NMR and potentiometric studies of 1,4,7-triazacyclononane-*N,N',N''*-tris(methylenephosphonate monoethylester) and its complexes with metal ions

Istvan Lazar*, Ravichandran Ramasamy, Erno Brücher*
Department of Chemistry, University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688 (USA)

Carlos F. G. C. Geraldes
Department of Chemistry, University of Coimbra, 3000 Coimbra (Portugal)

and A. Dean Sherry**
Department of Chemistry, University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688 (USA)

(Received November 25, 1991; revised February 4, 1992)

Abstract

1,4,7-Triazacyclononane-*N,N',N''*-tris(methylenephosphonate monoethylester) (NOTPME) and its complexes with the trivalent lanthanide cations and divalent calcium, magnesium, zinc and cadmium have been examined by potentiometry and NMR. The ^{31}P NMR spectra of the lanthanide–NOTPME complexes show single resonances characteristic of symmetrical chelated species at low concentrations (below 0.1 mM) but multiple resonances at higher concentrations. This tendency of the neutral complexes to form unsymmetrical, perhaps oligomeric, species is dependent upon the size of the Ln^{3+} and the solution pH. The alkaline earth and divalent transition metal cation complexes do not show this same behavior. The stability constants of the Ln(NOTPME) complexes were found to be considerably smaller than their respective Ln(NOTA) complexes.

Introduction

The polyaza macrocyclic carboxylate and phosphonate ligands have attracted considerable interest because the structural, equilibrium and kinetic behavior of their metal ion complexes differ considerably from those of their non-cyclic analogs. In recent years, the lanthanide complexes of macrocyclic polyaza polyphosphonate ligands have been used as shift reagents for biological systems [1] and as potential contrast agents in magnetic resonance imaging [2]. It was also demonstrated that the polyaza polyphosphonate ligands could be used as ^{31}P NMR indicators to report intracellular divalent cation concentrations in biological systems [3].

The complexation properties of 1,4,7-triazacyclononane-*N,N',N''*-triacetate (NOTA) and 1,4,7-triazacyclononane-*N,N',N''*-tris(methylenephosphonate) (NOTP) (see structures in Fig. 1) with metal ions have been examined in some detail [4–9]. Although the phosphonate chelate appears to form more stable complexes with the lanthanides than does the acetate chelate, the highly charged $Ln(NOTP)^{3-}$ complexes also form protonated species at physiological pH values and tend to aggregate in solution by some yet undescribed mechanism [7]. In an attempt to understand better the complexation properties of the triazacyclononane macrocycles, we have now prepared the phosphonate monoethyl ester derivative of NOTP and examined some of its metal binding properties.

Experimental

Materials

1,4,7-Triazacyclononane, paraformaldehyde, diethylphosphite and activated carbon Darco G-60 were purchased from Aldrich Chemical Company. $MgSO_4$ was from Mallickrodt, sodium hydroxide and benzene from J. T. Baker, and diethyl ether from Fisher Scientific. All chemicals were of highest purity and were used without further purification. Solutions of $ZnCl_2$, $CdCl_2$, $MgCl_2$ and $CaCl_2$ were standardized complexometrically.

*Present address: Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, H-Y010 Debrecen 10, Hungary.
**Author to whom correspondence should be addressed.

