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Electrochemical quantification of high-affinity halide binding by a steroid-based receptor

Robert A. W. Dryfe,*[a] Simon S. Hill,[a] Anthony P. Davis,*[b] Jean-Baptiste Joos[b] and Edward P. L. Roberts[c]

[a] *Dept. of Chemistry, University of Manchester Institute of Science & Technology, PO Box 88, Manchester, UK M60 1QD. E-mail: robert.dryfe@umist.ac.uk; Fax: +44 161 200 4559*
[b] *School of Chemistry, Univ. of Bristol, Cantock's Close, Bristol, UK BS8 1TS*
[c] *Dept. of Chemical Engineering, University of Manchester Institute of Science & Technology, PO Box 88, Manchester, UK M60 1QD*

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The strength of binding between a cholapod receptor and halide anions is quantified using voltammetry at the liquid/liquid interface, revealing very high affinities and size-selectivity peaking at chloride.

The control of ion concentration gradients across cell membranes is critical to living organisms.[1] Organic molecules which perturb those gradients can thus have profound biological effects.[2] Among such molecules, an important sub-class are the electroneutral "shuttle" transporters typified by the K^+-selective Valinomycin.[3] These compounds locate in membranes, extract ions from one aqueous phase to form charged but lipophilic complexes, then move across the membrane and release their substrates into the second aqueous phase. The great majority of electroneutral ion shuttles are cation transporters, but recently it has emerged that synthetic anion-transporting counterparts are also possible. The cholapod anion receptors **1** are derived from an inexpensive natural product, cholic acid.[4] Their steroidal backbone confers preorganisation and lipophilicity, while control of H-bond donor power allows tuning to very high affinities. Studies in liposomes have shown that cholapods such as **2** transport chloride, nitrate and, to a lesser extent, bicarbonate across bilayer membranes, without cotransport of cations, and probably *via* the shuttle mechanism outlined above.[5,6]

The synthetic nature of the ligands employed is relevant because they can be used readily for two-phase extraction into organic media, as is the case reported herein. A key property of ion shuttles is their affinity for their targets, expressed as the association constant K_a within the membrane [eqn. (1), H = host, $G^{\pm}$ = ionic guest].

$$H + G^{+/-} \rightleftharpoons HG^{+/-} \quad K_a = [HG^{+/-}]/[H][G^{+/-}] \qquad (1)$$

This quantity needs to be regulated such that it is high enough for extraction to take place, but not too high to prevent release. It is, however, non-trivial to measure directly. Most techniques must be applied in the presence of counterions and thus relate to *salts* rather than the isolated ions. Even "non-coordinating" counterions can associate with their partners in non-polar solvents and cause significant effects.[7]

One technique which can give K_a as defined in eqn. (1) is voltammetry at the interface between two immiscible electrolyte solutions (ITIES). Polarisation of the ITIES induces the transfer of charge from one electrolyte phase to the other. The technique can therefore be used to probe the transfer of ions between aqueous and organic phases, facilitated by a receptor initially present in the organic phase. The method has been quite widely used for cation recognition[8] but scarcely for anion binding.[9] In particular, it has not been employed for receptors with the high affinities which (it seems) are required for anion transport. We now report the application of voltammetry at the ITIES to **3**, an exceptionally powerful member of the cholapod family.[4a] The measurements provide new selectivity data and reveal unprecedented affinities to halide anions.

Receptor **3** was dissolved in 1,2-dichloroethane (DCE), the solvent most widely employed for electrochemistry at the ITIES.

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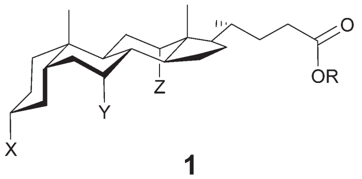

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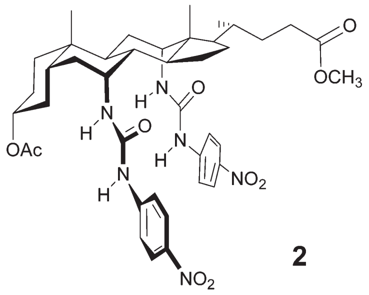
X, Y, Z contain H-bond donors



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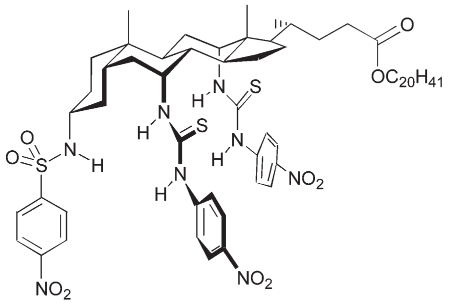

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The initial cell composition can be expressed as:† $Ag_{(s)}|AgTPBCl_{(s)}|0.015$ M BTPPA TPBCl, 1×10^{-3} M **3**, 1×10^{-3} M TRA $TPBCl_{(DCE)}||1$ M K $G_{(aq)}|AgG_{(s)}|Ag_{(s)}$ where the double bar denotes the polarised interface. The silver salt appropriate to the halide (G) was employed as the aqueous phase reference electrode. A tetraalkylammonium tetrakis(4-chlorophenyl)borate salt (TRA TPBCl) was added to the organic phase as an internal reference, against which the half-wave potentials of the facilitated process were measured. In the case of chloride and fluoride, TRA was tetrapropylammonium. For bromide, the transfer of tetrapropylammonium was found to interfere with the facilitated anion transfer, thus TRA represents the more hydrophilic tetraethylammonium in this case.

