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A cobalt(II) complex with unique paraSHIFT responses to anions†

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ParaSHIFT agents have shown promise in detecting chemical targets in biological systems by magnetic resonance, but few studies have used transition metal complexes for this purpose. Here we report our investigations into $CoMe_6$trenCl (tren = tris(2-aminoethyl)amine) as a paraSHIFT agent. The paramagnetic region of the ^{1}H NMR spectrum shows characteristic spectral profiles in the presence of fluoride, acetate, lactate and citrate in aqueous solution. These distinctive NMR shifts of each anion are maintained even in mixtures of anions.

The focus of medical research has shifted from primarily questions of structure to those of chemistry, and there is therefore a need for imaging techniques that report on the chemical environments of tissues and cells. Magnetic resonance imaging (MRI) is one of the most powerful imaging techniques, offering particularly high spatial resolution, and recent research efforts have therefore focussed on MRI protocols that can provide molecular information as well as structural. This has precipitated the development of responsive MRI contrast agents, which change the relaxation of the bulk water protons upon chemical stimuli.[1,2] Such systems are limited, however, by the concentration dependence of the response, due to the high backgrounds observed arising from both the intrinsic properties of tissue and in particular the residual relaxation effect of the contrast agent even in its inactivated form.[3,4]

We and others have addressed this challenge by utilising transition metals that can be converted from diamagnetic to paramagnetic forms upon external stimulus. For example, in Fe(II) systems the switch from the low spin diamagnetic to high spin paramagnetic form was achieved upon ligand-centred chemical reduction or enzymatic activity,[5–7] while we have shown that the biologically-tuned reduction of Co(III) to Co(II) gives similar off-to-on effects.[8] Another solution has been to use the same diamagnetic to paramagnetic switch to alter the readily-exchangeable protons of transition metal complexes, which can transfer the relaxation to the bulk water by a mechanism termed paramagnetic chemical exchange saturation transfer (paraCEST).[9] This has been utilised in a variety of transition metal complexes, including Fe(II),[10,11] Ni(II),[12] and cobalt,[13] with the main challenge being the intrinsically low "per-molecule" sensitivity of these agents.[14–16]

An alternative approach is to use magnetic resonance spectroscopy (MRS), which observes resonances other than the bulk water signal. In order to avoid the background from the protons of water and fat, the observed shift must be distinct from the endogenous diamagnetic region of ^{1}H NMR.[17] This can be achieved by probing a nucleus that is not typically present in biological systems, such as ^{19}F. Responsive agents of this type have been prepared by incorporating this nuclide in paramagnetic systems.[18–21] Clinical application of these agents may be limited by sensitivity challenges, and the less routine availability of detection coils suitable for ^{19}F.[17] Alternatively, to achieve improved sensitivity and minimised background signal, and compatibility with existing MRI instrumentation, a strategy involving paramagnetically-shifted ^{1}H signals (paraSHIFT) can be employed. By this method, pH and/or temperature-responsive complexes of a number of lanthanoids, including ytterbium,[22,23] praseodymium,[24] thulium,[25] and dysprosium[17] have been developed and their utility in biological systems has been confirmed, with the detection limit as low as tens of μM.[26]

While lanthanoid complexes predominate literature on shift agents, it has long been known that first row transition metal ions can be applied to paramagnetic ^{1}H NMR spectroscopy.[27,28] However, transition metal complexes for paraSHIFT imaging applications have been reported only recently, with Fe(II) and Co(II) systems utilised as temperature sensors.[29] In line with our interest in cobalt systems, we sought to identify a scaffold that could sense a chemical target. We report here our observations of the differential response of paramagnetically-shifted protons on a cobalt complex upon binding to different anions.

