

# $^{43}\text{Ca}$ NMR Relaxation Times and Quadrupole Coupling Constants for Some Small Calcium Complexes

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The  $^{43}\text{Ca}$  spin-lattice relaxation times have been measured for the calcium complexes with EDTA, EGTA and a cyclic ligand. The  $^{43}\text{Ca}$  quadrupole coupling constant was calculated for each complex, using correlation times calculated from  $^{13}\text{C}$  relaxation times. An increase in the quadrupole coupling constant by a factor of four is found on going from the Ca-EDTA to the Ca-EGTA complex. This is interpreted as being due to differences in the symmetry of the complexes.

$^{43}\text{Ca}$  NMR studies have up to recently been very scarce<sup>1–3</sup> although the calcium ion is recognised to be of importance in many physiological processes.<sup>4</sup> The calcium ion with a spin  $I \neq 0$ ,  $^{43}\text{Ca}$  ( $I = 7/2$ ) has a very low natural abundance (0.14 %) and also a sensitivity that is less than for *e.g.*  $^{13}\text{C}$ .<sup>5</sup> This explains the lack of interest shown in the past. However, very recently a few studies have appeared in which enriched  $^{43}\text{Ca}$  was used to study calcium–protein interaction.<sup>8–12</sup> It has even been possible to observe the  $^{43}\text{Ca}$  NMR signal from calcium strongly bound to some small proteins.<sup>13</sup>

The interpretation of  $^{43}\text{Ca}$  NMR data is somewhat hampered by the lack of knowledge about the  $^{43}\text{Ca}$  NMR relaxation time,  $T_1$ , for various systems. Therefore a study of the spin-lattice relaxation time of  $^{43}\text{Ca}$  bound to various small ligands has been initiated. The following complexes for which the metal exchange is slow on the NMR time scale have been studied: Ca-EDTA (EDTA-ethylenedi-

aminetetraacetic acid), Ca-EGTA [EGTA-ethylene-glycol-bis( $\beta$ -ethylamine)- $N,N'$ -tetraacetic acid], Ca-I.

## EXPERIMENTAL

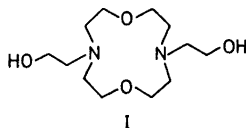
Ligand I was a gift from Prof. J. Dale (Ref. 15) and EDTA and EGTA were obtained from Merck and Sigma, respectively.

The  $^{43}\text{Ca}$  NMR studies were carried out at 17.16 MHz with a home made spectrometer with a magnetic field of 6T using horizontally arranged 17 mm O.D. samples. All samples were made up from  $^{43}\text{Ca}$  enriched (60 %<sub>o</sub>, Oak Ridge Natl. Lab., USA) calcium perchlorate in water solutions. The calcium concentration varied from 0.5 to 4 mM and the ligand concentration was 2–4 mM. The  $^{43}\text{Ca}$   $T_1$  measurements were made using the inversion recovery method, using 10 000 pulse sequences with a  $180^\circ$  pulse length of 36  $\mu\text{s}$  and a delay between the sequences of at least 3 times  $T_1$ .

$^{13}\text{C}$  NMR measurements were made on a Varian XL-100 spectrometer using *ca.* 40 mM concentration of complexes with natural abundance calcium in 12 mm O.D. NMR tubes. The  $T_1$  measurements were made using the fast inversion recovery method<sup>16</sup> with 10 000 pulse sequences, a  $180^\circ$  pulse width of 180  $\mu\text{s}$ , and a delay between the sequences of 0.5 s. All  $T_1$  values were obtained from a non-linear least squares fit to the experimental data, using three adjustable parameters. The given uncertainties are three times the standard deviation.

