ditopic receptors for the specific recognition of various targeted nucleotides. To date, we have mostly focused on the use of systems based on nucleobase-substituted oligopyrrolic macrocycles, such as sapphyrin and calix[4]-pyrroles. In the present study, we describe a new set of metalloporphyrin–nucleobase conjugates and tests of their use as potential nucleotide-recognizing ISE sensing elements (Fig. 1).

Results and discussion

In an initial series of experiments, PVC/*o*-NPOE membrane electrodes containing no lipophilic additive were tested for their response to nucleotides. Such membranes, which did, however, contain the cytosine–metalloporphyrin conjugates, were found to demonstrate: *i*) a high potentiometric sensitivity toward 5′-CMP (slopes of −27 and −34 mV/decade beginning from 10^{-3} M for **2** and **3**, respectively), *ii*) a sub-Nernstian response toward 5′-GMP and 5′-UMP beginning from 10^{-5} M, and *iii*) a weak response toward 5′-AMP and 5′-TMP (Fig. 2).

A comparison of the overall anion responses (ΔmV) of the PVC-membrane electrodes doped with receptors **2** and **3** showed an increasing potentiometric response within the series: 5′-AMP < 5′-TMP < 5′-GMP < 5′-UMP < 5′-CMP. It should also be noted that the potentiometric response of the membranes based on the Co(II)-porphyrin **2** was far cleaner than that of the membranes based on Zn(II)-porphyrin **3**. While a variety of factors could account for the latter findings, they could also be providing a "hint" as to which portions of the receptor are most important in terms of recognizing nucleotide(s).

In receptors **2** and **3**, two types of interactions (or some combination thereof) were considered likely to provide the basis for nucleotide recognition: *i*) hydrogen-bonding between the complementary nucleobases present in the receptor and the targeted nucleotide guest and *ii*) direct coordination, wherein the nucleotide guest binds as an axial ligand to the Lewis acidic metal center of the metalloporphyrin. In an effort to determine whether the latter mode is likely to be important for nucleotides

DOI: 10.1039/b400880d

Table 1 Association constants (M^{-1}) for the interaction of 3′,5′-bis(*O*-triisopropylsilyl) substituted deoxyribonucleosides and zinc octaethylporphyrin, as reported by Ogoshi *et al.*[20]

Nucleoside derivatives	Adenosine	Guanosine	Cytidine	Thymidine
Association constant[a]	600 (100)	150 (50)	80 (50)	—

[a] In CH_2Cl_2, at 15 °C. Standard deviations are given in parentheses. In the case of thymidine, an appreciable absorption change was not observed. Data taken from ref. 20.

