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Interaction of $[Ln(DO2A)(H_2O)_{2-3}]^+$ and $[Ln(DO2P)(H_2O)_{2-3}]^-$ with phosphate, acetate and fluoride anions in aqueous solution

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Abstract

Mixed complexation of the lanthanide(III) chelates of the 1,7-disubstituted tetraazacyclododecane macrocycles DO2A and DO2P with phosphate, acetate and fluoride anions was studied in aqueous solutions using high resolution ^{1}H and ^{31}P NMR spectroscopy. The $[La(DO2A)(H_2O)_{2-3}]^+$ and $[Ce(DO2A)(H_2O)_{2-3}]^+$ chelates readily decompose in aqueous solutions containing phosphate anions. $[Nd(DO2A)(H_2O)_{2-3}]^+$ and $[Eu(DO2A)(H_2O)_{2-3}]^+$ chelates remain stable in an acetate environment. Association constants for the 1:1 adduct were obtained via ^{1}H NMR titrations. In a fluoride environment, $[La(DO2A)(H_2O)_{2-3}]^+$ was found to be unstable, in contrast to $[Eu(DO2A)(H_2O)_{2-3}]^+$, for which the association constant for the 1:1 adduct was calculated. Chelate formation, as well as mixed complexation with fluoride and phosphate was studied for $[Eu(DO2P)(H_2O)_{2-3}]^-$. The ^{31}P chemical shifts of $[Eu(DO2P)(H_2O)_{2-3}]^-$ indicate that the DO2P macrocycle forms an 'in-cage' complex with binding through all four nitrogens. The $[Eu(DO2P)(H_2O)_{2-3}]^-$ chelate gradually decomposes in a phosphate environment, but remains stable in an fluoride environment.

Keywords: Nuclear resonances; Lanthanides; Rare earths; MRI contrast agents

1. Introduction

In the last decennium, macrocyclic lanthanide chelates were successfully introduced in the field of biomedicine. Some applications of these compounds are: the use of Gd(III) and Dy(III) chelates as contrast agents for magnetic resonance imaging (MRI) [1–4], Eu(III) and Tb(III) as fluorescent labels for immunoassay [5,6], Eu(III) complexes as hyperfine shift reagents [7] and Yb(III) chelates as in vivo temperature reporters [8]. In order to use lanthanides as contrast agents, one needs to obtain thermodynamically stable and kinetically inert chelates, as free Ln(III) ions are too toxic at the concentrations used in MRI. Furthermore, interaction through complexation of these chelates with anions present in blood may promote their decomposition and alter their relaxation behaviour. The lanthanide(III) chelates of the 1,7-disubstituted tetraazacyclo-dodecane macrocycles DO2A (1,4,7,10-tetraazacyclododecane-1,4,7,10-1,7-bis(acetic acid)) and DO2P (1,4,7,10-tetraazacyclododecane-1,4,7,10-1,7-bis-(methylenephosphonic acid)) (see structure of these ligands in Fig. 1), $[Ln(DO2A)(H_2O)_{2-3}]^+$ and $[Ln(DO2P)(H_2O)_{2-3}]^-$, respectively, have been characterized in solution, showing an inner-sphere which is quite open to mixed ligand complexation [10–13]. Thus, in this article we present a study of their mixed complexation with several anions of interest in aqueous solution, using high resolution ^{1}H and ^{31}P NMR spectroscopy.

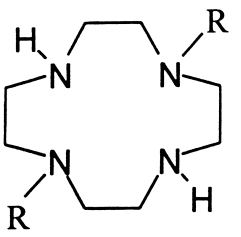

Fig. 1. Representation of the chemical structures DO2A and DO2P. DO2A, R = $CH_2CO_2^-$; DO2P, R = $CH_2PO_3^{2-}$.

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2. Experimental

Lanthanide(III) chlorides (Aldrich, 99.9%), sodium fluoride and sodium acetate (Sigma, 99.9%) and sodium dihydrogen phosphate (Merck, 99.9%) were used as obtained. The DO2A and DO2P ligands were synthesized as previously described [9,13]. All ^{1}H and ^{31}P NMR spectra were recorded on a Varian Unity 500 spectrometer operating at 499.824 and 202.3 MHz, respectively, in D_2O (99.8% D from Sigma) solutions. ^{31}P NMR spectra were measured with broad-band proton decoupling. The pH of the solutions was adjusted before each measurement with DCl and CO_2-free NaOD (from Sigma) using a Crison MicropH 2002 pH-meter with an Ingold 405-M5 combination electrode. TSP (sodium-3-trimethylsilylpropionate-2,2-3,3-d_4) and 90% H_3PO_4 were used as external references for the ^{1}H and 31P NMR spectra, respectively. The temperature precision of the experiments was ± 0.5°C.

