Gadolinium Complexes as Contrast Agents for Liver NMR Imaging*

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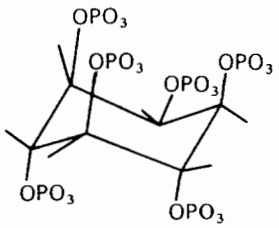
The effects of paramagnetic ions on nuclear magnetic resonance have been extensively studied [1–3]. In particular, the lanthanide ion Gd(III), owing to long electron spin correlation times ($\tau_s > 10^{-10}$), is currently used to enhance nuclear magnetic relaxation rates. In this system Gd(III) represents one of the most efficient contrast agents for NMR imaging as revealed by water line broadening.

A series of potential ligands was considered in order to reduce the toxicity exhibited by the free paramagnetic ions and to achieve a better selectivity towards individual organs. Particular attention was devoted to the liver. In this context, myoinositol hexakis-phosphate (phytate) plays an important role:



[–12]

In fact at present it is widely used in nuclear medicine as the ^{99m}Tc–phytate complex. Such complexes bind Ca^{2+} in the bloodstream and undergo phagocytosis by Küpfer cells.

In order to gain more insight into the relationship between the solution structure of Gd(III)–phytate complexes and water relaxivity (involved in the NMR contrast imaging), model Gd(III)–phytate complexes at different molar ratios and at different pH values were studied. In fact, shortening of the nuclear spin rates induced by the paramagnetic Gd(III) ion was expected to yield both kinetic and structural information for the ligand and also information about the efficiency of the relaxing system related to the water exchange kinetics in the solvation sphere.

In vivo images of rats have been performed by a 0.5 T Gyroscan imager.

Experimental

Stock solutions of phytate (Sigma) and $GdCl_3$ were prepared in H_2O at the same ionic strength and the pH was adjusted to extreme values by DCl or NaOD. Preparation of samples as well as pH adjustments were carried out under a nitrogen atmosphere in order to prevent misleading relaxation time measurements. The NMR experiments were performed with a Varian XL-300 spectrometer operating in the FT mode at 300 MHz for ^{1}H NMR and 121.45 MHz for ^{31}P NMR. Longitudinal relaxation times (T_1) were measured by using the inversion recovery pulse sequence (180–t–90).

The T_1 values were calculated by exponential regression analysis of the magnetization temporal dependence by using the spectrometer computer. The error in a single measurement was given as the 95% confidence limits of the regression analysis.

NMR images were obtained under the following conditions: SE-multislices were performed with repetition time (TR) = 400 ms and echo time (TE) = 30 ms. A 12-cm diameter surface coil was used with a field of view of 200 nm; an average slice thickness of 5 mm was used on two free induction decays (FIDs).

Results and Discussion

The ^{31}P NMR spectrum of phytate at pH 7 is reported in Fig. 1. In the pH range examined (5–7) phytate is in the monoanionic form and shows spectral patterns and chemical shifts which depend on pH values. At pH = 5 the ^{31}P NMR proton de-

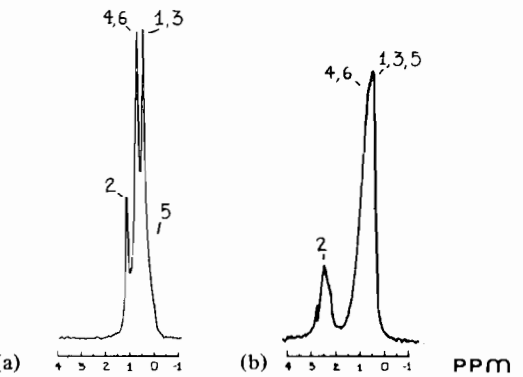


Fig. 1. ^{31}P NMR spectrum at pH 7 of (a) phytate and (b) phytate–Gd(III) complex.

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coupled spectrum of myoinositol hexaphosphate gives rise to three signals that are in the ratios 1:2:3 due to high concentration (150 mM) and attributed, as previously reported [4] and going from downfield to upfield respectively, to phosphate on C-2, to the phosphate groups on C-4 and C-6 and to the phosphate groups on C-1, C-3 and C-5. Over a larger pH range (4–10) the peaks shift and overlapping occurs because of broadening induced by the variation of the relaxation times of the phosphorus atoms involving exchange phenomena. At pH 6 and 7 three lines are still present and are assigned as for pH 5. These features prompted us to study the trend of the ^{31}P NMR relaxation times in order to clarify the behaviour of the phosphate groups as a function of pH and the complexing properties of phytate towards Gd(III).