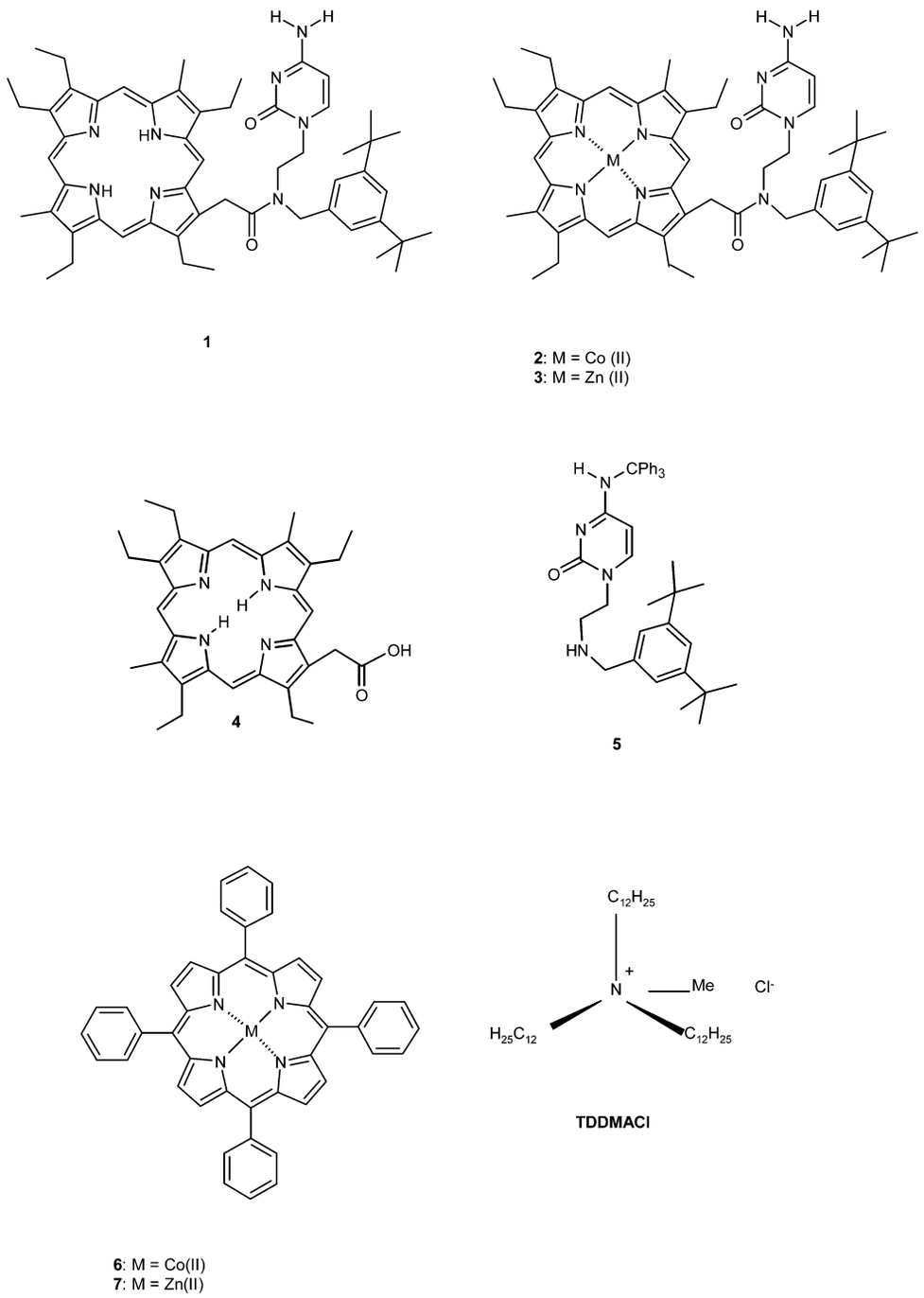

Fig. 1 Compounds used in this study.

interacting with cytosine–metalloporphyrin conjugates in a membrane phase, we started by considering the association constants reported by Ogoshi *et al.*, corresponding to the interaction of zinc octaethylporphyrin with 3′,5′-bis(*O*-triisopropylsilyl)deoxyribonucleosides in dichloromethane at 15 °C (Table 1).[20]

Analysis of Ogoshi's results reveals that the zinc(II)-containing metalloporphyrin ring has a greater preference for purine nucleobases (*i.e.*, adenine and guanine) than for pyrimidine-type nucleobases (*e.g.*, cytosine). Other studies also support the notion that in the absence of a competing axial ligand, metalloporphyrins containing a soft Lewis acidic metal center are capable of interacting strongly with various amine-type ligands,[21–25] in particularly, nucleobases.[20,26] To the extent that such conclusions may be generalized, they lead to the consideration that, in the absence of other effects, ISE containing metalloporphyrins would show selectivities dominated by specific nucleobase metal coordination interactions. In the present