0020-1693/92/$5.00

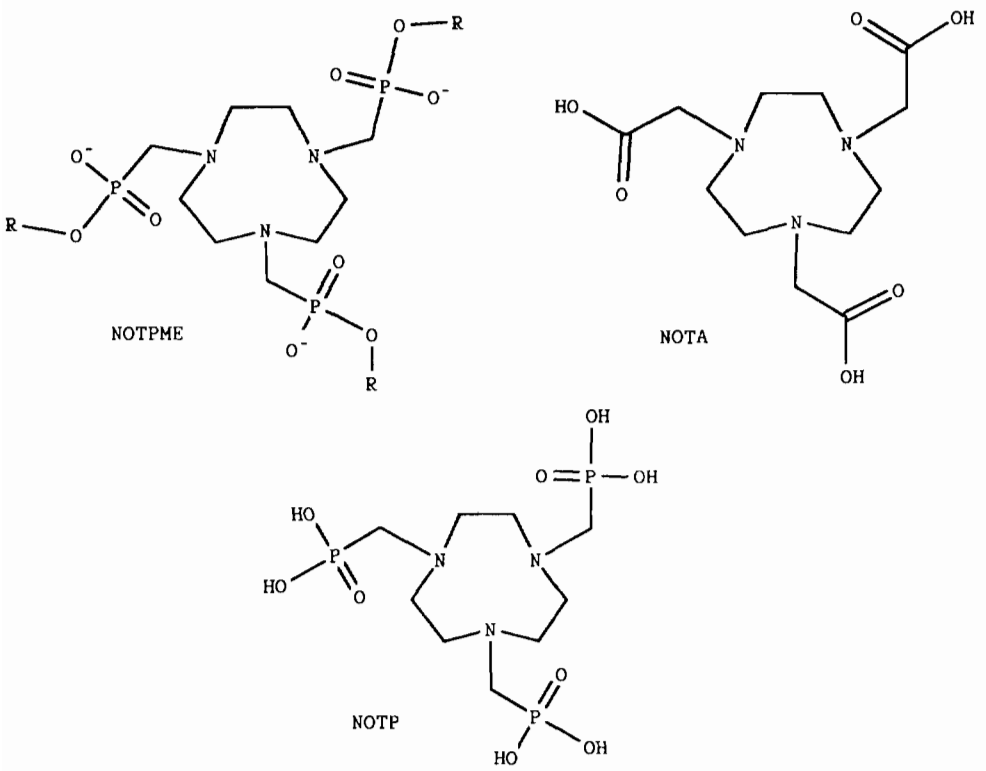

Fig. 1. Structure of NOTPME (R = CH_3CH_2), NOTP and NOTA.

Synthesis of NOTPME

1,4,7-Triazacyclononane (1.91 g, 14.71 mmol) and diethylphosphite (7.018 g, 16.94 mmol, 15% excess) were dissolved in 125 ml of benzene and heated to reflux. Anhydrous paraformaldehyde (1.727 g, 30% excess) was added in small portions to this refluxing mixture while the benzene–water azeotropic mixture was removed by distillation. After addition of paraformaldehyde was complete, the entire solution was boiled for 30 min and then evaporated to obtain a yellow viscous oil. The oil was dissolved in 150 ml anhydrous diethyl ether and dried with anhydrous $MgSO_4$ overnight. $MgSO_4$, along with a white precipitate which formed, were filtered off and discarded. The filtrate was decolorized with activated carbon and filtered. The filtrate was evaporated in vacuum to obtain a viscous oil of 1,4,7-triazacyclononane-*N*,*N*′,*N*″-tris-(methylenephosphonate diethylester) (NOTPDE) 96% yield (9.46 g, 16.33 mmol). ^{1}H NMR, $CDCl_3$, δ (ppm): 1.33 (t, 18H, $-CH_3$), 2.97 (s, 12H, N–CH_2), 3.00 (d, 6H, P–CH_2), 4.13 (p, 12H, O–Ch_2).

NOTPDE (9.44 g, 16.29 mmol) was mixed with 5.0 ml of water and sodium hydroxide pellets (2.5 g) under vigorous stirring. The mixture was refluxed for approximately 15 min. 3.0 ml of water were added and the resulting hot solution was cooled to room temperature. The mixture was cooled on ice for 15 min and hexagonal crystals which formed were filtered off on a pressure filter funnel (70–100 mm porosity) using dry nitrogen gas. The crystals were washed with ice-water, with ethanol–ether mixtures (1:1, 2 ml; 1:10, 6 ml) and finally with anhydrous ether (3×10 ml) and dried to constant weight in high vacuum at 40 °C over phosphorus pentaoxide. Pure Na_3NOTPME (3.87 g, 6.90 mmol, 42.4%) is a hygroscopic solid, soluble in water, methanol and absolute ethanol and sparingly soluble in chloroform. ^{1}H NMR (DCl + D_2O/TSP): 4.10 (p, 6H, O–CH_2), 3.65 (s, 12H, N–CH_2, 3.52 (d, 6H, P–CH_2), 1.31 (t, 9H, $-CH_3$). ^{13}C NMR (D_2O, ref.: MeCN = 1.30): 61.08, 60.95 (O–C), 54.44, 54.31 (C_{ring}), 53.43 (d, N–C–P, J(CP) = 146 Hz), 16.61, 16.49 ($-CH_3$).

NMR measurements

^{1}H NMR data were collected using either a JEOL FX-200 NMR spectrometer or a General Electric GN-500 NMR spectrometer. ^{31}P NMR shifts for NOTPME and its complexes were measured using 85% H_3PO_4 as an external standard. Probe temperatures were accurate to ±0.5 °C.