Fig. 1 shows the response obtained from the above cell in the presence of chloride. Two peaks are visible within the voltammetric window defined by the cell. Significantly, feature **A** towards the right of the potential window, is only observed in the presence of the receptor **3**, and is thus attributed to the facilitated transfer of chloride across the water/DCE interface. The left-hand wave, **B**, is only observed in the presence of tetrapropylammonium, hence is assigned to the transfer of this ion across the ITIES, in agreement with earlier work on its interfacial transfer.[10]

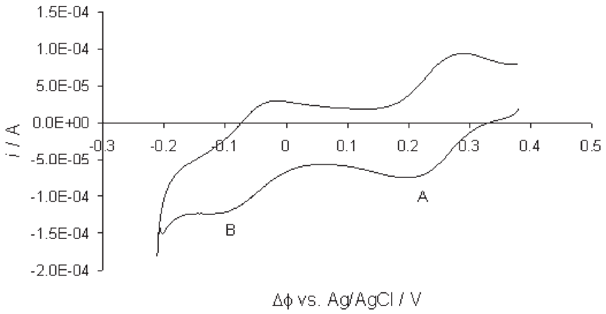


Fig. 1 Voltammetry obtained using cell defined in the text with G = Cl, TRA = tetrapropylammonium and dilution of the aqueous phase to give 0.13 M KCl.

A similar voltammetric response was observed in the presence of fluoride, with feature **A** only being seen on addition of receptor **3**, indicating that the binding of **3** to fluoride is sufficiently strong to extract this extremely hydrophilic ion from the aqueous phase (see Fig. 2). Likewise, the introduction of bromide ions to the aqueous phase gave voltammetric responses given in Fig. 3, which are similar to those of Figs. 1 and 2, implying that facilitated transfer of this ion was also possible. The position of the facilitated transfer wave was found, in each case, to vary as a function of the anion concentration (see Fig. 3).

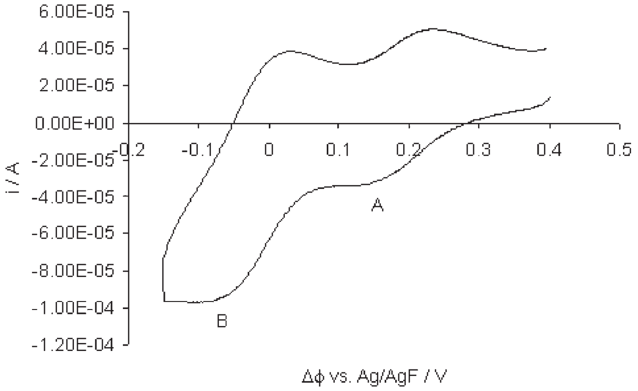


Fig. 2 Voltammetry obtained using cell defined in the text with G = F, TRA = tetrapropylammonium and dilution of the aqueous phase to give 0.13 M KF.

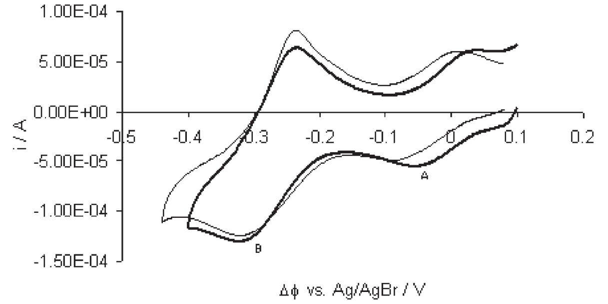


Fig. 3 Voltammetry obtained using cell defined in the text with G = Br, TRA = tetraethylammonium and dilution of the aqueous phase to give 0.60 M KBr (bold line) and 0.22 M KBr (narrow line).

Quantification of binding can be achieved by analysis of the dependence of the voltammetric half-wave potential,[8] $\Delta\phi_G^{1/2}$, which for 1 : 1 stoichiometry should be related to the aqueous phase concentration of the anion, $c_{G,aq}$, by eqn. (2):

$$\Delta\phi_{G}^{1/2} = \Delta\phi_{G}^{o'} - \frac{RT}{zF}\ln(c_{G,aq}K_{a,org}) \qquad (2)$$

Since voltammetric experiments were performed with the halide in excess over the receptor, where $\Delta\phi^{o\prime}_G$ refers to the formal transfer potential of ion G from water to the organic medium, K_a is the association constant in that medium [eqn. (1)]. The other symbols have their usual meanings.