## RESULTS

$^{43}\text{Ca}$  NMR spectra from solutions of one of the ligands (EDTA, EGTA or ligand I) and an excess of  $\text{Ca}^{2+}$  showed two signals, when a sufficiently



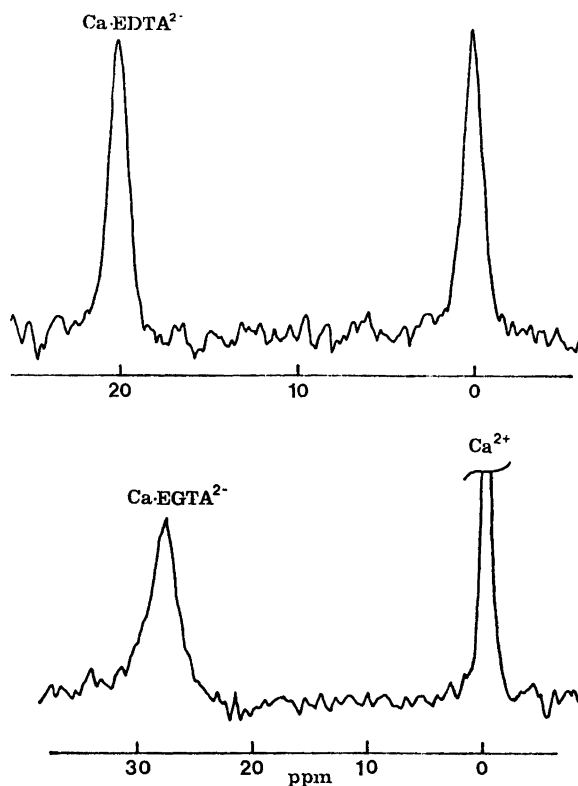


Fig. 1.  $^{43}\text{Ca}$  NMR spectrum at 17.16 MHz from a solution containing 2mM  $^{43}\text{Ca}$  and 1 mM of either EGTA or EDTA.  $10^4$   $70^\circ$  pulses, with a 1 s repetition time, were accumulated for each spectrum.

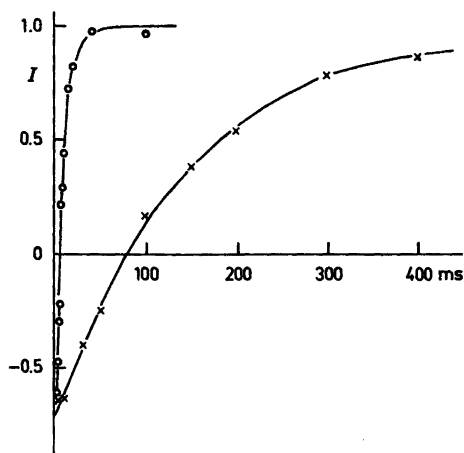


Fig. 2. The  $^{43}\text{Ca}$  signal intensity as a function of the delay time in the inversion recovery experiment.  $I = I_0(1 - k \exp(-\tau/T_1))$ . The solid curves are the calculated best fit.  $\times$ , Ca-EDTA;  $\circ$ , Ca-EGTA.

high pH (8, 10 and 10 for EDTA, EGTA and ligand I, respectively) was used. Even at elevated temperature no broadening of the signals could be detected, showing that the exchange between free and complexed calcium ions is very slow (Fig. 1). For solutions with total calcium and ligand concentrations of 4 mM the spin lattice relaxation time was measured using the inversion recovery method. (Fig. 2) The resulting relaxation times, as well as the chemical shifts, are given in Table 1.

In order to obtain the correlation times of the various complexes, the carbon-13 spin lattice relaxation times for all the carbons, except the carbonyles, were measured for solutions that had a complex concentration of 40 mM. The large difference in concentration between the  $^{43}\text{Ca}$  and  $^{13}\text{C}$  solutions was a compromise due to the high cost of  $^{43}\text{Ca}$  and the sensitivity of  $^{13}\text{C}$ , however a doubling of the concentration for the  $^{13}\text{C}$  experiment had no significant effect on the  $^{13}\text{C}$   $T_1$ 's. The correlation times,  $\tau_c$ , was calculated from

Table 1. Chemical shifts and relaxation times of <sup>43</sup>Ca in some complexes.

Ligand	δ <sup>b</sup> (ppm)	T <sub>1</sub> (ms)	χ <sup>a</sup> (MHz)
EGTA	27.9	7.1 ± 1.4	2.1
EDTA	20.0	150 ± 10	0.5
I	4.7	22 ± 7	1.4

<sup>a</sup>Quadrupole coupling constants defined as  $\chi = \frac{e^2qQ}{h} \left(1 + \frac{\eta}{3}\right)^{\frac{1}{2}}$ . <sup>b</sup>Chemical shift from 1 mM Ca(ClO<sub>4</sub>)<sub>2</sub> in H<sub>2</sub>O, positive to higher frequency.

$$1/T_1 = \gamma_H^2 \gamma_C^2 \hbar^2 r^{-6} \tau_c$$

where γ<sub>H</sub> and γ<sub>C</sub> are the gyromagnetic ratio for protons and carbon-13, respectively, ħ is Planck's constant and r the proton-carbon bond distance (1.09 Å). The measured relaxation times and the calculated correlation times are summarized in Table 2.