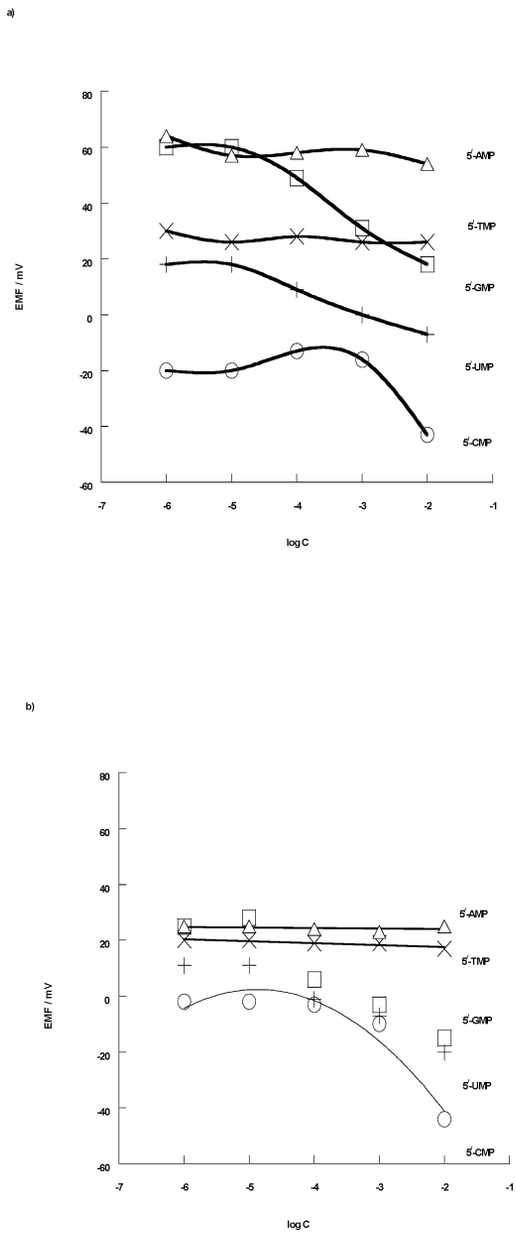

Fig. 2 Potentiometric response towards the nucleotides: 5′-AMP (Δ), 5′-CMP (○), 5′-GMP (□), 5′-TMP (×) and 5′-UMP (+) observed for PVC-membranes based on **2** a) and **3** b) in the absence of a lipophilic additive.

instance, *i.e.*, with receptors **2** and **3**, such axial ligation effects would be expected to be the most important selectivity determining factors in the absence of other competing binding modes, or in the limit where the nucleobase-derived Watson–Crick recognition effects are small. The question we sought to address, therefore, was whether the latter would represent important selectivity determinants and, if so, under what conditions.

Under the conditions of our interfacial ISE experiments, we found as a general rule that the PVC-membranes based on the cytosine metalloporphyrin conjugates **2** and **3** displayed a preference for pyrimidine over purine nucleotides in the absence of a lipophilic additive (**TDDMACl**). In other words, under these conditions, the pyrimidine nucleotides (5′-CMP; 5′-UMP, although not, however, 5′-TMP) engendered a greater potentiometric response than the corresponding purine nucleotides (5′-AMP; 5′-GMP). While it is difficult to discuss the selectivity of ion-selective electrodes in the absence of a Nernstian response towards the tested analytes, it is important to note that our conclusion that the selectivity of cytosine-substituted metalloporphyrins is larger for 5′-CMP than for 5′-GMP is additionally supported by results obtained using the matched potential method. The matched potential method was officially recommended by the IUPAC when the interfering ions and/or the primary ion do not satisfy the Nernstian condition.[27] Using this latter method, the selectivity factor, $k^{sel}_{5'\text{-UMP/J}}$ (J represents interfering ions, in this case a competing nucleotide) was found to follow the sequence:

2: 5′-AMP (−0.3) < 5′-GMP (0.1) < 5′-CMP (0.75)
3: 5′-AMP (−0.3) < 5′-GMP (−0.1) < 5′-CMP (0.2)

(using 5′-UMP at 10^{-4} M as the background, the concentration of the interfering nucleotides was varied over the range $n \times 10^{-3}/n \times 10^{-4}$ M).

The above findings are not consistent with what one would infer from the Ogoshi study and led to the consideration that factors other than nitrogen-centered metal–nucleotide axial ligation or Watson–Crick base pairing (which would favor 5′-GMP) were acting to regulate the selectivity in the absence of a lipophilic additive.

Among the interactions that could serve to regulate the selectivity observed in the absence of a lipophilic additive, those involving axial ligation were still thought to be the most important. However, here it is important to appreciate that binding modes other than direct nitrogen-centered co-ordination could be important. For instance, binding of water to the metalloporphyrin core (as an axial ligand), could reduce or obviate the effect of direct metalloporphyrin–nucleobase interaction. Such effects, which are not likely to be important under the carefully controlled, anhydrous conditions associated with the Ogoshi study, could be quite significant under the interfacial ISE membrane conditions. Another effect, not controlled in the Ogoshi study, is the metal–phosphate axial ligation effect. The phosphate oxyanion is a potential hard ligand and could compete with what are presumably softer nucleobase nitrogen donors for the metal centers present in **2** and **3**.

The importance of porphyrin metal core "deactivation" and, indeed, more generally the importance of metalloporphyrin axial ligand interactions, in terms of regulating binding affinity and ISE membrane selectivity in systems such as **2** and **3**, was tested by exploring the preference, if any, the cytosine-substituted Co(II)-porphyrin **2** displayed towards nucleobase and phosphates. Using as analytes sodium dihydrophosphate and diphenylphosphate, this receptor was found to display the expected anionic responses, with sensitivities of −45 and −38 mV/decade with a linear range of 10^{-3}–10^{-2} and 10^{-5}–10^{-2} M being recorded for the dihydrophosphate and diphenylphosphate salts, respectively. By contrast, when adenine phosphate was chosen as the targeted analyte, a cationic response was observed (sensitivity: +38 mV/decade; linear range: 10^{-3}–10^{-2} M). These findings lend important support for the notion that this particular receptor can interact with a nucleotide *via* axial ligation to both its constituent nucleobase and phosphate subunits.