In our previous studies of cobalt-tris-2-pyridylmethylamine systems, we observed a large paramagnetic shift of almost 100 ppm for some ligand protons.[8] In order to maximise this

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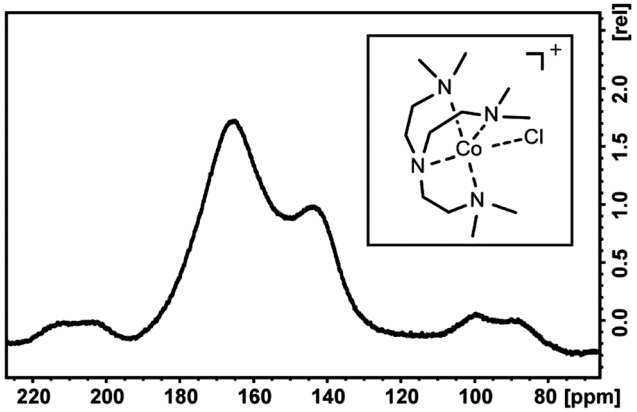

Fig. 1 Paramagnetic ^{1}H NMR region of CoMe$_6$TrenCl (10 mM, structure inset) in D_2O at 9.4 T.

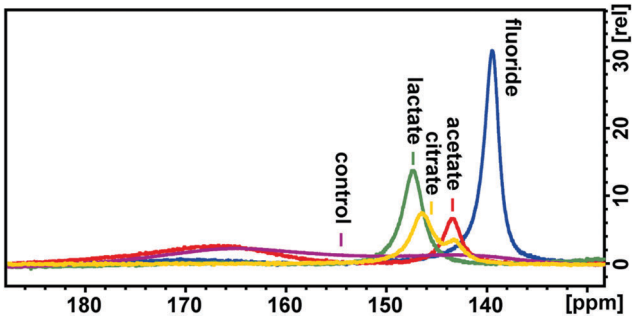

Fig. 2 Paramagnetic ^{1}H NMR of CoMe$_6$TrenCl (1 mM) upon addition of various anions (lactate, citrate, acetate and fluoride) that induce unique chemical shifts of the methyl protons of CoMe$_6$TrenCl (H_2O : D_2O 90 : 10, pH 6.5, 9.4 T).

signal, we sought a ligand bearing a large number of equivalent protons. Cobalt(II) complexes of tris(2-aminoethyl)amine (tren) have previously been widely studied.[30–32] We identified the hexa-*N*-methyl analogue of this system (CoMe$_6$TrenCl) (Fig. 1 inset) as an appropriate model complex, as it has up to 18 equivalent protons. This complex has previously been studied for its photophysical and binding properties, and has demonstrated potential utility as an atom-transfer radical-polymerisation (ATRP) radical mediator[33] and single molecule magnet.[34] In addition, the observed effects of different anions on the ligand field of the Co(II) centre in the solid state indicated that this complex may be responsive to anions in solution.[30]

We prepared CoMe$_6$TrenCl following a reported procedure (Scheme S1, ESI†).[35] ^{1}H NMR spectroscopy of the complex revealed the presence of significantly-shifted broad proton signals at 180–130 ppm (Fig. 1). In order to identify the origin of these main signals, we prepared the (d_3-Me)$_6$Tren ligand and complex. We observed the clear loss of the signals in the 180–130 ppm region compared to the non-deuterated analogue prepared by the same synthetic method (Fig. S1, ESI†), confirming that these signals arise from the methyl groups.

Given the previously-reported interactions of the complex with anions,[30] we sought to investigate the effects of various anions on the paramagnetic region. Interestingly, we observed a distinctive spectral profile within the 180–130 ppm region for each added anion (Fig. 2), indicating that the paramagnetic environment of the methyl groups is diagnostic of the anion bound.

Of the anions tested, fluoride showed the sharpest and most intense signal, which increased upon the gradual addition of the anion (Fig. 3a). By monitoring peak intensity (Fig. 3b), we were able to establish a weak 1 : 1 binding (29 ± 7 M^{-1}). We could also monitor the ratio of the peaks at 140 and 165 ppm, which demonstrates a linear dependence on fluoride concentration up to 40 mM (Fig. S2, ESI†). Importantly, this correlation is independent of the cobalt concentration, enabling the quantification of F^- in unknown samples. This binding affinity was consistent with that obtained from absorption spectroscopy, in which fluoride addition led to increases in the peaks at 455 and 635 nm, and a concomitant decrease at 605 nm (Fig. 3c and Fig. S3, ESI†).