3. Results and discussion

3.1. Effect of phosphate on $La(DO2A)^+$ and $Ce(DO2A)^+$ chelates

The effect of increasing concentration of phosphate on the $[La(DO2A)(H_2O)_{2-3}]^+$ chelate was measured using a ^{1}H NMR titration in D_2O at 80°C and pH 7.5 (80°C because at lower temperatures the spectra are very broad due to intramolecular conformational averaging, pH 7.5 because at this condition, the solution contains no free La(III)). Fig. 2 shows the successive ^{1}H NMR spectra of $[La(DO2A)(H_2O)_{2-3}]^+$ titrated with increasing concentrations of phosphate at pH 7.5. It can be seen that the stepwise addition of phosphate causes a decrease of the acetate (3.46 ppm) and ring (3.18, 2.97, 2.84 and 2.63 ppm) peaks of $[La(DO2A)(H_2O)_{2-3}]^+$ [9]. Three new peaks appear when phosphate is added, and sharpen with increasing additions. They are assigned to the acetate protons (3.28 ppm) and the ethylenediamino protons (3.02 and 3.18 ppm) of the free DO2A ligand [9]. At $[La(DO2A)(H_2O)_{2-3}]^+$:phosphate ratio of 1.3 almost all the complex initially present is decomposed and La(III) phosphate precipitates. Thus, the presence of phosphate destabilises the $[La(DO2A)(H_2O)_{2-3}]^+$ complex, leading to replacement of DO2A by phosphate in the La(III) coordination sphere. This indicates that the complex may not be stable enough in biological media, limiting its possible biomedical application. In fact, the thermodynamic stability constants of the $[Ln(DO2A)(H_2O)_{2-3}]^+$ chelates are relatively low ($\log K_{ML}=10.94$ (La), 12.99 (Eu), 13.16 (Lu) [8]). The same picture holds for $[Ce(DO2A)(H_2O)_{2-3}]^+$ when increasing amounts of phosphate are added. Here again the chelate proved to be unstable, as was evidenced by ^{1}H NMR titration data: adding phosphate to $[Ce(DO2A)(H_2O)_{2-3}]^+$ resulted in a fast decomposition of the complex and precipitation of Ce(III) phosphate.

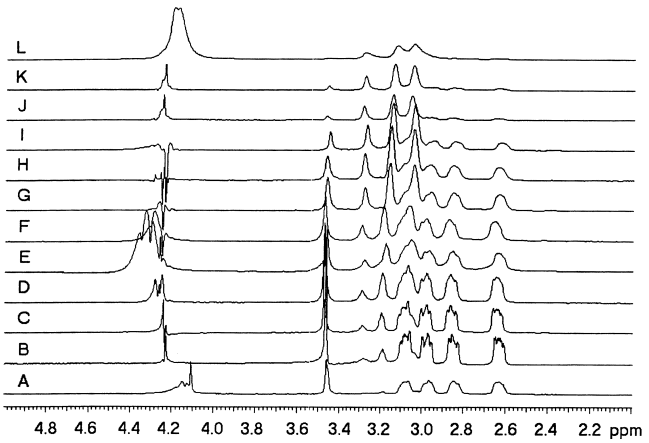

Fig. 2. ^{1}H NMR spectra of 2.5 mM $La(DO2A)^+$ (pH 7.5, 80°C), with increasing phosphate concentrations (mM): (A) 0.0, (B) 0.25, (C) 0.5, (D) 0.75, (E) 1, (F) 1.25, (G) 1.5, (H) 1.75, (I) 2.25, (J) 2.5, (K) 2.75, (L) 3.25.