Swift and Connick derived the relaxation time in a paramagnetic solution from a rigorous solution of the Bloch equation [5]. If the chemical exchange from the coordination sphere controls the relaxation process of the ligand nuclei, the observed relaxation rate T_{iobs}^{-1} ($i = 1, 2$) is related to the exchange rate of the ligand:

$$T_{iobs}^{-1} - T_{iF}^{-1} = T_{ip}^{-1} = f\tau_m^{-1} \qquad (i = 1, 2) \quad (1)$$

where T_{iF} is the relaxation rate in the absence of metal ions; f is the fraction of the ligand present in the coordination sphere and τ_m^{-1} is the rate constant for dissociation of the ligand from the metal coordination sphere.

If the chemical exchange is rapid, the relaxation process is controlled by dipole–dipole or scalar coupling T_{iM} mechanism in the first or second coordination sphere of the involved ion:

$$T_{ip}^{-1} = fT_{iM}^{-1} \qquad (2)$$

The general Solomon–Bloembergen expression [6] for T_{iM}^{-1} provides a very useful way of obtaining structural information from T_{iM}^{-1} values of different nuclei within the ligand molecule. The same kind of information can also be obtained when the chemical exchange is extremely low, but in such cases the relaxation process is controlled by a T_{iM} mechanism involving the outer-sphere ligand molecules exchanging by a diffusion-controlled process.

Equation (1) is applicable if the plot is linear with a negative slope; eqn. (2) is applicable if the plot is linear with the positive slope being the outer sphere T_{iM} mechanism relevant in the low temperature region. Alternatively the T_{2p}^{-1}/T_{1p}^{-1} can be considered in fact in the exchange-controlled region $T_{2p}^{-1}/T_{1p}^{-1} = 1$. When the value is different, T_{2p}^{-1}/T_{1p}^{-1} can be determined by eqn. (2), whereas the T_{2p}^{-1} value can still be determined by τ_m^{-1}.

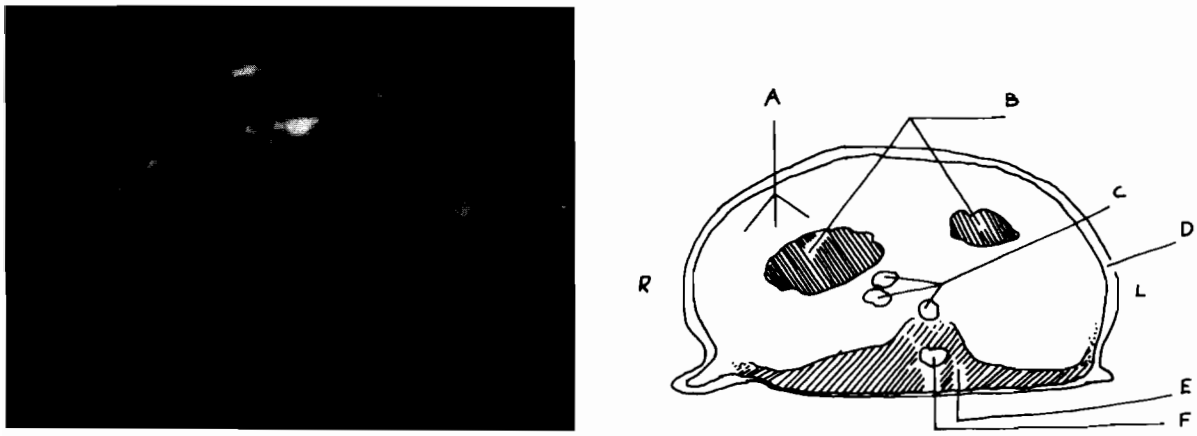
The measured ^{31}P NMR T_1 relaxation times of the phosphorus groups of phytate reveal a general lowering as the pH rises from 5 to 7. Such a variation is more evident for the phosphate groups on C-4 and C-6. The same behaviour is shown in the examined pH range from the corresponding phosphorus atoms when the complexation with Gd(III) occurs. The effect is the same for all the phosphorus atoms, indicating the presence of rapid exchange between complexation sites. Confirmation was obtained by preliminary T_2 values being similar to T_1 values and indicating, as reported above, the presence of a chemical exchange.

The effect due to Gd(III) ions is high (0.2 s for a Gd(III) concentration of 10^{-6} M in 0.150 M phytate) and the extensive broadening of the resonances of all the phosphorus atoms can be taken as a reliable proof of the structure involving two Gd(III) ions for one molecule of phosphate (see Table I).

