Paramagnetic complexes as novel NMR pH indicators

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The remarkable pH dependence of the chemical shift separation (7.0 ± 0.1 ppm/pH unit) observed between the outer ^{1}H NMR resonances on the spectrum of the paramagnetic complex [Yb(dotp)]$^{5-}$ [H_8dotp = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetrakis-(methylenephosphonic acid)] in the pH range 5.0–7.5 at 39 °C enables this compound to be considered as a potential NMR probe for *in vivo* pH measurements.

Accurate measurements of pH *in vivo* still represents an issue of importance since it has been recognized that important pathologies, such as tumour and ischemic lesions, may be accompanied by changes in this parameter with respect to the surrounding normal regions.[1] Several NMR procedures have been devised to this purpose. For instance, intracellular pH may be easily determined by measuring the ^{31}P NMR chemical shift of the endogenous inorganic phosphate,[2] whereas extracellular pH monitoring requires the addition of a suitable exogenous probe. To this regard, Frenzel *et al.*[3] proposed the use of a fluorine containing compound 3-[*N*-(4-fluoro-2-trifluoromethylphenyl)sulfamonyl]propionic acid the ^{19}F NMR spectrum of which is characterized by a significant pH dependence of the chemical shift difference between the trifluoromethyl and the aromatic *p*-F signals with a sensitivity of 5.3 ppm (pH)$^{-1}$ and a measuring range between pH 6.6 and 8.0. Another pH-sensitive indicator is provided by 3-aminopropyl phosphonate, for which the ^{31}P NMR signal has titration end points of δ 24.32 ± 0.01 (acid) and δ 21.10 ± 0.01 (base).[4]

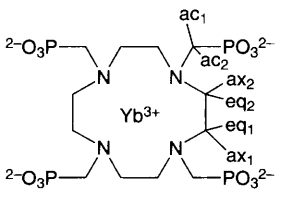
It is known that paramagnetic complexes of transition metals or lanthanide(III) ions having short electronic relaxation times (*ca.* 10^{-11} to 10^{-13} s) are able to induce large paramagnetic shifts of nearby nuclei.[5] We found that these compounds may represent excellent NMR pH indicators provided that they contain functionalities whose pK_a values fall in the pH range of interest. In fact, the NMR resonances of a suitable paramagnetic complex cover a much wider chemical shift region than those of the corresponding diamagnetic systems and hence they are much more sensitive to subtle structural and electronic variations.

As a representative example of this class of compounds we report here the results obtained with the ytterbium(III) complex of the macrocyclic ligand H_8dotp [1,4,7,10- tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetrakis(methylenephos- phonic acid)].[6]

The corresponding gadolinium(III) complex has been studied in detail[7,8] for its remarkable relaxation properties as a potential MRI contrast agent, whereas the analogous thulium(III) and dysprosium(III) derivatives have been used as shift reagents to differentiate extra- and intra-cellular signals of NMR active cations of biological importance in a number of *in vitro* and *in vivo* applications.[6,9] H_8dotp acts as an octadentante ligand towards lanthanide(III) ions by utilizing the four nitrogen atoms of the macrocyclic ring and four oxygen atoms of the pendant methylenephosphonate groups. At ambient temperature the lanthanide(III) complexes are stereochemically rigid on the NMR timescale, and display D_4 symmetry and show a single resonance in their ^{31}P NMR spectra, three equally intense methylenic (two for the macrocycle and one for the pendant arms) resonances in the ^{13}C NMR spectra and six signals of equal intensity (four for the ethylenic ring protons and two for the methylenic phosphonate arms) in the proton NMR spectra.[10] At 39 °C and pH = 8.1, the ^{1}H NMR spectrum† of [Yb(dotp)]$^{5-}$ (Fig. 1) covers a spectral region of *ca.* 140 ppm and the resonances show different linewidths according to the different distances of the corresponding protons from the metal centre. The assignment of the peaks has been carried out by comparison with the spectrum of [Yb(dota)]$^-$ (H_4dota = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid)[11] and confirmed by a quantitative analysis of the dipolar shifts.[10] At basic pH values there are five residual negative charges on the complex, located on the eight uncoordinated oxygen atoms of the phosphonate groups, which progressively decrease when the pH of the solution is lowered. The stepwise addition of H$^+$ ions causes large changes in the chemical shift of all the resonances.‡ From the NMR titration curve relating to proton ac_1 we calculated the following pK_a values:

$$[Yb(Hdotp)]^{4-} \rightleftarrows [Yb(dotp)]^{5-} + H^+ \quad pK_{a4} = 7.76$$
$$[Yb(H_2dotp)]^{3-} \rightleftarrows [Yb(Hdotp)]^{4-} + H^+ \quad pK_{a3} = 6.56$$
$$[Yb(H_3dotp)]^{2-} \rightleftarrows [Yb(H_2dotp)]^{3-} + H^+ \quad pK_{a2} = 5.33$$
$$[Yb(H_4dotp)]^{-} \rightleftarrows [Yb(H_3dotp)]^{2-} + H^+ \quad pK_{a1} = 3.86$$

In the aim of providing an accurate pH sensitive probe for *in vivo* applications, it is advantageous to simply consider the