3.2. Effect of fluoride on $[La(DO2A)(H_2O)_{2-3}]^+$ and $[Eu(DO2A)(H_2O)_{2-3}]^+$ chelates

The addition of fluoride to a solution of $[La(DO2A)(H_2O)_{2-3}]^+$ at pH 7.5 and 80°C causes free DO2A ligand to appear, as is evidenced by the growing intensity of free ligand ^{1}H NMR peaks around 3.28, 3.18 and 3.02 ppm, as well as a decrease of the concentration of the initial complex. This indicates an interaction of the anion with the cationic $[La(DO2A)(H_2O)_{2-3}]^+$ complex, which destabilises it via transmetallation. As the $[La(DO2A)(H_2O)_{2-3}]^+$: fluoride ratio reaches 0.5, a significant part of the complex is already dissociated and La(III) fluoride precipitates. However, the effect of F^- is weaker than that of phosphate.

The ^{1}H NMR spectra of $[Eu(DO2A)(H_2O)_{2-3}]^+$ in the presence of fluoride show a similar behaviour for some of its peaks as for acetate binding (cfr. infra): the peaks at -7.8 ppm (peak 'I'), -5.2, -1.4 and -0.6 ppm all shift to higher frequencies as the fluoride concentration increases.

Fig. 3 shows the shift of peak 'I' as a function of fluoride concentration. It can be used to calculate the association constant of the mixed complex:

$$K_{ass} = [[Eu(DO2A)(H_2O)_{1-2}]^+(F^-)]/[F^-][[Eu(DO2A)(H_2O)_{1-2}]^+] \quad (1)$$

Assuming 1:1 complex formation and a weak interaction, a plot of $1/d$ versus added fluoride concentration gives a straight line with slope $1/(K_{ass}\cdot D)$ and intercept $1/D$. D is the total change of the shift and d is the difference of the shift between the initial value and the

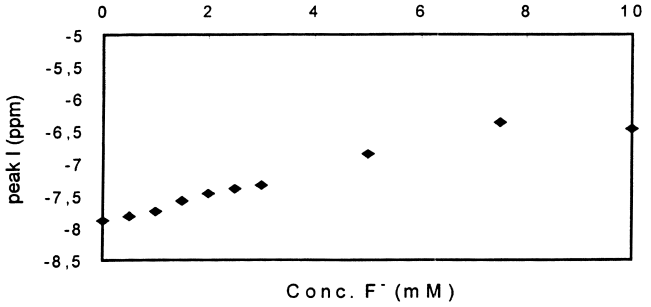

Fig. 3. Shift dependence of peak 'I' (−7.8 ppm) for 5 mM $Eu(DO2A)^+$ (pH 7.5, 80°C) with increasing fluoride concentration.

value obtained at a given fluoride concentration. The value obtained for K_{ass} is 90±4 l/mol. Note that the simplest chemical model is used here, to fit the data. We did not take into account formation of complexes with two fluoride anions in the first coordination sphere.

3.3. Effect of acetate on the $[Nd(DO2A)(H_2O)_{2-3}]^+$ and $[Eu(DO2A)(H_2O)_{2-3}]^+$ chelates

The successive ^1H NMR spectra of $[Nd(DO2A)(H_2O)_{2-3}]^+$ titrated with acetate at pH 10.4 and 80°C show almost no shift of the free acetate peak, indicating only a weak interaction between the chelate and the acetate anion. The signals of free and bound acetate are in fast exchange and the fraction of bound acetate is small. Thus, the shift is dominated by the free ligand fraction. The peaks from the complex are somewhat shifted due to the binding of acetate within the first coordination sphere of $[Nd(DO2A)(H_2O)_{2-3}]^+$. This can be ascribed to the interaction between $[Nd(DO2A)(H_2O)_{2-3}]^+$ and acetate in the mixed complex(es) $[Nd(DO2A)(acet)_n(H_2O)_m]$.

The first association constant of acetate with $[Nd(DO2A)(H_2O)_{2-3}]^+$ was derived using

$$K_{ass} = [Nd(DO2A)(acet)(H_2O)_{1-2}]]/[[Nd(DO2A)(H_2O)_{2-3}]^+][acet] \quad (2)$$

The value for the association constant, obtained using the same approximations as for $[Eu(DO2A)(H_2O)_{2-3}]^+$ titrated with fluoride, is 175±15 l/mol.

In the successive ^1H NMR spectra of $[Eu(DO2A)(H_2O)_{2-3}]^+$ titrated with acetate at pH 7.5 and 80°C, two peaks of $[Eu(DO2A)(H_2O)_{2-3}]^+$ at −7.8 (peak 'I') and −5.2 ppm show a downfield shift as more acetate is added. The peaks at −1.4 and −0.6 ppm gradually overlap, as the first peak shifts upfield and the second shifts downfield, resulting in a very broad signal. The signal for free acetate appears initially at 1.5 ppm and moves slowly to 1.8 ppm as more free acetate is present. Thus, the acetate signal has a downfield paramagnetic shift, indicating that it binds into the first coordination sphere of $[Eu(DO2A)(H_2O)_{2-3}]^+$.