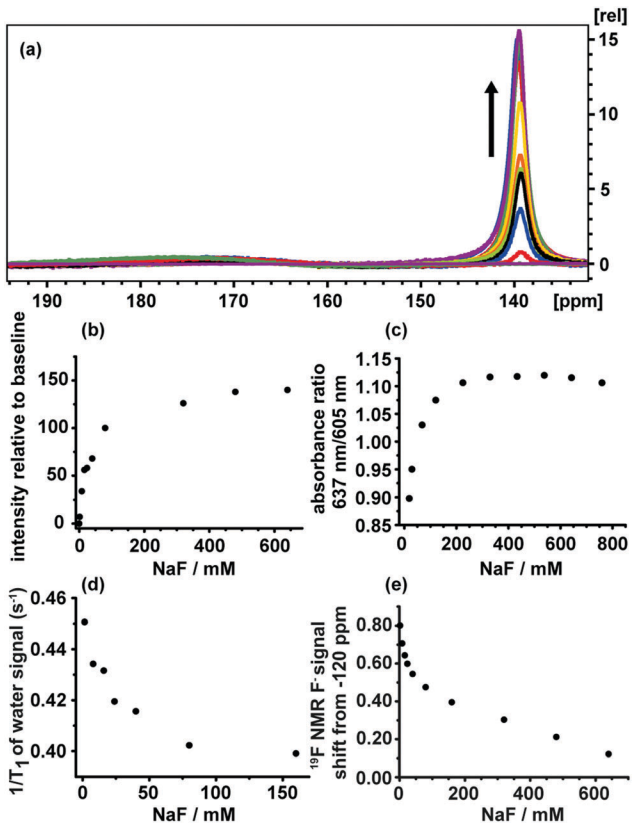

Fig. 3 Investigations of the interaction of NaF with CoMe$_6$Tren (H_2O : D_2O 90 : 10, pH 6.5, 9.4 T). (a and b) Sodium fluoride binding affinity determination by paramagnetic ^{1}H NMR (1 mM CoMe$_6$Tren), (c) UV-Visible absorption ratio between peak intensities at 637 nm and 605 nm of CoMe$_6$Tren (5 mM), (d) water longitudinal relaxation rate by inversion recovery sequences in the presence of CoMe$_6$Tren (1 mM), and (e) ^{19}F NMR shift of NaF with varying [NaF] and fixed CoMe$_6$Tren (1 mM), relative to NaF signal at −120.05 ppm without CoMe$_6$Tren.

An alternative method of studying binding events to a paramagnetic centre, which has been extensively utilised to investigate metalloprotein–ligand interactions, is to monitor the T_1 relaxation of the bulk water signal.[36] In our case, the relaxivity decreased upon the addition of F^-, consistent with a lower accessibility of water molecules to the paramagnetic metal

Table 1 Association binding constants of fluoride to $CoMe_6TrenCl$ calculated by different methods

Method	K_a (M^{-1})
T_1	31 ± 6
UV	30 ± 4
pNMR intensity	29 ± 7
^{19}F NMR	34 ± 5

centre (Fig. 3d). We were also able to monitor the binding event by observing the chemical shift of the fluoride anion in ^{19}F NMR (Fig. 3e and Fig. S4, ESI†). The presence of $CoMe_6TrenCl$ caused a downfield shift in the ^{19}F NMR spectrum relative to F^- alone. This peak then shifted upfield with subsequent addition of excess fluoride. The binding affinities calculated for all of these methods were in agreement, within error (Table 1). In terms of sensitivity, it is possible to detect 1 mM F^- using a 1 mM solution of cobalt complex, with a total experiment time of approximately 1 minute (signal : noise ratio >4; Fig. S5a, ESI†).

We then investigated the binding properties of the complex towards lactate, acetate and citrate (Fig. 4 and Fig. S6, ESI†). Acetate showed similarly weak binding to fluoride, with lactate an order of magnitude weaker. By contrast, the binding affinity of citrate is markedly tighter, and its spectral form includes two signals at shifts consistent with those of both lactate and acetate-bound forms (Fig. 2). Taken together, this might indicate a cooperative mode of binding for citrate.

As for fluoride, the sensitivity of the complex is sufficient to detect, within 1–20 min, 1 mM of these anions (signal : noise ratio >4), which correspond to the reported levels of lactate,[37] acetate[38] and citrate[39] in biological fluids (Fig. S5b–d, ESI†). Significantly, citrate, which shows the tightest binding of the three offers the best signal : noise ratio (>9), and requires the shortest experimental acquisition time (approximately 1 minute; Fig. S5d, ESI†).