As a further means of assessing nitrogen *vs.* phosphate ligation effects, and to establish a base-line for understanding the role, if any, the appended cytosine hydrogen bond receptor functionality present in **2** and **3** might play in regulating the observed ISE selectivity, control experiments involving zinc(II) tetraphenylporphyrin and cobalt(II) tetraphenylporphyrin were carried out (Table 2).

The PVC-membranes derived from Co-tetraphenylporphyrin showed the same ability to sense both nucleobase and various phosphate species (dihydrophosphate, diphenylphosphate) as did their cytosine substituted analogues. Likewise, the membranes containing Zn-tetraphenylporphyrin demonstrated the same weak response towards simple phosphorylated species seen for the functionalized derivative, while demonstrating only a cationic response towards nucleobases. In the case of the

Table 2 Potentiometric responses of PVC-membranes based on cytosine-substituted Co(II)- and Zn(II)-porphyrins (**2** and **3**) and their unsubstituted analogues (**6** and **7**) towards simple phosphate-type analytes

Analyte	**2**	**6**	**3**	**7**
$H_3PO_4/H_2PO_4^-$	−45 (10^{-3}–10^{-2})	−36 (10^{-3}–10^{-2})	[a]	[a]
Diphenylphosphate	−38 (10^{-5}–10^{-2})	−42 (10^{-3}–10^{-2})	[a]	[a]
Adenine phosphate	+38 (10^{-3}–10^{-2})	+41 (10^{-3}–10^{-2})	[a]	+60 (10^{-3}–10^{-2})

[a] No detectable response.

