DOI: 10.1002/cphc.200800412

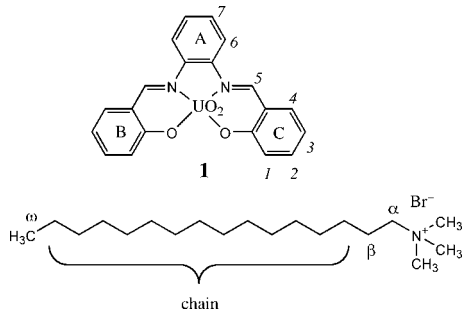
Fluoride Binding in Water: A New Environment for a Known Receptor

Massimo Cametti,*[a, b] Antonella Dalla Cort,[a] and Kristin Bartik[b]

The attention that the scientific community is devoting to the recognition and sensing of the fluoride anion has greatly increased in the last few years. Fluoride has found many uses in various industrial processes and in medical applications, especially in dental care. However, it is nowadays believed that fluoride can have adverse health effects and the advisability of water fluoridation, a process that has been implemented in several countries, is currently a controversial subject.[1] The search for a receptor able to bind fluoride efficiently and selectively in water is consequently a worthwhile albeit challenging task.

Many examples of anion receptors, presenting different design and binding paradigms, and which work in organic solvents or organic–water mixtures, have been reported.[2,3] Nevertheless a last challenge still remains: the vast majority of these receptors fail to confront the unique properties of water. This is especially true for fluoride binding. Apart from hexaprotonated azacryptands,[4] high affinity and selectivity for fluoride are still unattained.

Salophen–UO_2 complexes (Scheme 1) have been extensively studied and are known to bind hard Lewis bases in organic solvents and in the solid state.[5] We envisaged that the hard Lewis acid character of the UO_2 center could suffice for the binding of F^- in an extremely challenging solvent such as water. The UO_2 complex **1** is not soluble in water, but we observed that in the presence of cationic surfactant cetyltrimethylammonium (CTABr) micelles, it dissolves up to millimolar concentrations so that a clear yellow solution is obtained. We report here our study of the anion binding ability of **1** under conditions where the highly ordered CTABr micelles play the role of carrier for the receptor molecules. Structural information pertaining to the system, obtained by NMR relaxivity and NOE measurements, are also presented.



Scheme 1. Chemical formulae and numbering for the Salophen–UO_2 receptor **1** and for CTABr.

UV/Vis spectral changes of **1** in a 50 mM CTABr water solution, observed upon addition of potassium fluoride, are shown in Figure 1. The titration data points were found to fit well to a 1:1 binding isotherm with a binding constant of $10800 \pm 800\ M^{-1}$. This value is, as far as we know, the highest ever recorded for fluoride by a neutral receptor in water. A similar well-behaving 1:1 binding isotherm fits the experimental data for sulphate, acetate and phosphate for which binding constants of 140 ± 20, 260 ± 16 and $3100 \pm 170\ M^{-1}$, respectively, were obtained (Supporting Information).[6] These are apparent affinity constants as, in the absence of titrants, the fifth equatorial coordination site of the UO_2 center, where the binding with the guest anion occurs, is usually occupied by a solvent molecule. However, with a large excess of Br^- counterion present (50 mM) it cannot be excluded that, prior to the addition of a guest anion, Br^- is coordinated to the UO_2 center instead of a water molecule. No binding was detected with Cl^- and I^-, and NO_3^-.

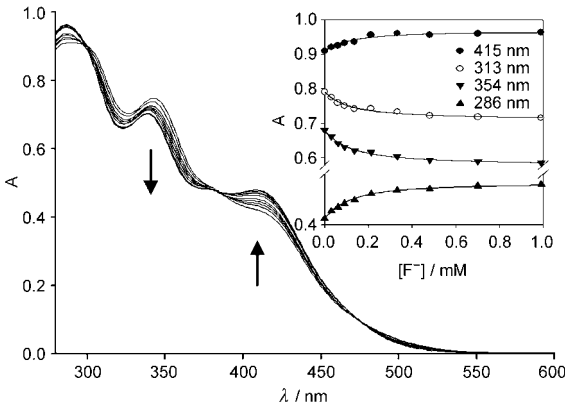


Figure 1. UV/Vis spectral changes of a 4×10^{-5} M solution of 1 (H_2O, 50 mM CTABr, 25 °C) upon addition of KF. The inset shows the spectral changes at selected wavelengths, lines represent the parametric adjustment of a 1:1 binding isotherm to the experimental data points.