The correlation times calculated from the <sup>13</sup>C T<sub>1</sub> data and the <sup>43</sup>Ca relaxation times were used to calculate the quadrupole coupling constant, χ, for <sup>43</sup>Ca in the various complexes from

$$1/T_1 = \frac{2\pi^2}{49} \cdot \chi^2 \tau_c$$

where T<sub>1</sub> is the <sup>43</sup>Ca spin lattice relaxation time,

$$\chi = \frac{e^2qQ}{h} \cdot \left(1 + \frac{\eta^2}{3}\right)^{\frac{1}{2}}$$

(the quadrupole coupling constant), and the other parameters are as defined above.

### DISCUSSION

Very little is known about the effect of various ligands on the chemical shift of <sup>43</sup>Ca. In contrast to the effect of a carboxyl group on the <sup>113</sup>Cd chemical shift, which is upfield, Robertson *et al.*<sup>6</sup>

Table 2. Carbon-13 relaxation times (mean values) and correlation times for some Ca-complexes.

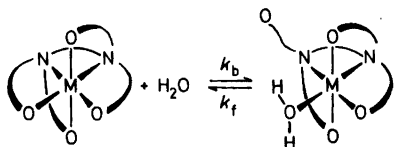
Ligand	T <sub>1</sub> (s)	τ <sub>c</sub> (s)
EGTA	0.29 ± 0.05	8.0 × 10 <sup>-11</sup>
EDTA	0.4 ± 0.05	5.8 × 10 <sup>-11</sup>
I	0.42 ± 0.1	5.6 × 10 <sup>-11</sup>

have reported a downfield shift of 2 ppm due to complexation with Z-D-Gla-D-Gla-OMe. This, however, also contradicts the results reported by Lutz *et al.*, who found an upfield shift for both formate and lactate solutions.<sup>2</sup>

The chemical shifts of <sup>43</sup>Ca in the complexes studied in the present work (Table 1) are in agreement with the accepted effect of a nitrogen ligand on the <sup>113</sup>Cd chemical shift.<sup>13</sup> However, as expected the <sup>43</sup>Ca shifts are smaller than the <sup>113</sup>Cd shifts. These <sup>43</sup>Ca shifts are so small that they will probably not be very important, especially not in studies of protein-metal interactions, because the linewidth of the signals from protein bound <sup>43</sup>Ca are of the order of 10<sup>3</sup> Hz (~60 ppm at our field).<sup>13</sup>

As can be seen from Table 1 there is a significant variation in the <sup>43</sup>Ca relaxation rate in the various complexes. The <sup>43</sup>Ca relaxation can safely be assumed to be totally dominated by the quadrupolar relaxation. Reasonable estimates of the relaxation rate due to other mechanisms result in rates that are at least a factor of 100 slower than the observed ones. Therefore the differences in the relaxation rates among the complexes can be explained by either a difference in the quadrupole coupling constant, χ, and/or a difference in the correlation time, τ<sub>c</sub>.

The <sup>13</sup>C relaxation rates show clearly that the overall correlation times sensed by the carbon nuclei are very similar for all three complexes (Table 2), ruling out this as a possible explanation for the observed variation in the <sup>43</sup>Ca relaxation rate. However, there could still exist a local mobility in the Ca-EDTA complex which would not be sensed by the carbons, but would strongly affect the calcium nucleus. There is some evidence that the Ca-EDTA complex in water solution is a mixture of two forms,<sup>17-22</sup> where the metal is either penta- or hexa-coordinated to the EDTA ligand, as illustrated by Scheme 1.



Scheme 1.