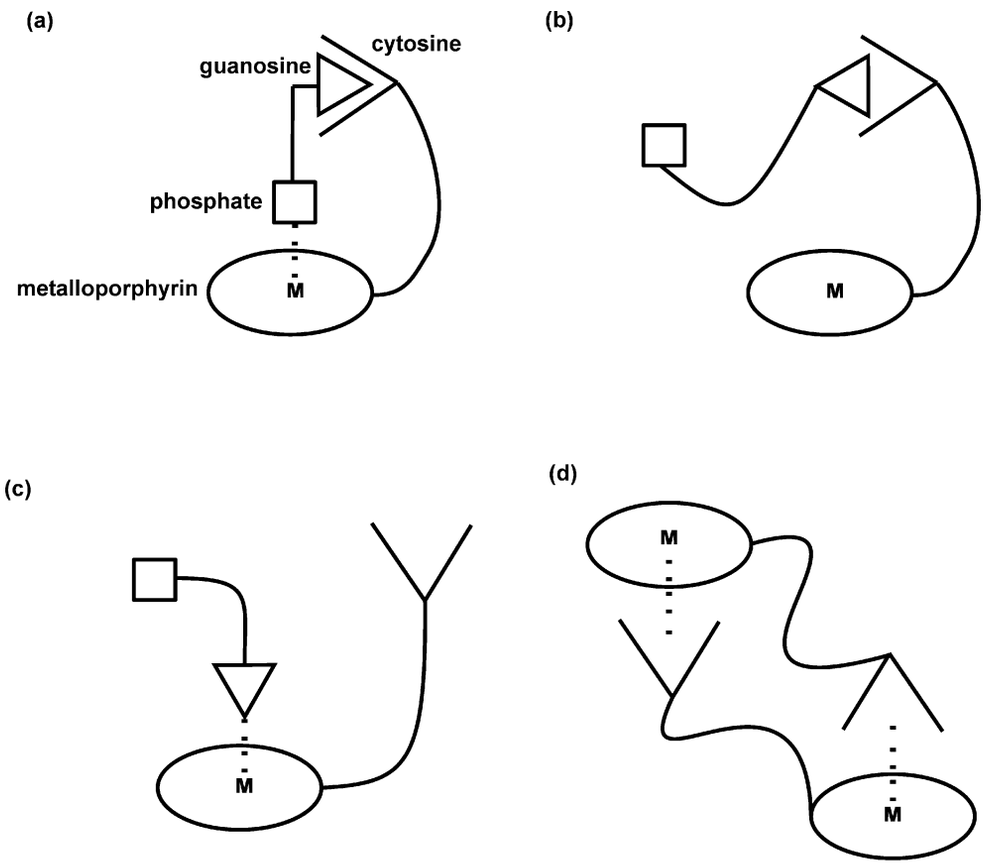

Fig. 3 Schematic representation of the various proposed interactions: (a) expected ditopic binding of a targeted nucleotide, (b) axial ligation to the metal (only), with no hydrogen bonds; (c) binding of a nucleotide substrate *via* only hydrogen bonds, and (d) "head-to-tail" dimer formation involving only the receptor.

various nucleotides, the following selectivity order was observed for the unsubstituted metalloporphyrins:

6: 5′-TMP (0.00) < 5′-GMP (0.22) < 5′-CMP (0.25) < 5′-UMP (0.38)

7: 5′-AMP (−0.31) < 5′-GMP (−0.13) < 5′-TMP (0.00) < 5′-CMP (0.08) < 5′-UMP (0.61),

where the values in parentheses refer to the selectivity factor ($k^{sel}_{5'\text{-TMP/J}}$, J represents a competing nucleotide). As detailed in the Experimental Section, because of the lack of Nernstian response seen for the nucleotides in question, comparisons of selectivity were made using the matched potential method. Specifically, using 5′-TMP at 10^{-4} M as the background, the concentration of the interfering anion was varied (up to $n \times 10^{-3}/n \times 10^{-4}$ M) and the corresponding response recorded.

Thus, in contrast to their cytosine-substituted metalloporphyrin analogues, the simple unsubstituted systems display a greater preference for 5′-UMP than for 5′-CMP. Further, for the unsubstituted systems **6** and **7**, the greatest distinction between purine and pyrimidine analytes was observed in the case of Zn(II)-tetraphenylporphyrin. While not fully determined by the present experiments, presumably these observations reflect the greater importance of nucleobase axial ligation, as opposed to phosphate-based metal binding, in terms of determining both the extent of the ISE response and the inherent nucleotide selectivities.

That a difference was seen between **2** and **3** and their respective unfunctionalized controls provides support for the notion that substitution with a nucleobase could serve to impart a degree of nucleotide selectivity (Fig. 3a). Here, the more hydrogen bonds the cytosine–substituent interaction is capable of supporting, the greater the observed ISE signal should be. In particular, if such effects are important, the following selectivity order might be expected: 5′-AMP (× 1) < 5′-TMP (× 2) ~ 5′-UMP (× 2) ~ 5′-CMP (× 2) ≤ 5′-GMP (× 3) (the values in parentheses correspond to the number of the possible hydrogen bonding interactions) (Fig. 3a, b). Of course, such a prediction is predicated on the hydrogen bonding interactions being the sole determinant of selectivity with axial ligation, clearly an important factor, being completely ignored. This is clearly an over-simplification hand, since a strong predilection for nucleobase axial ligation is expected to reduce the selectivity of a nucleobase-substituted metalloporphyrin recognition element. Specifically, strong ligation effects are expected to "wash out" the effects of Watson–Crick base pairing (Fig. 3c).

Another complication that has to be considered is that self-recognition, resulting in "head-to-tail" interactions between separate molecules of **2** or **3** under the conditions of the

membrane-based experiments, could lead to the formation of dimers or higher order aggregates (Fig. 3d). The formation of such species, for which some support comes from mass spectrometric analyses of **3** (*cf.* Experimental Section), would necessarily reduce the ability of the nucleobase porphyrin conjugates to function as nucleotide selective ISE sensing elements.

Knowledge of the operative mechanism of the metalloporphyrin based sensors is critical since optimization of membrane selectivity is highly dependent on the incorporation of additional membrane components. Indeed, it has recently been demonstrated that the presence of lipophilic electrically charged additives improves the potentiometric behavior of certain anion-selective electrodes, including ones prepared with metalloporphyrins and cobyrinates.[28–30] The type of additive required depends on the carrier mechanism involved. It is known that the oxidation state of the metal center within the metalloporphyrin structure can dictate the possible mechanism for the interaction of anions with such a receptor at the sample/membrane interface.[31] Porphyrins with metal(II) centers can function *via* a neutral carrier mechanism,[32] as such, their response sensitivity could be counter cation limited. For this reason, neutral metalloporphyrin-based membranes containing lipophilic cationic additives should exhibit improved selectivity.[30] In the present instance, involving the cytosine substituted porphyrins, **2** and **3**, the use of a lipophilic additive is expected to be beneficial since it would reduce the polarity of the environment and facilitate charge compensation. In doing so, it should enhance the importance of the Watson–Crick base pairing interactions, which should lead to selectivity for 5′-GMP in those cases where these latter are sufficiently large.