The association constant was calculated using the NMR titration data for peak 'I' and the same approximations as mentioned before:

$$K_{ass} = [Eu(DO2A)(acet)(H_2O)_{1-2}]/[acet][[Eu(DO2A)(H_2O)_{2-3}]^+] \quad (3)$$

The value thus obtained for K_{ass} is 46±3 l/mol.

3.4. Effect of phosphate on the $[Eu(DO2P)(H_2O)_{2-3}]^-$ chelate

Fig. 4 shows the successive ^{31}P NMR spectra of 10 mM $[Eu(DO2P)(H_2O)_{2-3}]^-$ titrated with increasing amounts of phosphate at pH 10.1 and 25°C (pH 10.1 because the signals then become sharper). Initially, peaks I (+73 ppm, 'in-cage' $[Eu(DO2P)(H_2O)(OH)]^{2-}$), II (+48 ppm, 'in-cage' $[Eu(DO2P)(H_2O)_2]^-$) and V (−56 ppm, mixed $[Eu(DO2P)(HDO2P)]^{4-}$) are prominent [11]. Addition of phosphate results in the appearance and growth of two new peaks with shift values of +5 ppm (peak IV, free phosphate) and +18 ppm (free DO2P), while peak II disappears, peak I stays constant and peak V sharpens and increases. The appearance of free DO2P is due to the dissociation of the 'in-cage' $Eu(DO2P)^-$ complex in which no hydroxyl groups are present. This is evidenced by the observation that the two other peaks, at −56 and +73 ppm, show no changes. The changes observed for peak V could result from replacement of HDO2P by phosphate in the mixed complex, forming a new mixed complex $[Eu(DO2P)(PO_4)]^{4-}$ with a ^{31}P shift coinciding with the previous one (−56 ppm).

3.5. Effect of fluoride on the $[Eu(DO2P)(H_2O)_{2-3}]^-$ chelate

Fig. 5 shows the successive ^{31}P NMR spectra of 15 mM $[Eu(DO2P)(H_2O)_{2-3}]^-$ titrated with fluoride at pH 10.1 and at 25°C. As the fluoride:$[Eu(DO2P)(H_2O)_{2-3}]^-$ ratio increases, peak III (+18 ppm, free DO2P) initially decreases, to increase again at a ratio of 1:1. Peak IV (+1 ppm, 'out-of-cage' $[Eu(DO2P)^-]$ complex) disappears, peak V (−56 ppm, mixed $[Eu(DO2P)(HDO2P)]^{4-}$ complex), gains intensity and sharpens, peak I (+73 ppm, 'in-cage' $[Eu(DO2P)(H_2O)(OH)]^{2-}$) is not affected and peak II (+48 ppm, 'in-cage' $[Eu(DO2P)(H_2O)_2]^-$ complex) splits into two signals of equal intensity. This splitting is probably due to replacement of a water molecule by the fluoride anion in the first coordination sphere of the 'in-cage' complex, forming a $[Eu(DO2P)^-(H_2O)F]$ species either with two different ^{31}P environments for the two phosphonates or two isomers. Note that this study is only qualitative, as the data obtained