Plots of $\Delta\phi_G^{1/2}$ *versus* the natural logarithm of the concentration of the anions employed are given in Fig. 4. The constants in the above equation imply that a 25 mV/decade gradient should result from the plots of Fig. 4 for halides at room temperature. The experimentally measured values are 24 mV/decade, 28 mV/decade and 25 mV/decade for fluoride, chloride and bromide transfer, respectively. Furthermore, the intercept of the plots depends on the formal transfer potential of the ion and its association constant with **3**. The formal transfer potentials of fluoride, chloride and bromide from water to DCE have been determined previously, from extra-thermodynamic assumptions, to be −0.598 V, −0.529 V and −0.446 V, respectively.[10,11] Normalising the experimental data, by comparing the observed values for transfer of the reference ions with those reported for these ions in the literature,[10,12] leads to values of 1×10^{12} ($\pm 0.5 \times 10^{12}$) M^{-1}, 5×10^{12} ($\pm 2 \times 10^{12}$) M^{-1} and 2×10^{11} ($\pm 0.5 \times 10^{11}$) M^{-1} for the association of **3** with fluoride, chloride and bromide respectively, in DCE.

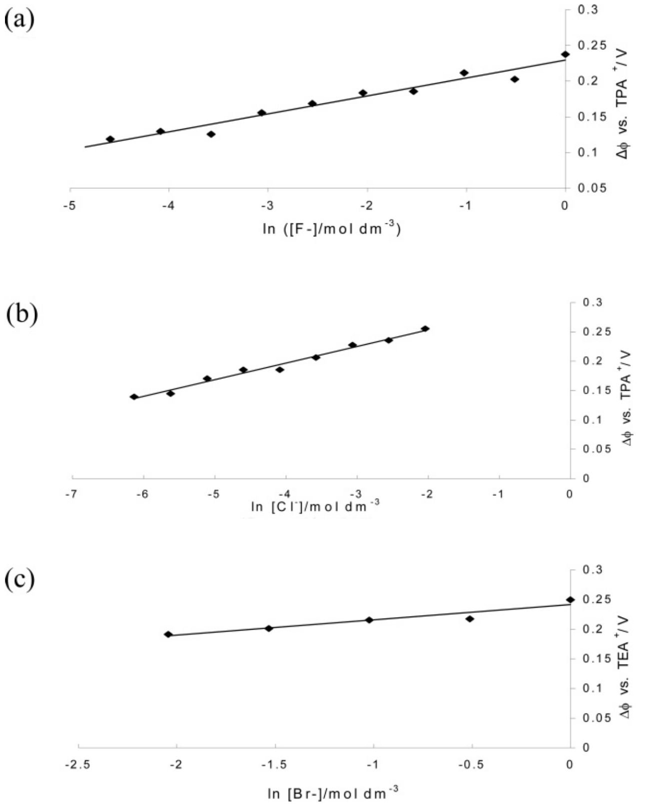
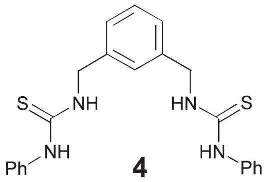


Fig. 4 Plots of observed half-wave potential for anion transfer as a function of the natural logarithm of anion concentration for (a) G = fluoride, (b) G = chloride and (c) G = bromide.

Three conclusions may be drawn from these results. Firstly, the remarkable affinity of **3** for halide anions is confirmed. The above K_a's are even higher than those measured previously for $Et_4N^+Cl^-$ and $Et_4N^+Br^-$ in $CHCl_3$ (10^{11} and 3×10^{10} M^{-1} respectively),[4a] probably due to the decrease in solvent polarity. Indeed, the K_a for chloride is the highest yet reported to an electroneutral receptor. Previous voltammetric studies, on receptors **4–6**, gave values lower by 7–8 orders of magnitude.[9a–c] Secondly, receptor **3** is shown to possess apical size-selectivity, with K_a passing through a maximum at Cl^-. Finally the successful application of voltammetry at ITIES to this high-affinity receptor bodes well for future exploitation in anionophore development.



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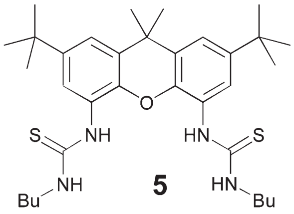

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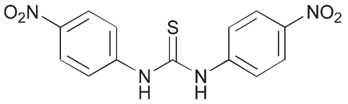

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Notes and references

† G represents the halides employed: fluoride, chloride and bromide. The halide concentrations were repeatedly reduced by 60% using successive dilutions of the aqueous phase with pure water. The organic phase also contained the electrolyte bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate (BTPPA TPBCl). Silver wires oxidised in the appropriate salt were employed as reference electrodes in each phase, with Pt gauzed employed as counter electrodes. The ITIES was polarised with a four-electrode potentiostat (Autolab PGSTAT 30, supplied by Eco-Chemie, Utrecht, Netherlands). The cell was made from glass, with an interfacial area of 0.78 cm^2.

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