As a test of the above expectations, we investigated the effect of adding a lipophilic positive charged additive (*i.e.*, **TDDMACl**) into the ISE membranes containing receptor **2**. Furthermore, to test whether this latter system was indeed acting as an efficient ditopic receptor for 5′-GMP, we decided to use this latter nucleotide as the targeted substrate.

The optimal molar ration of the ionic additive was found to be 10 mol% with respect to the total ionophore concentration present in the membrane (Fig. 4). As compared to the membranes made up without any cationic additive, those membranes containing 10 mol% **TDDMACl** demonstrated considerably higher sensitivity (in particular, −26 and −29 mV/decade in the concentration range 10^{-3}–10^{-2} M for 5′-AMP and 5′-GMP, respectively) and selectivity for 5′-GMP over 5′-AMP ($K^{Pot.}_{5'\text{-GMP}/5'\text{-AMP}} = 0.045$). It is interesting to note that the resulting value $K^{Pot.}_{5'\text{-GMP}/5'\text{-AMP}}$ is approximately one order greater than that reported by Umezawa *et al.* ($K^{Pot.}_{5'\text{-GMP}/5'\text{-AMP}} = 0.45$).[9] An increase in the **TDDMACl** content from 50 mol% to 150 mol% led to a significant decrease in selectivity; under these latter conditions, the membrane began to function as an ordinary ion-exchanger (Fig. 4b). However, this same drop off in selectivity serves to highlight the fact that under appropriately chosen conditions (*e.g.*, 10 mol% **TDDMACl**), the cytosine-substituted metalloporphyrin systems described in this report, in particular, the Co(II)-containing complex **2**, can be made to function as signaling agents for their Watson–Crick complement, 5′-GMP.

Conclusion

In summary, we have described a new class of ditopic receptors, based on the covalent linking of a nucleobase, cytosine in the present instance, to a metalloporphyrin. While a number of competing interactions serve to complicate the use of these systems as sensing elements in ISEs, under appropriately chosen conditions (10 mol% **TDDMACl** as a lipophilic additive; use of cobalt(II) as the coordinated porphyrin metal center), good selectivities for the Watson–Crick complementary analyte, 5′-GMP, can be obtained. The present work thus

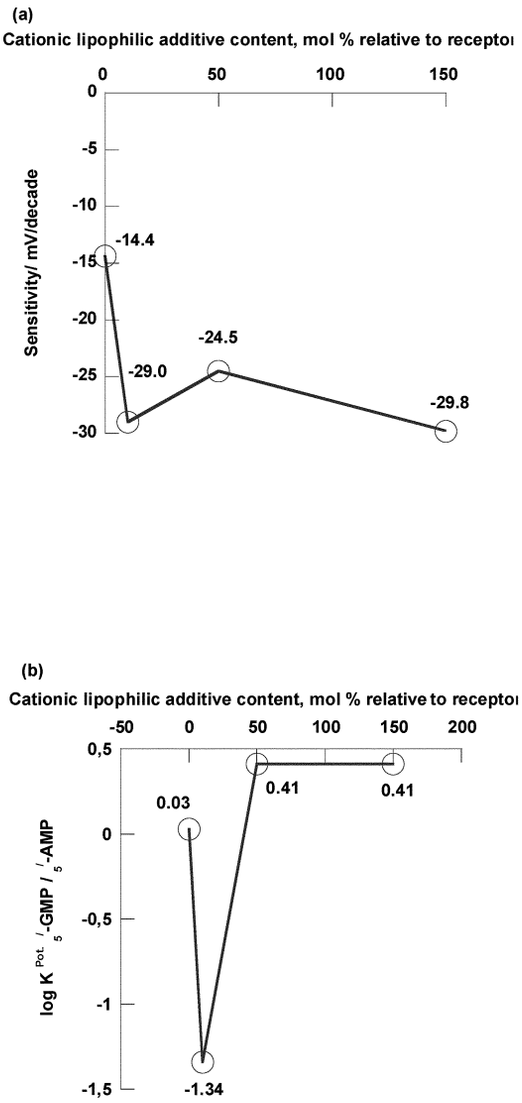

Fig. 4 Influence of lipophilic additive content on (a) sensitivity and (b) potentiometric selectivity coefficients ($K^{Pot.}_{5'\text{-GMP}/5'\text{-AMP}}$) of a PVC-membrane based on cytosine-substituted Co(II)-porphyrin conjugate **2** toward 5′-GMP.

underscores the utility of a basic, molecular recognition inspired approach to sensor design but also serves to highlight the difficulties associated with extrapolating simple well-defined solution phase-based results into the more "real world" conditions associated with ISE sensor development.