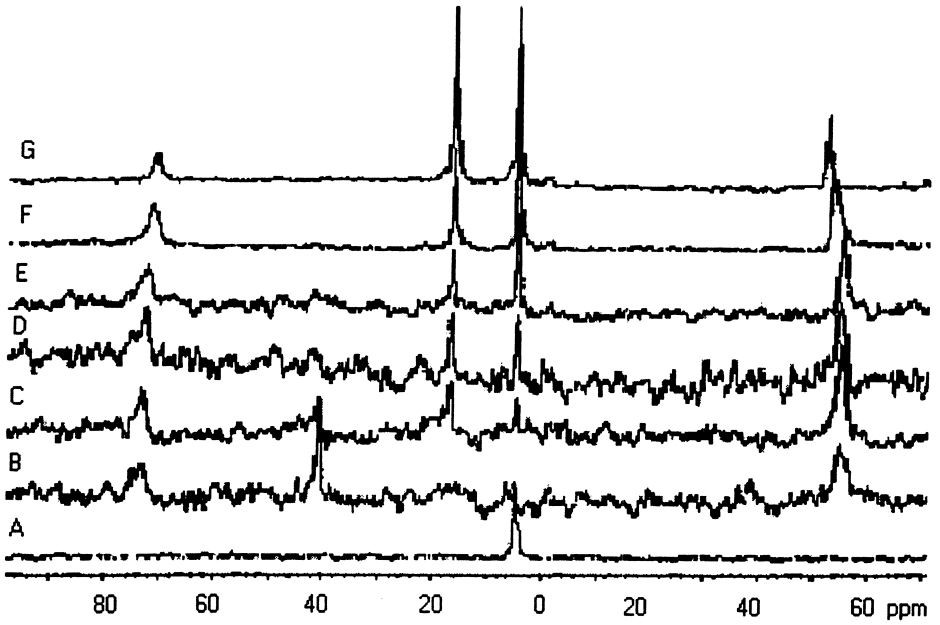

Fig. 4. ^{31}P NMR spectra of 10 mM Eu(DO2P)$^{-}$ titrated with phosphate (pH 10.1, 25°C): (A) 80 mM phosphate, (B) Eu(DO2P)$^{-}$, (C–G) Eu(DO2P)$^{-}$ in phosphate: (C) 1 mM; (D) 2 mM; (E) 3 mM; (F) 5 mM; (G) 10 mM.

are not suitable for quantitative analysis (not enough data points, poor S/N ratio, complex chemical model).

4. Conclusion

This NMR study indicates that the presence of phosphate anions has a clear influence on the $[La(DO2A)(H_2O)_{2-3}]^{+}$ and $[Ce(DO2A)(H_2O)_{2-3}]^{+}$ macrocyclic chelates, leading to dissociation. The presence of fluoride destabilises the $[La(DO2A)(H_2O)_{2-3}]^{+}$, $[Eu(DO2A)(H_2O)_{2-3}]^{+}$ macrocyclic chelates. The much weaker binding acetate anion forms mixed complexes with the $[Nd(DO2A)(H_2O)_{2-3}]^{+}$ and $[Eu(DO2A)(H_2O)_{2-3}]^{+}$ chelates. For the $[Eu(DO2P)(H_2O)_{2-3}]^{-}$ chelate, addition of phosphate or fluoride results in the dissociation of the complex and the formation of mixed complexes. The least affected complex is the 'in-cage' $[Eu(DO2P)(H_2O)(OH)]^{2-}$ species.

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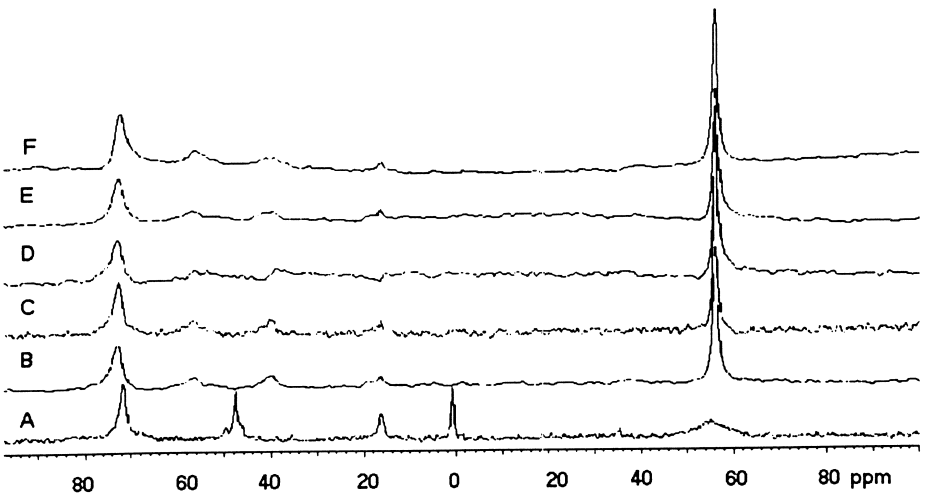

Fig. 5. ^{31}P NMR spectra of 15 mM Eu(DO2P)$^{-}$ titrated with fluoride (pH 10, 1°C), (A) Eu(DO2P)$^{-}$, (B–F) Eu(DO2P)$^{-}$ in fluoride: (B) 2 mM; (C) 3 mM; (D) 4 mM; (E) 5 mM; (F) 7 mM.

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