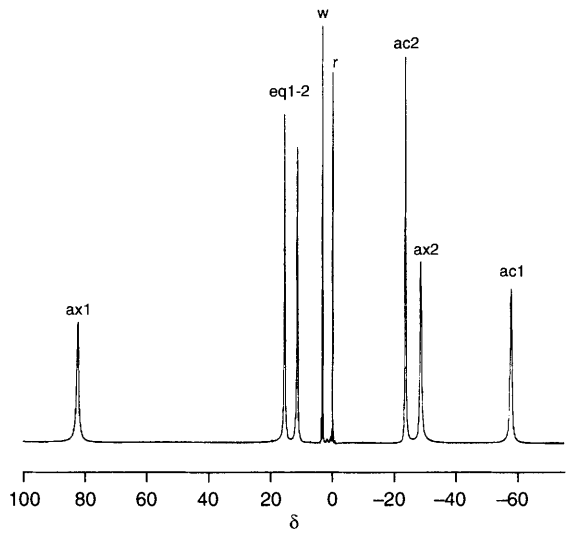


Fig. 1 90 MHz ^{1}H NMR spectrum of Na_5[Yb(dotp)] (25 mmol dm^{-3}) in D_2O at 39 °C and pH 7.1. The resonances indicated by w and r refer to the solvent and *tert*-butyl alcohol (1% internal reference; δ = 0), respectively.

chemical shift separation between a selected pair of resonances rather than to reference the position of a given peak to a pH-independent absorption. For instance, the dependence of ($\delta_{ax1} - \delta_{ae1}$) with pH appears to be linear (R = 0.999) in the range pH 5.0–7.5, with a slope of 7.0 ± 0.1 ppm $(pH)^{-1}$ (Fig. 2). As far as '*in vivo*' applications are concerned, potential sources of error in the pH determination have to be carefully checked. These can be basically related to a chemical shift dependence from: (*i*) local concentration of the complex, (*ii*) ionic strength and (*iii*) temperature. The value of ($\delta_{ax1} - \delta_{ac1}$) was found to be constant within the limits of the experimental error (±0.15 ppm), for solutions of Na_5[Yb(dotp)] in the concentration range 5.0–80 mmol dm^{-3} at 39 °C and pH = 7.4. Thus, changes in the concentration of the paramagnetic probe appear to have a negligible effect on the observed ^{1}H shifts. Next, we checked the dependence of the proton shifts upon the interactions with cations by measuring (at 39 °C and pH 8.0), ($\delta_{ax1} - \delta_{ac1}$) as a function of NaCl concentration up to a 30-fold excess with respect to the metal complex. The chemical shift difference showed a very limited linear decrease [0.003 ppm $(mmol)^{-1}$] with increase of concentration of Na^+, indicating a negligible effect of ionic strength on the derived pH value. Furthermore, we found that the ($\delta_{ax1} - \delta_{ac1}$) separation measured in blood serum (pH 7.4, 39 °C) is identical to that reported in Fig. 1 for the same pH value. Finally, we measured the temperature dependence of ^{1}H chemical shifts at three different pH values and found that there was a maximum change of 0.5 ppm $(°C)^{-1}$ for the difference ($\delta_{ax1} - \delta_{ac1}$). Thus, the uncertainty introduced in the pH determination, relative to the data plotted in Fig. 2, is ≤0.1 pH unit for each °C.

In conclusion, $[Yb(dotp)]^{5-}$ appears to be a prototype of a new class of pH indicators for *in vivo* applications and is characterized by a high sensitivity, ease of measurement and high accuracy. Furthermore, the use of a paramagnetic compound appears much more advantageous with respect to diamagnetic systems. These advantages may be summarized as follows: (*i*) good sensitivity of ^{1}H nuclei further enhanced by the very short relaxation times of the paramagnetic species which allows the aquisition of a large number of transients; (*ii*) the large chemical shift range of ^{1}H resonances of paramagnetic complexes avoids any interference with absorptions from endogenous substances which fall in the diamagnetic region; (*iii*) there is no need to use an internal reference to quote the shifts of the pH-dependent resonance (as in the case of the 3-aminopropylphosphonate[4]) because the ^{1}H NMR spectrum of these complexes provides suitable pairs of resonances whose separation is pH dependent; (*iv*) the remarkable pH sensitivity may allow the determination of very small pH changes, once other parameters such as temperature are independently controlled.

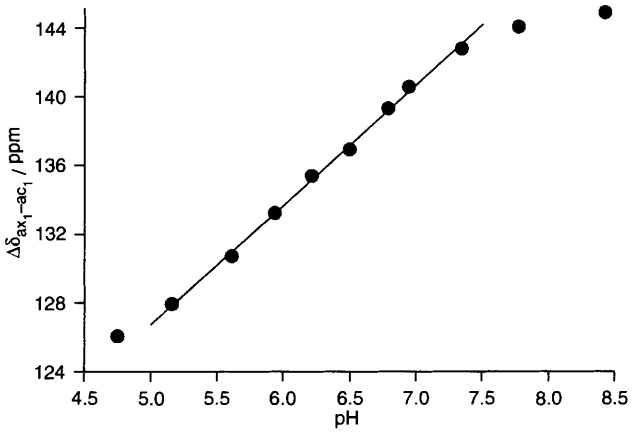


Fig. 2 pH dependence of the chemical shift difference (Δδ) between ax1 and ac1 protons of Na_5[Yb(dotp)] measured at 39 °C for a 25 mmol dm^{-3} solution in D_2O

Finally, these results suggest that it might be possible to exploit the pH dependence of a given resonance to obtain pH-dependent images *via* standard chemical shift imaging procedures.

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Footnotes

† The spectrum was acquired by using a spectral width of 18000 Hz, 16 K data points (digital resolution 2.2 Hz per data point), 10 μs pulse length (40°) and a delay of 600 ms between pulses.

‡ A dependence on the solution pH of the ^{31}P NMR chemical shift for $[Eu(dotp)]^{5-}$ (ref. 8) and $[Dy(dotp)]^{5-}$ (ref. 6) have been reported.

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