Experimental

Materials

All reagents were of the highest grade commercially available and used without further purification. Poly(vinyl chloride) high molecular weight (PVC), 2-nitrophenyl octyl ether (*o*-NPOE), tridodecylmethylammonium chloride (TDDMACl), tetrahydrofuran (THF; stored over 3 Å molecular sieves) were purchased from Fluka (Switzerland). Adenosine 5′-monophosphate sodium salt (5′-AMP), cytidine 5′-monophosphate disodium salt (5′-CMP), guanosine 5′-monophosphate disodium salt (5′-GMP), thymidine 5′-monophosphate sodium salt (5′-TMP), uridine 5′-monophosphate disodium salt (5′-UMP), 2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES), cobalt and zinc tetraphenylporphyrins were from Sigma-Aldrich Chemie (Steinheim, Germany). Adenine phosphate salt and diphenylphosphate were obtained from Sigma and Aldrich Chem. Co. (Germany), respectively. Distilled water was used to prepare buffer and standard solutions.

Electrode preparation and ISE measurements

Ion-selective membranes were prepared in accord with the procedure described in ref. 12. In the present study, 0.7 ml THF was used to dissolve approximately 100 mg of a mixture containing 3 wt% of the receptor in question, 22 wt% PVC, and 75 wt% *o*-NPOE. The resulting membranes, obtained following evaporation as before, were mounted on an electrode body (Crytur, Czech Republic). The influence of cationic sites on the potentiometric characteristics of these membranes was assessed by studying not only these membranes but ones containing, in addition, 10, 50 and 150 mol% TDDMACl relative to the receptor (incorporated at 3 wt% level, as noted above). Control electrodes, containing just Co-tetraphenylporphyrin **6** and Zn-tetraphenylporphyrin **7**, were prepared and studied as reported previously.[12] EMF measurements were performed using a digital voltammeter, Model M1T330 (Metra s.p., Blansko, Czech Republic), and in accord with the following cell assembly: Hg | Hg_2Cl_2 | 3 M KCl || 0.1 M HEPES–NaOH pH 6.6 || sample | modified PVC-membrane | 0.1 M KCl | AgCl | Ag. All potentiometric analyses were carried out at ambient temperature. The pH was monitored using a glass electrode Type 01-29 B (Labio Prague, Czech Republic) and a Type OP-205/1 pH-meter (Radekis, Budapest, Hungary). In the studies of potentiometric response and anion selectivity, working solutions of the analytes in question were prepared by diluting concentrated stock solutions with 0.1 M HEPES adjusted to pH 6.6 with NaOH. Calibration curves were constructed by plotting the potential *vs.* the logarithm of the concentration of the anion present in the buffer solution. Anion concentrations rather than activities were used because it is difficult to estimate activity coefficients in the zwitterionic buffer. Before starting the ISE studies, the electrodes were soaked overnight in HEPES buffer (0.1 M HEPES, adjusted to pH 6.6 by the addition of NaOH as above) in the presence of the analyte. Potentiometric selectivity coefficients (log $K^{Pot.}_{I,J}$) were then determined by the separate solution method,[27] with the primary (I) and interfering (J) ion concentrations being 1.0×10^{-2} M for both the PVC membranes containing the receptors (*e.g.*, **2**) and the analyte (*e.g.*, 5′-GMP). In certain instances, especially those wherein a non-Nernstian response was observed, the selectivity factor ($k^{sel}_{I,J}$) was measured using the matched potential method.[27]

Instrumentation for receptor characterization

NMR spectra were obtained using a General Electric QE-300 spectrometer at the University of Texas at Austin. High resolution fast atom bombardment mass spectra (HR FAB MS) were recorded at the University of Texas at Austin Department of Chemistry and Biochemistry MS Facility using a VG ZAB-2E instrument.