The large affinity for fluoride found under these conditions is remarkable, especially if we consider that in MeOH, in which **1** is soluble, this binding constant drops to $360 \pm 20\ M^{-1}$ (Supporting Information). Had the receptor been soluble in pure water, an even less efficient binding could have been expect-

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cphc.200800412.

ed. This indicates that the micellar environment has an influence, not only on the solubility of **1**, but also on its affinity for anions. This situation may be reminiscent of what happens in proteins, where the binding site is frequently buried within a hydrophobic pocket in the protein structure, thus providing a less challenging environment for binding than bulk water. For these reasons, the location of the UO_2 complex within the micellar system is of particular interest. The micellar environment should provide **1** with a suitable location in which it can diminish its interaction with the bulk water and become soluble, but at the same time, the receptor must have access to the target anions in the bulk. Indeed, the permeability of the micelles to fluoride must be very low, and it is unlikely that a deeply buried receptor could be accessible to F^-.

Table 1. Relaxivity values (Φ, $mM^{-1}s^{-1}$)[a] for the CTABr protons derived from T_1 and $\Delta\nu_{1/2}$ measurements using paramagnetic $[Cr^{III}(CN)_6]^{3-}$.

		$N(CH_3)_3$	α-CH_2	β-CH_2	ω-CH_3
$\Phi(T_1)$	CTABr	57 ± 4	57 ± 3	40 ± 1	3.4 ± 0.2
	1·CTABr	77 ± 8	63 ± 2	45 ± 1	3.2 ± 0.1
$\Phi(\Delta\nu_{1/2})/\pi$	CTABr	103 ± 7	81 ± 6	57 ± 3	10 ± 6
	1·CTABr	162 ± 8	141 ± 7	100 ± 15	10 ± 6

[a] Average of three repetitions, [CTABr] = 50 mM, [**1**] = 1–1.5 mM.

Table 2. Relaxivity values (Φ, $mM^{-1}s^{-1}$)[a] for the receptor 1 protons derived from T_1 and $\Delta\nu_{1/2}$ measurements using paramagnetic $[Cr^{III}(CN)_6]^{3-}$.

	H1	H2	H3	H4	H5	H6	H7
$\Phi(T_1)$	120 ± 4	57 ± 2	27.2 ± 0.2	13.6 ± 0.2	9.7 ± 0.2	10.0 ± 0.1	8.2 ± 0.1
$\Phi(\Delta\nu_{1/2})/\pi$	160 ± 6	120 ± 8	56 ± 2	39 ± 2	50 ± 1.5	30 ± 2	24 ± 2

[a] Average of three repetitions, [CTABr] = 50 mM, [**1**] = 1–1.5 mM.

Structural information pertaining to the spatial relationship between the micelle and the UO_2–salophen receptor **1** can be obtained from NMR relaxivity and Nuclear Overhauser Effect (NOE) measurements. The longitudinal and transverse relaxation rates ($1/T_1$ and $1/T_2$, respectively) of the nuclei of a molecular system can be enhanced by the presence of a paramagnetic species. The paramagnetic relaxation enhancement (PRE) effect is inversely proportional to the sixth power of the unpaired electron–nuclei distance (r^{-6}) and is proportional to the concentration of the paramagnetic species.[7a,b] Quantitative information can be derived from the relaxivity, Φ (expressed in $mM^{-1}s^{-1}$), which is the slope of the dependence of the relaxation rates on the concentration of the paramagnetic species.[7c–e] We measured the relaxation time T_1 using the inversion recovery pulse sequence; transverse relaxivity data were obtained by monitoring the width of the signals at half height, $\Delta\nu_{1/2}$. Experiments were run at non-buffered neutral pH, using $K_3[Cr^{III}(CN)_6]$ as the paramagnetic species.[8]

The spherically ordered surfactant monomers, which form CTABr micelles, expose their most hydrophilic part to the bulk water. The Cr^{III} ion confined in the water environment should enhance the relaxation rates of the CTABr nuclei closer to the bulk to a greater extent. Consequently, we expect the PRE effect to decrease in the following manner: $(CH_3)_3N > \alpha > \beta >$ chain $> \omega$-Me. The comparison of the Φ values obtained for the receptor protons (see the Supporting Information for assignment using a COSY spectrum) with those obtained for the CTABr protons should yield information on the location of the receptor in the micellar system. Given that the reported micellar aggregation number for 50 mM CTABr is 145,[9] a concentration of **1** in the 1–1.5 mM range corresponds to an approximate 3–4:1 receptor-to-micelle ratio.

