Pergamon

0277-5387(95)00172-7

Polyhedron Vol. 14, No. 23-24, pp. 3579-3583, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0277-5387/95 $9.50+0.00

LANTHANIDE-INDUCED SHIFT AND RELAXATION RATE STUDIES OF THE AQUEOUS COMPLEXATION OF CITRATE

YUAN CHUNBO* and LIU JUZHENG

National Laboratory of Molecular & Biomolecular Electronics, Department of Chemistry and Chemical Engineering, Southeast University, Nanjing 210096, P. R. China

and

ZHAO DAQING, WU YIJIE and NI JIAZUAN

Laboratory of Rare Earth Chemistry & Physics, Changchun Institute of Applied Chemistry, Academia Sinica, Changchun 130022, P. R. China

(*Received* 17 *January* 1995; *accepted* 11 *April* 1995)

Abstract—The aqueous complexation of lanthanide ions with citrate in pH 7.4 solution has been investigated with use of the lanthanide-induced shift and paramagnetic relaxation rate enhancement methods. The results show that citrate coordinates via hydroxyl and central carboxylate groups with lanthanide ions and forms 1 : 2 (Ln/cit) isostructural complexes through the lanthanide series. A new possible coordination geometry deduced from our experimental data is suggested and discussed.

The application of the lanthanide complex, Gd–DTPA, as a magnetic resonance imaging reagent in clinical practice[1-3] has stimulated studies on the toxicity of lanthanide complexes[4] and gadolinium(III) *in vivo* speciation.[5] For the important role of gadolinium(III) citrate complex in Jackson's computer model,[5] the aqueous complexation of gadolinium(III) citrate complex had been studied at near physiological pH values by using potentiometric titration and NMR spectroscopic methods.[6] A coordination model was described in which citrate coordinated with lanthanide ions via hydroxyl and two carboxyl groups and formed a 1 : 1 (Ln/cit) complex. One of the terminal carboxyl groups was involved in the complexation.

We have been interested for some time in NMR investigations on the complexation behaviour of lanthanide ions in blood plasma with low-molecular-weight ligands for clarifying the interactive mechanism of lanthanide ions when they are introduced into biological liquids. Lanthanide paramagnetic-induced shift and enhancement of relaxation rate methods have proved to be powerful tools in the elucidation of dynamic solution conformation of simple carboxylate[7,8] and amino acid molecules.[9] We show here some evidence which excludes the terminal carboxylate groups in complexation in pH 7.4 solution.

EXPERIMENTAL

Potassium citrate ($K_3C_6H_5O_7 \cdot H_2O$, A.R.) and lanthanide oxides (99.9%) were products of Beijing Chemical Plant. D_2O (99.8%), deuterochloride (DCl) and sodium deuteroxide (NaOD) were obtained from Aldrich Chemical Co. Inc. All other reagents were reagent grade.

$LnCl_3$ solutions were prepared by dissolving Ln_2O_3 in DCl solution and eliminating the excess acid. The pH of these solutions was around 5.4. The concentrations were determined by EDTA titrations.

^{13}C NMR spectra were recorded on a Varian

---

* To whom correspondence should be addressed.

Unity 400 spectrometer, using a 5 mm standard sample tube at temperature of 298 K. Tert-butylalcohol was used as an internal reference. The pH of 0.5 mol dm^{-3} potassium citrate was adjusted to pH 7.4 with addition of DCl or NaOD solutions. Relaxation time (T_1) values were measured by using the inversion recovery sequence. The T_1 values reported here are for deoxygenated samples with mean values of three measurements.

RESULTS AND DISCUSSION

Lanthanide-induced shifts

The chemical shift in the lanthanide complex is given by[10] $\delta_M = \delta_{CF} + \delta_{DIP} + \delta_{FC}$, where δ_{CF} is diamagnetic in origin and is always negligible in the case of small molecule complexes. δ_{DIP} is the dipolar contribution to the chemical shift and δ_{FC} is the Fermi contact contribution.