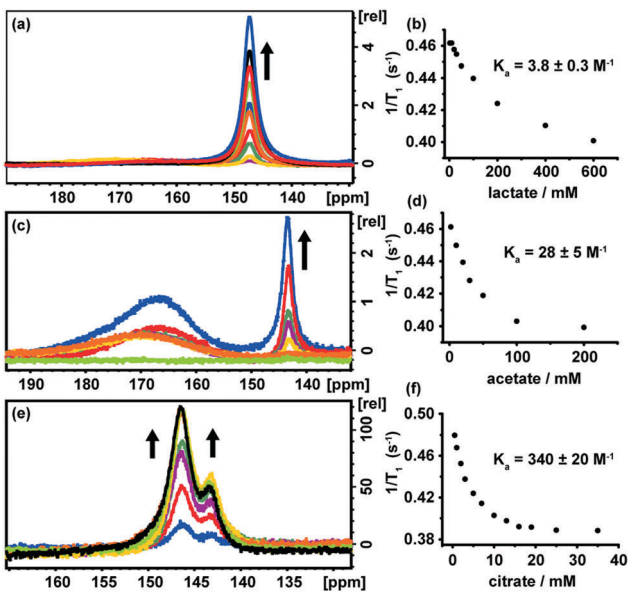

Fig. 4 Paramagnetic ^1H-NMR spectra and the results of corresponding longitudinal relaxation studies of $CoMe_6trenCl$ (1 mM) with the addition of lactate (a and b), acetate (c and d), and citrate (e and f), (H_2O : D_2O 90 : 10, pH 6.5, 9.4 T).

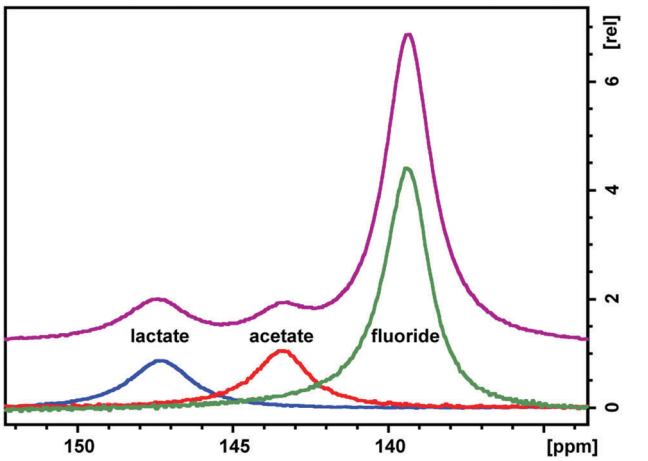

Fig. 5 Paramagnetic region of ^1H NMR spectrum of $CoMe_6Tren$ (1 mM, H_2O : D_2O 90 : 10, pH 6.5, 9.4 T) in the presence of a 1 : 1 : 1 mixture of lactate, acetate and fluoride (50 mM; purple). Spectra of the complex in the presence of single anions (50 mM) are also shown (blue – lactate, red – acetate and green – fluoride).

Competition studies with fluoride and lactate show that lactate can be displaced upon fluoride addition (Fig. S7, ESI†), confirming the reversibility of binding. Interestingly, even in mixtures of anions, the unique signal for each anion is preserved, rather than an average signal (Fig. 5). These results demonstrate that this system is uniquely poised to simultaneously detect several different anions in mixtures.

In summary, we have demonstrated that the model paramagnetic $CoMe_6TrenCl$ complex can be used to report on the presence of a variety of anions in aqueous solutions by observing the distinct chemical shift of the *N*-Me protons. Significantly, this system contributes an alternative method for fluoride detection, currently predominated by irreversible reaction-based probes.[40] Furthermore, the unique spectral fingerprint for each individual anion opens up the possibility for "multicolour" magnetic resonance. Future investigation into the detailed mechanism of binding for each anion will enable the rational design of novel scaffolds with varied selectivities and binding affinities towards applications in environmental and biological imaging.

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