The plots of the paramagnetic contribution to the longitudinal relaxation rate of the different protons of the CTABr and **1**·CTABr systems, as a function of the Cr^{III} salt concentration, all show the expected linear behaviour (Supporting Information). The longitudinal relaxivities $\Phi(T_1)$ derived from these plots are reported in Tables 1 and 2. The CTABr relaxivities follow the expected trend. The polar head methyls and the α-methylene signals possess the same value. The relaxivity becomes smaller for the β-protons and it is more than an order of magnitude smaller for the ω-methyl (Table 1). The presence of receptor **1** has the effect of increasing, and differentiating the slopes for the $N(CH_3)_3$ and α-protons. The relaxivity of the β signal is also slightly increased, while the value is unaltered for the ω-methyl. The $\Phi(T_1)$ values for the protons of **1** decrease in the following order: H1 > H2 > H3 > H4 > H5,H6 > H7 (Table 2). H1 and H2 are the most affected signals, with a clear predominance of H1 over H2, while, from H3 on, the effect fades away.

The plots of $\Delta\nu_{1/2}$ of the CTABr (50 mM) proton signals versus the concentration of added Cr^{III} salt all show the expected linear behaviour (Supporting Information). The $N(CH_3)_3$ protons exhibit the highest $\Phi(\Delta\nu_{1/2})/\pi$ value and the $\Phi(\Delta\nu_{1/2})/\pi$ values of the other signals decrease in the expected order: $\alpha > \beta > \omega$-Me (Table 1).[10] When **1** is present, as with the $\Phi(T_1)$ measurements, significantly higher values are observed for the protons at the interface [the $(NCH_3)_3$ methyls and α and β protons] than in the absence of **1** (Supporting Information). The effect of the Cr^{III} salt on the $\Delta\nu_{1/2}$ of the receptor proton signals is clearly visible in the spectra reported in Figure 2. A greater progressive broadening of the H1 and H2 protons of the receptor, corresponding to the highest $\Phi(\Delta\nu_{1/2})/\pi$ values, is clearly noticeable. The $\Phi(\Delta\nu_{1/2})/\pi$ values for the protons of **1** are reported in Table 2 and follow the same general trend observed with the $\Phi(T_1)$ values, namely H3 > H5 > H4 > H6 > H7. These PRE measurements allow us to picture receptor **1** as adopting a preferential position at the interface of the micellar system, with its H1 and H2 protons facing the aqueous bulk and ring A pointing towards the interior. The fact that the Φ values for the CTABr polar head methyls and the α and β methylenes protons are 1.5–2 times higher in the presence of **1** than in its absence deserves a comment. We speculate that the packing of the surfactant molecules is less compact at the interface in the presence of the salophen–UO_2 complex, easing the access of the paramagnetic ion deeper into the micelle.

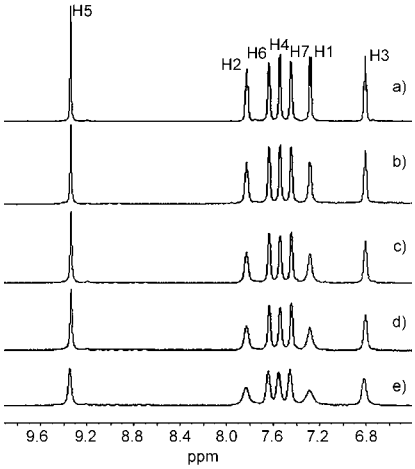

Figure 2. a) 600 MHz ^{1}H NMR spectrum of a 1.5 mM solution of **1** in water in the presence of 50 mM CTABr, and b–e) spectra of **1** upon addition of increasing amounts of $K_3[Cr^{III}(CN)_6]$ (0–0.1 mM).