Paramagnetic lanthanide ion-induced shift. The ^{13}C NMR spectrum of 0.5 mol dm^{-3} potassium citrate (pH 7.4), together with the spectra which show the effects of increasing the concentration ratio $[Pr^{3+}]/[cit]$, are shown in Fig. 1. The assignments of ^{13}C NMR lines of citrate were based on a standard spectrum. The ^{13}C lines of citrate all showed a significant chemical shift to lower field, together with line broadening in the presence of various amounts of Pr^{3+}. The praseodymium-induced shifts in each nucleus of citrate at different concentration ratios are tabulated in Table 1. The presence of Pr^{3+} changed the chemical shifts of the C(3) and C(6) atoms greatly, while changes in chemical shift of C(1,5) and C(2,4) were less than that of C(3) and C(6). In particular, changes in chemical shift of C(3) were always the greatest, suggesting strongly that the 3-hydroxyl group was involved in the complexation with lanthanide ions. This is in accord with the results of potentiometric titrations.[6] The fact that the chemical shift of C(1,5) changed much less than that of C(3) and C(6), and even less than that of C(2,4), is surprising and contrary to the classical view which regarded citrate as a tridentate ligand in the complexation with lanthanide ions, using the three carboxylate groups. These data for Pr^{3+}-induced shifts can be explained in only two ways. (1) C(1,5) is far away from the lanthanide ion in comparison to C(3) and C(6), i.e. two terminal carboxylate groups are not bonding directly with lanthanide ion and are not involved in the complexation. (2) The δ_{DIP} and δ_{FC} contribution to the C(1,5) shifts may be of opposite sign and thereby make their Pr^{3+}-induced shift anomalously low. To clarify completely which case is responsible for the situation may be dependent on the separation of δ_{DIP} and δ_{FC} contributions to the lanthanide-induced shifts of each nucleus. Unfortunately, for the citrate ligand, which has many sites of complexation with lanthanide ions, it

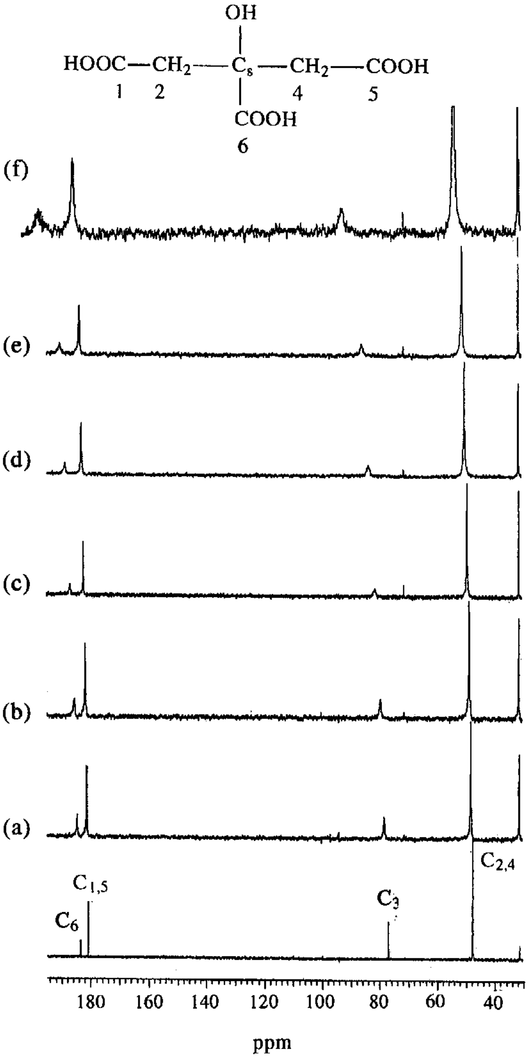

Fig. 1. The ^{13}C NMR spectra of citrate ligand showing the effects of increasing $[Pr^{3+}]/[cit]$, where $[Pr^{3+}]/[cit]$ is: (a) 1 : 40; (b) 1 : 16; (c) 1 : 9; (d) 1 : 5.3; (e) 1 : 4; (f) 1 : 2.