Synthesis and characterization

General procedure for preparing cytosine–metalloporphyrin conjugates, 1–3. Compounds **1**–**3** were prepared *via* amide bond formation starting from the porphyrin carboxylic acid **4**, prepared according to the procedure of ref. 33, and the protected aminoethylcytosine **5**.[13,34] Thus, **4** (210 mg; 3.98×10^{-4} mol) was dissolved in 5 ml of dry dimethylformamide (DMF) and cooled to 0 °C. To this solution was then added 0.75 ml diisopropylcarbodiimide. Stirring was continued at 0 °C for one hour, before the mixture was allowed to warm to RT. At this point, **5** (465 mg; 7.78×10^{-4} mol) was added along with 5 mg 1-hydroxybenzotriazole (HOBT) and 30 mg 4-dimethylaminopyridine (DMAP). The reaction mixture was then stirred in the dark for 30 hours under argon, before the DMF was removed *in vacuo*. The resulting residue was dissolved in dichloromethane (DCM) and subjected to column chromatography over silica gel, using DCM containing 0–5% methanol (MeOH) as the eluent. Isolation of the appropriate fraction, followed by removal of the solvent yielded the protected version of conjugate **1** in 89% yield. This intermediate product was not isolated or characterized but rather was immediately subjected to deprotection using the procedure described earlier;[8] product **1** was obtained by crystallization from MeOH–DCM diethyl ether in 92% yield. This cytosine–porphyrin conjugate was used as a precursor for the corresponding Co(II) and Zn(II) metal complexes, **2** and **3**; these latter were prepared in quantitative yield by heating the metal free form, **1**, with the appropriate acetoacetonates in chloroform–methanol 1 : 1 at reflux for 4 hours.

Cytosine–porphyrin conjugate 1. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.21 (s, 4H, CH), 10.14 (s, 4H, CH), 10.13 (s, 4H, CH), 10.11 (s, 4H, CH), 6.72 (d, 1H, cytosine C^{6}H), 4.75 (d, 1H, cytosine C^{5}H), 4.44 (br t, 2H, CH_2CH_2CON), 4.1 (t, 8H, $4 \times CH_3CH_2$), 4.75 (br s, 2H, N–CH_2-bis(*t*-Bu)phenyl), 3.669, 3.665, 3.605 ($3 \times$ s, 9H, $3 \times CH_3$), 3.38 (br, 2H, CO–N–CH_2CH_2–N–cyt), 3.249 (br t, 2H, CH_2CH_2CON), 3.139 (br, 2H, CO–N–CH_2CH_2–N–cyt), 1.931, 1.89 ($2 \times$ br q, 12H, $4 \times CH_3CH_2$), 1.25 (s, 18H, *t*-Bu), −3.792 (s, 2H, $2 \times$ NH). ^{13}C NMR (125 MHz, $CDCl_3$ with 5% CD_3OD) δ 12.30, 18.42, 19.22, 20.59, 22.95, 30.42, 32.10, 35.86, 46.65, 48.56, 54.09, 76.37, 91.96, 97.28, 120.54, 122.20, 136.20, 144.98, 152.17, 163.40, 173.74. HR FAB MS: calcd. for $C_{55}H_{71}N_8O_2$: 875.569999; found 875.569265. Elemental analysis: calcd. for $C_{55}H_{70}N_8O_2$ (875.20): C 75.48, H 8.06, N 12.80; found C 75.23, H 8.18, N 12.60%.

Receptor 2. HR FAB MS: calcd. for $C_{55}H_{68}N_8O_2Co$: 931.479722; found: 931.478546. Elemental analysis: calcd. for $C_{55}H_{68}N_8O_2$ (932.11): C 70.87, H 7.35, N 12.02; found C 70.63, H 7.12, N 11.89%.

Receptor 3. HR FAB MS: calcd. for $C_{55}H_{68}N_8O_2Zn$: 936.475669; found: 936.476541. Elemental analysis: calcd. for $C_{55}H_{68}N_8O_2Zn$ (938.57): C 70.38, H 7.30, N 11.94; found C 70.10, H 7.12, N 11.69%. Dimer of **3**, as inferred from the HR FAB mass spectrum: Calcd for $C_{110}H_{137}N_{16}O_4Zn_2$: 1873.969164; found: 1873.956841.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant no. CEZ: MSM 223400008), the Grant Agency of the Czech Republic (grant nos. 203/02/0933, 203/02/0420), the EU (grant QLRT-2000-02360), and the National Institutes of Health (grant no. GM 58907).

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