NOE experiments[11] were undertaken on the **1**·CTABr system. The NOE effects observed for the receptor protons upon inversion of three different proton signals of the CTABr micelle are shown in Figure 3.[12] When the signals of the CTABr methyls

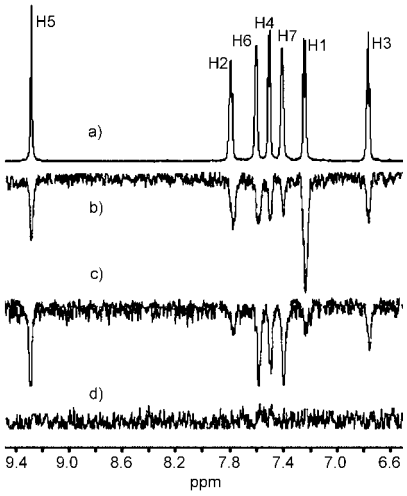

Figure 3. a) 600 MHz ^{1}H NMR spectrum of a 1.5 mM solution of **1** in water in the presence of 50 mM CTABr; NOE effect on the receptor proton signals when inverting b) the $N(CH_3)_3$, c) the chain and d) the ω-methyl signals of CTABr.

exposed to the bulk are inverted (Figure 3b) the most affected receptor signal is that of the H1 proton. A different pattern of enhancements is observed when the signal corresponding to the chain protons is inverted (Figure 3c). In this case the most affected signals are those of the H4, H6 and H7 protons. The signals of the H5 and H3 protons show a NOE in both experiments. No NOE is detected when the terminal ω-methyl is inverted (Figure 3d). The NOE and PRE experiments give complementary information which match satisfactorily.

The receptor molecule probably maintains a certain degree of freedom within the micelle. Consequently, the observed Φ values and NOE correspond to an average for the different environments which the receptor experiences. Since there is only one set of signals for **1** in the NMR spectrum, the exchange dynamics must be fast with respect to the chemical shift time scale. However, from the data collected, it is apparent that receptor **1** has a preferential position and orientation.

In conclusion, the work presented herein demonstrates that, in the presence of CTABr micelles, receptor **1** becomes soluble in water and it is able to bind fluoride with the highest affinity ever reported to date for a neutral receptor. The salophen–UO_2 complex has a preferential location and orientation at the micelle–water interface with its H1 and H2 protons pointing towards the bulk water, as highlighted by NOE and PRE experiments. We feel that the protocol followed for this investigation can be usefully applied to other receptors not soluble in water which exhibit high affinity, in organic solvents, for fluoride or other anionic species. We are currently working on the design of new salophen–UO_2/micelle systems with enhanced selectivity for fluoride.

Experimental Section

CTABr, TBAF, KF, KOAc, KH_2PO_4, KNO_3, K_2SO_4 and $K_3[Cr^{III}(CN)_6]$ were Aldrich analytical grade and used with no further purification. Receptor **1** was available from a previous investigation.[5b] The **1**·CTABr samples were prepared in the following manner: 5–10 mg of **1** were added to a 50 mM CTABr solution at room temperature. The solution, which turned yellow after a few minutes (thus confirming the solubilization of **1)** was maintained under stirring for 30 min. The solution was then filtered through Acrodisc filters (0.2 μm, Nylon membrane). For NMR experiments Aldrich D_2O 99.98% was used. For UV/Vis experiments Fluka UV spectroscopy water was used.

All the NMR experiments were performed on a 600 MHz Varian spectrometer. For all the experiments fresh solutions were prepared. Longitudinal relaxation times, T_1, at different concentrations of $K_3[Cr^{III}(CN)_6]$ were measured using the standard inversion recovery method and data were processed using the Varian software. The diamagnetic and paramagnetic contribution to the relaxation rates are additive in nature. $\Phi(T_1)$ relaxivity plots ($1/T_1$p versus Cr^{III} concentration) for each proton were fitted by a one-parameter linear equation whose slope corresponds to the relaxivity $\Phi(T_1)$ value [Eq. (1)]:

$$\left(\frac{1}{T_1}\right)_p = \left(\frac{1}{T_1}\right)_{obs} - \left(\frac{1}{T_1}\right)_d = (T_1)[Cr^{III}] \quad (1)$$