On the basis of these results, NMR imaging experiments were carried out on rats before and after injection of Gd(III)–phytate solutions. Gd(III)–phytate and phytate solutions at pH = 7 were intravenously (i.v.) injected into six pairs of rats (at the dose of 10 mg/kg) and studied by a 0.5 T Gyroscan NMR imager. Liver images were obtained at 10, 15

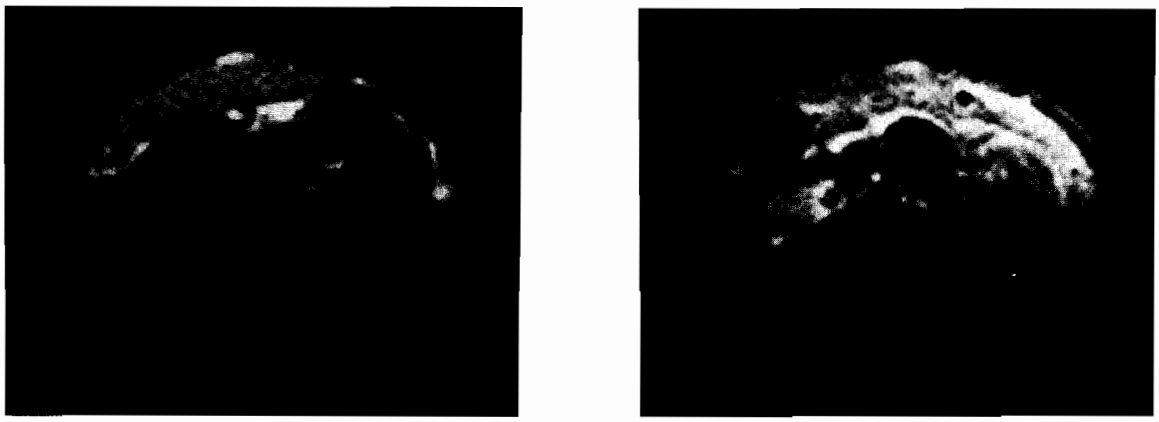
TABLE I. ^{31}P NMR Spin-lattice Relaxation Times (T_1) of Phytate (150 mM) in H_2O and Phytate (150 mM)–Gd(III) (10^{-6} M) in H_2O (Temp. = 295 K, T_1 in s)

OPO_3^{2-} groups							
Phytate				Phytate–Gd(III)			
n	pH			n	pH		
	5	6	7		5	6	7
2	1.032	0.894	0.739	2	0.633	0.592	0.549
4, 6	1.087	0.909	0.567	4, 6	0.727	0.607	0.416
1, 3, 5	0.883	0.782	0.676	1, 3, 5	0.569	0.541	0.501



(a) (b)

Fig. 2. ^{1}H NMR spin–echo images of rat liver: before the injection of Gd(III)–phytate (10 mg/kg) (a); and 15 min after the injection of the Gd(III)–phytate. (a) Cross-image of rat abdomen. (b) Sketch of the upper image: (A) liver; (B) gut; (C) vessels; (D) skin; (E) muscle; (F) spinal cord.



(a) (b)

Fig. 3. (a) Cross-image of rat abdomen prior to contrast injection. (b) Cross-image of rat abdomen following contrast injection (Gd–phytate 10 mg/kg), performed 15 min after injection.

and 20 min after the i.v. administration of the complexes and showed a remarkable contrast enhancement attributable to the Gd(III) complexes.

In Fig. 2(a) a transversal slice of rat abdomen before contrast injection obtained by a spin–echo sequence is shown. Figure 2(b) represents a sketch of the abdomen where different anatomical areas are indicated. Figures 3(a) and 3(b) are relative to a rat abdomen prior and following contrast agent injection, respectively. The Figures show slight differences owing to the positioning of the rat before and after the contrast injection. Both images are shown under the same conditions of window and level. In Fig. 3(b), the liver exhibits a consistent intensity enhancement, further indicating a remarkable effect of Gd(III)–phytate solutions.

Experiments are in progress to investigate contrast enhancement induced by phytate–Gd(III) complexes entrapped in liposomes.

References

1 J. P. Jesson, in G. N. La Mar, W. de W. Horrocks, Jr. and R. H. Holm (eds.), 'NMR of Paramagnetic Molecules', Academic Press, New York, 1973.
2 E. de Boer and H. van Willigen, *Prog. NMR Spectrosc., 2,* 111 (1967).
3 D. R. Eaton and W. D. Phillips, in J. S. Waugh (ed.), 'Advances in Magnetic Resonance', Vol. 1, Academic Press, New York, p. 103.
4 L. R. Isbrant and R. P. Oertel, *J. Am. Chem. Soc., 102,* 344 (1980).
5 T. J. Swift and R. E. Connik, *J. Chem. Phys., 37,* 307 (1962).
6 N. Niccolai, E. Tiezzi and G. Valensin, *Chem. Rev., 82,* 359 (1982).