Table 1. The praseodymium-induced shift Δ δ (ppm) of 0.5 mol dm^{-3} K_3cit at different molar ratios ρ

ρ	C(2,4)	C(3)	C(1,5)	C(6)
1 : 40	0.44	1.34	0.44	1.02
1 : 16	0.90	2.48	0.80	1.90
1 : 9	1.63	4.58	1.36	3.30
1 : 5.3	2.51	6.62	2.09	5.05
1 : 4	3.43	8.85	2.82	6.85
1 : 2	6.19	15.79	5.15	13.01

is very difficult to separate the contributions of δ_{DIP} and δ_{FC} to the induced shifts. However, the contributions of δ_{DIP} and δ_{FC} to lanthanide-induced shifts will vary along the series of Ln^{3+} complexes, especially between light series and heavy series. Therefore, it is possible to distinguish the above two cases by measuring the lanthanide-induced shifts of each carbon nucleus in the presence of heavy lanthanide ions. Some heavy lanthanide ion-induced shifts were measured and are tabulated in Table 2. For the strongly broadening effects of heavy lanthanide ions on the ^{13}C lines, the chemical shifts of citrate ligand were measured at 0.5 mol dm^{-3} K_3cit with 50 mmol dm^{-3} lanthanide ions. The data in Table 2 exhibit the same trend of lanthanide-induced shifts in each carbon nucleus of citrate ligand in the presence of different heavy lanthanide ions as that in the presence of Pr^{3+} ion, i.e. $C(3) > C(6) \gg C(2,4) > C(1,5)$, demonstrating that heavy lanthanide ions coordinate with citrate in the same manner as with Pr^{3+}. The observation of C(3) and C(6) consistently having greater values along the series of Ln^{3+} complexes is further evidence of metal ions binding in their vicinity, while the two terminal carboxylate groups are not involved in the complexation. Aqueous titration [titrations were carried out as follows: (1) adding 0.5 mol dm^{-3} K_3cit gradually to 25 cm^{-3} 0.25 mol dm^{-3} Pr^{3+} solution; (2) adding 0.25 mol dm^{-3} Pr^{3+} to 0.5 mol dm^{-3} K_3cit solution] showed that in pH 7.4 solutions, the 1 : 1 lanthanide complexes are insoluble complexes which will redissolve in K_3cit solution to form 1 : 2 Ln/cit soluble complexes.

Complex formation shift of diamagnetic La^{3+}. The ^{13}C NMR chemical shifts of the citrate in the presence of various molar ratios of La^{3+} are tabulated in Table 3. Complexation of La^{3+} was accompanied by relatively minor perturbations to the chemical shifts in comparison with Pr^{3+}. Such changes upon binding a diamagnetic metal ion are to be expected and can generally be explained as the result of perturbation of electron density about the carbon by La^{3+}. It is interesting to note that chemical shift changes of C(3) and C(6) nuclei upon binding of La^{3+} are opposite to those of C(1,5) and C(2,4) nuclei. The chemical shifts of C(3) and C(6) shifted to low field, whereas C(2,4) and C(1,5) went to high field. The changes in chemical shift of C(3) and C(6) may be easily explained by the perturbation of La^{3+} to the electron density of the carbon atoms, for there is a positive electrostatic field around the La^{3+} ion which may attract the electron clouds of carbon atoms near the metal ion. Therefore, the coordinated carbon atoms would be deshielded. The different behaviour of C(2,4) and C(1,5) atoms in chemical shift upon binding with diamagnetic La^{3+} ion in comparison with C(3) and C(6) atoms suggest a different behaviour of these atoms in the complexation with La^{3+} ion. Under our experimental conditions at pH 7.4, lanthanide ions coordinated with citrate ligands to form 1 : 2 (Ln^{3+}/cit) complexes in which one hydrated lanthanide ion coordinates with two citrate ligands and generates a coordination sphere which involves two 3-hydroxyl and two 3-carboxylate groups with some water molecules.