Transverse relaxation times T_2 were derived form the width of the signals at half height, $\Delta\nu_{1/2}$, as shown in Equation (2):

$$\Delta\nu_{1/2} = \left(\frac{1}{\pi T_2}\right) \quad (2)$$

These were measured by Lorentzian line deconvolution analysis of the ^{1}H NMR spectra applying a LB factor of 0.2 for receptor signals and 10 for CTABr signals. The large LB factor used for the micelle signals is necessary to avoid the analysis of the complex α-CH_2, and β-CH_2 signals. The terminal ω-methyl is a well-defined triplet. In this case no significant difference was found when applying the deconvolution analysis and taking into account the multiplicity of

the signals (LB = 0.2) or when simply considering a single broad line (LB = 10).

The $\Phi(\Delta\nu_{1/2})$ relaxivity plots were fitted by a two-parameter linear equation whose slope correspond to the relaxivity $\Phi(\Delta\nu_{1/2})$ value as shown in Equation (3). No subtraction of the LB factor from the measured $\Delta\nu_{1/2}$ was performed.

$$\Delta\nu_{1/2} = \left(\frac{1}{\pi T_2}\right)_{obs} = \left(\frac{1}{\pi T_2}\right)_{d} + \left(\frac{1}{\pi T_2}\right)_{p} = \left(\frac{1}{\pi T_2}\right)_{d} + \frac{(\Delta\nu_{1/2})}{\pi}[Cr^{III}] \quad (3)$$

NOE experiments were run using a double-pulsed-field-gradient-spin-echo (DPFGSE) pulse sequence.[13] Selective inversion was achieved by using a *iburp2* pulse shape.

All UV/Vis titrations were carried out at 25 °C in non-buffered solution using a Perkin–Elmer Lambda 35 spectrometer. No correction for acid–base equilibria of the anions was made. In a typical experiment small aliquots of fresh, concentrated solution of the potassium salt of a given anion (F^-, AcO^-, $H_2PO_4^-$, NO_3^-, SO_4^{2-}, Cl^- and I^-) in water in the presence of 50 mM CTABr were added directly in the UV/Vis cuvette to 2.1 mL of 50 mM CTABr solution containing receptor **1** ([**1**] = 0.2–1 × 10^{-4} M). After each addition the UV/Vis spectrum was recorded and the absorbance was corrected for the small dilution.

See the Supporting Information for UV/Vis titration plots, for the COSY spectrum of **1**·CTABr and for the $\Phi(T_1)$ and $\Phi(\Delta\nu_{1/2})$ plots.

Acknowledgements

The authors thank the "Communauté française de Belgique" (ARC 2002-7 n° 286 and 289) and ULB Bureaux des Relations Internationales. M.C. thanks COST for a STSM. We thank Prof. L. Mandolini for discussions and Prof. M. Luhmer for the help to conduct the NOE measurements.

Keywords: fluorides · micelles · NMR spectroscopy · paramagnetic relaxation enhancement (pre) · salophen-UO_2

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[8] In the concentration range used (< 0.2 mM), the Cr^{III} salt does not interact directly with CTABr micelles, nor with **1**, as proved by the absence of any changes in the chemical shift of the CTABr ^{1}H-signals in water and of the receptor ^{1}H-signals in a DMSO–water 1:1 mixture.

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[10] The water signal is not much affected by the presence of Cr^{III}, and has a $\Phi(\Delta\nu_{1/2})/\pi$ value in the range of 5–10 $mM^{-1}s^{-1}$, whether CTABr is present or not.

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Received: July 2, 2008
Published online on ■■ ■■, 2008

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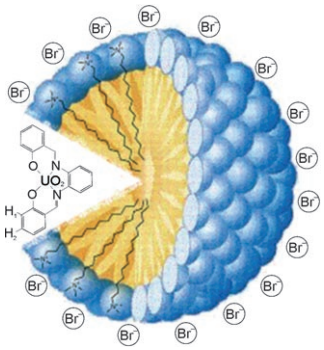
Binding in a micelle: In the presence of CTABr micelles (see picture), the salophen–UO_2 complex **1** binds fluoride in water with the highest affinity ever recorded for a neutral receptor ($K \approx$ ca. $10^4\ M^{-1}$). The receptor's location and its orientation within the micellar system are determined by PRE and NOE NMR experiments.



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