In the penta coordinated complex the sixth site on the metal is occupied by a water molecule. It could well be anticipated that the water exchange might be fast without affecting the carbon correlation time. Such a water exchange could be coupled to a nitrogen inversion, which for the Pb-EDTA complex has been shown to be fast on the NMR time scale.<sup>23</sup> Very recently Harada *et al.*<sup>22</sup> used an ultrasonic absorption method to measure the water exchange rate in the alkaline earth complexes with EDTA. They found a rate constant,  $k_f$ , of  $3.6 \times 10^7 \text{ s}^{-1}$  for the Ca EDTA complex. Since this exchange is much slower than the inverse of the rotation correlation time of the complex the effective symmetry brought about by the water exchange cannot be the cause of the difference between the relaxation behaviour of  $^{43}\text{Ca}$  in Ca-EDTA and Ca-EGTA.

Consequently, we must assume that the observed longer relaxation time for  $^{43}\text{Ca}$  in the Ca-EDTA complex, as compared to the other two complexes, is caused by an effective symmetry in the Ca-EDTA complex, resulting in a field gradient that is smaller than for the other complexes. It is not unreasonable to assume that small changes in the geometry of the complex might have a pronounced effect on the field gradient. Maybe it is even so that this is the type of variation one should expect.

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## REFERENCES

1. Bryant, R. G. *J. Am. Chem. Soc.* 91 (1969) 1870.
2. Lutz, O., Schwenk, A. and Uhl, A. *Z. Naturforsch. A* 30 (1975) 1122.
3. Lindman, B., Forsén, S. and Lilja, H. *Chem. Scr.* 11 (1977) 91.
4. Wasserman, R. H., Corradino, R. A., Carifoli, E., Kretsinger, R. H., MacLennan, D. A. and

Siegel, F. L., Eds., *Calcium Binding Proteins and Calcium Function*, North-Holland, New York 1977.

5. Lindman, B. and Forsén, S. In Harris and Mann, Eds., *NMR and the Periodic Table*, Academic, New York 1979.
6. Robertson, P., Jr., Hiskey, R. G. and Koehler, K. A. *J. Biol. Chem.* 253 (1978) 5880.
7. Farmer, R. M. and Popov, A. I. *Inorg. Nucl. Chem. Lett.* 17 (1981) 51.
8. Parello, J., Lilja, H., Cavé, A. and Lindman, B. *FEBS Lett.* 87 (1978) 191.
9. Reimarsson, P., Parello, J., Drakenberg, T., Gustavsson, H. and Lindman, B. *FEBS Lett.* 108 (1979) 439.
10. March, H. C., Robertsen, P., Jr., Scott, M. E., Koehler, K. A. and Hiskey, R. G. *J. Biol. Chem.* 254 (1979) 4628.
11. Andersson, T., Drakenberg, T., Forsén, S., Wieloch, R. and Lindström, M. *FEBS Lett.* 123 (1981) 115.
12. Andersson, T., Drakenberg, T., Forsén, S. and Thulin, E. *FEBS Lett.* 125 (1981) 39.
13. Andersson, T., Swärd, M., Drakenberg, T. and Forsén, S. *J. Am. Chem. Soc.* *In press.*
14. Forsén, S., Thulin, E. and Lilja, H. *FEBS Lett.* 104 (1979) 123.
15. Amble, E. and Dale, J. *Acta Chem. Scand. B* 33 (1979) 698.
16. Levy, G. C., Ed., *Topics in Carbon-13 NMR Spectroscopy*, Wiley, New York 1974, Vol. 1.
17. Bhat, T. R. and Krishnamurthy, M. *J. Inorg. Nucl. Chem.* 25 (1963) 1147.
18. Brunetti, A. P., Nancollas, G. H. and Smith, P. N. *J. Am. Chem. Soc.* 91 (1969) 4680.
19. Wilkins, R. G. and Yelin, R. E. *J. Am. Chem. Soc.* 92 (1970) 1191.
20. Higgins, W. E. C. and Samuel, B. *J. Chem. Soc. A* (1980) 1579.
21. Grant, M. W., Dodgen, H. W. and Hunt, J. P. *J. Am. Chem. Soc.* 93 (1971) 6828.
22. Harada, S., Funaki, Y. and Yasunga, T. *J. Am. Chem. Soc.* 102 (1980) 136.
23. Day, R. J. and Reilley, C. H. *Inorg. Chem.* 36 (1964) 1073.

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