Table 2. Some different heavy lanthanide-induced shifts, $\Delta\delta$ (ppm)

	Tb^{3+}	Ho^{3+}	Yb^{3+}	Lu^{3+}
C(6)	1.68	2.78	0.78	0.03
C(1,5)	0.22	0.0	0.196	−0.05
C(3)	1.51	2.03	0.60	0.12
C(2,4)	0.87	0.75	0.41	−0.15

Table 3. The lanthanide complex formation shift $\Delta\delta$ (ppm) of 0.5 mol dm^{-3} K_3cit at different molar ratios ρ

ρ	C(2,4)	C(3)	C(1,5)	C(6)
1 : 15	−0.19	0.29	−0.09	0.10
1 : 7.5	−0.41	0.68	−0.24	0.20
1 : 4	−0.68	1.17	−0.38	0.29
1 : 2	−0.92	1.60	−0.46	0.37

Relaxation time (T_1) *measurements*

The paramagnetic contribution to the relaxation rate of a given nucleus is given by[11]: $1/T_{1P} = P_M/T_{1M} = Kf(\tau_C)/r^6$, where P_M is the fraction of complexed ligand, T_{1M}^{-1} is the relaxation rate of the fully bound ligand, K is a constant characteristic of a given nucleus and $f(\tau_C)$ is a function of the correlation time that dominates the relaxation process. In the case of Gd^{3+}, there is a simple relation between relaxation time and the spatial distance from a given nucleus to Gd^{3+}, i.e. $(T_{1P})_1/(T_{1P})_2 = (r^6)_1/(r^6)_2$. The T_1 values of 0.5 mol dm^{-3} K_3cit in the presence of 5 mmol dm^{-3} Gd^{3+} were measured and the relative ratios of spatial distance from each nucleus to Gd^{3+} were calculated with $r_{C(6)}$ taken as 1 unit. These data are tabulated in Table 4. The distances of the two coordinated nuclei, C(3) and C(6), were shorter than those of C(1,5) and C(2,4). These data strongly support our conclusion drawn from lanthanide-induced shift

Table 4. Spin-lattice relaxation times (T_1, ms) and calculated relative distance ratios

	K_3cit/Gd^{3+}	r_a/r_b	$La(cit)_2/Gd^{3+}$	r_a/r_b
C(6)	6.79 ± 0.5		4.21 ± 0.5	
C(1,5)	16.87 ± 0.5	1.16	8.33 ± 0.4	1.12
C(3)	8.38 ± 0.3	1.04	6.33 ± 0.5	1.07
C(2,4)	26.42 ± 0.4	1.25	17.3 ± 0.4	1.27

data. The spatial distance between C(1,5) and Gd^{3+} is less than that of C(2,4), suggesting that two terminal carboxylate groups are directed towards the coordination centre, which may result from a hydrogen bond interaction of the terminal carboxylate with the coordinating water molecules. In order to investigate the aqueous structure of lanthanide–citrate complexes at high Ln/cit ratio, the T_1 values of $La(cit)_n$ (n is near 2) at each carbon nucleus in the presence of 5 mmol dm^{-3} Gd^{3+} were measured and are also tabulated in Table 4 with calculated relative ratios of spatial distance. Within experimental error, the relative ratios of distance of $La(cit)_2$ in the presence of Gd^{3+} were the same as those of citrate ligands in the presence of Gd^{3+}. This result implies that Gd^{3+} has identical complexation behaviour to La^{3+} in the complexation with citrate ligand at pH 7.4, and lanthanide–citrate complexes have the same coordination structure at low ratios of Ln/cit up to 1 : 2. The possible coordination geometry deduced from our experimental data is suggested in Fig. 2.

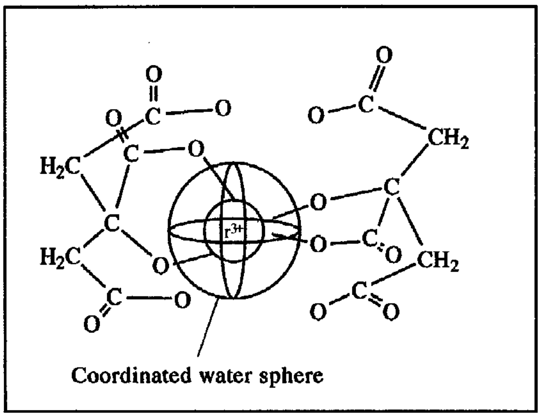

Fig. 2. The possible coordination geometry of $Ln(cit)_2$ complex in pH 7.4 solution. One lanthanide ion coordinates with two citrate ligands via a central hydroxyl group and a central carboxylate group and forms a coordination centre involving some water molecules. Two terminal carboxylate groups are not directly involved in the complexation but they are directed towards the coordination centre due to a hydrogen bond interaction between carboxylate groups and coordinated water molecules.

Recent studies on some ligands[7,8] have suggested that ligands do not form isostructural complexes with all lanthanide cations. Reuben and Elagavish, however, used relaxation data to show that sarcosine forms isostructural complexes with all lanthanide cations[12] and proposed[13] that the earlier Ln^{3+}–ligand structural changes resulted from a choice of improper models for the interpretation of LIS data. Relaxation time (T_1) measurements have been used to elucidate the aqueous complexation of lanthanide cations with L-proline.[14] Our data for relaxation times strongly demonstrate that the citrate ligand forms 1 : 2 Ln/cit isostructural complexes with lanthanide ions under experimental condition, using 3-hydroxyl and 3-carboxylate groups in the complexation. Two terminal carboxylate groups were not involved in the complexation. Our work shows again that the lanthanide-induced shift method combined with relaxation time measurements can provide valuable information of dynamic solution conformation of low-molecular-weight ligands.

Acknowledgement—This research was supported by National Foundation of Natural Science of China.

REFERENCES

1. M. Laniado, H. J. Weinmann, W. Schoerner, R. Felix and U. Spect, *Physiol. Chem. Phys. Med. NMR* 1984, **16**, 157.
2. H. J. Weinmann, M. Laniado and W. Mutzel, *Physiol. Chem. Phys. Med. NMR* 1984, **16**, 167.
3. G. K. Stimac, B. A. Porter, D. O. Olson, R. Gerlach and M. Genton, *Am. J. Neuoradiol.* 1988, **9**, 839.
4. R. B. Lauffer, *Chem. Rev.* 1987, **87**, 901.
5. G. E. Jackson, S. Wynchank and M. Woudenberg, *Magn. Reson. NMR* 1990, **16**, 57.
6. G. E. Jackson and J. du Toit, *J. Chem. Soc., Dalton Trans.* 1991, 1463.
7. B. A. Levine, J. M. Thorton and R. J. P. Williams, *J. Chem. Soc., Chem. Commun.* 1974, 669.

8. B. A. Levine and R. J. P. Williams, *Proc. R. Soc. Lond. Ser. A* 1975, **345**, 5.
9. A. D. Sherry and E. Pascual, *J. Am. Chem. Soc.* 1977, **99**, 5871.
10. J. Reuben and G. A. Elagavish, in *Handbook of the Physics and Chemistry of Rare Earths* (Edited by K. A. Gschneider Jr and L. Eyring), North Holland, New York (1979).
11. C. M. Dobson and B. A. Levine, *New Techniques in Biophysics and Cell Biology*, Vol. 3. Wiley, New York (1976).
12. G. A. Elagavish and J. Reuben, *J. Am. Chem. Soc.* 1978, **100**, 3617.
13. J. Reuben and G. A. Elagavish, *J. Magn. Reson.* 1980, **39**, 421.
14. M. Singh, J. J. Reynolds and A. D. Sherry, *J. Am. Chem. Soc.* 1983, **105**, 4172.