Potentiometric measurements

pH-potentiometric titrations were conducted at 25 °C using a Corning Ion Analyzer 250 pH meter, an Orion 8103 ROSS combination electrode and a Metrohm

665 Dosimat automatic burette (Brinkman Instruments). The hydrogen ion concentration was obtained from the measured pH values by the method suggested by Irving *et al.* [10]. Na_3NOTPME was dissolved in 0.1 M tetramethylammonium chloride, the pH adjusted to a low pH value with HCl and titrated with 0.098 M KOH. KOH was standardized by potentiometric titration against potassium hydrogen phthalate and stored under an N_2 atmosphere. The hydrogen ion activity coefficient and K_w were determined separately in these same salt solutions. The stability constants of all complexes reported in Table 1 were determined by potentiometric titration of solutions containing 1:1 ratio of metal and ligand. In all of the titrations, samples were covered by a layer of cyclohexane to exclude CO_2.

Protonation constants (K_{H_iL}) and stability constants (K_{ML} and K_{MHL}) are defined by the following equations:

$$K_{H_iL} = [H_iL]/\{[H_{i-1}L][H^+]\} \quad (1)$$

$$K_{ML} = [ML]/\{[M][L]\} \quad (2)$$

$$K_{MHL} = [MHL]/\{[ML][H^+]\} \quad (3)$$

The protonation and stability constants were obtained from the potentiometric data using a simplex non-linear algorithm [11] run on an IBM PC.

Results

Protonation studies

Potentiometric titration curves for NOTPME were obtained in 0.1 M tetramethylammonium chloride at 25 °C. Three protonation constants ($pK_1 = 11.8$, $pK_2 = 3.65$ and $pK_3 = 1.4$) were obtained from those data. The ^{31}P NMR spectrum of NOTPME was also measured as a function of pH (Fig. 2). The protonation constants obtained from these data were in general agreement with those obtained by potentiometry. The phosphorus shifts are very sensitive to protonation of the nitrogens in the polyaza ring, similar to that observed for the parent phosphonate NOTP [8]. The first two protonations (log $K = 11.8$, 3.65) result in ^{31}P shifts to low frequency which are consistent with protonation at two ring nitrogens [8]. This indicates that the first nitrogen protonations in NOTP and NOTPME are quite similar (log $K_1 = 12.1$ versus 11.8, respectively) while the second nitrogen protonations are dramatically different (log $K_2 = 9.4$ versus 3.65). These differences in pK_2 likely reflect differences in the ability of the phosphonate versus the phosphonate monoester side chains to form internal hydrogen bonds with the protonated nitrogens. This would be consistent with the greater basicity of the phosphonate oxygens in NOTP (protonation constants between 6.0 and 7.5) relative to the phosphonate ester oxygens in NOTPME (protonations below 1.4). The ^{31}P NMR spectra of NOTPME exhibited a single resonance over the entire pH range, indicating rapid exchange between all protonated species.

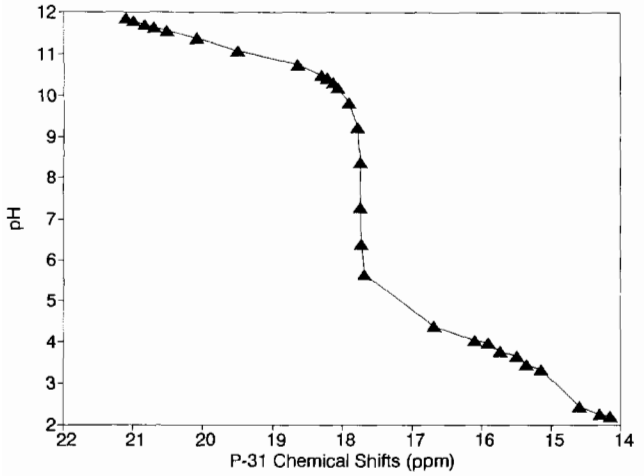

Fig. 2. pH dependence of the ^{31}P chemical shift of the ligand NOTPME in D_2O.

Complexation studies

Potentiometric titrations were also carried out for NOTPME in the presence of one equivalent of Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, La^{3+}, Ce^{3+}, Gd^{3+} and Y^{3+}. Each of these ions forms 1:1 complexes quite rapidly in solution. The stability constants derived from potentiometric titration data for a variety of metal ion–NOTPME solutions are summarized in Table 1 and compared with those available in the literature for the corresponding NOTP and NOTA [4, 6, 12–14] complexes. Of those ions studied, NOTPME forms the most stable complexes with Zn^{2+} and Cd^{2+} followed by the lanthanides, yttrium, Mg^{2+} and Ca^{2+}. This

TABLE 1. Comparison of metal ion stability constants for NOTPME and the ligands NOTP and NOTA

	log K_{ML} (log K_{MHL})		
	NOTPME[a]	NOTP	NOTA
Mg^{2+}	6.2(–)	11.0(5.4)[b]	9.69[d]
Ca^{2+}	5.1(–)	6.4(2.7)[b]	8.92[d]
Zn^{2+}	15.8(–)	24.9(18.3)[b]	18.3[e]
Cd^{2+}	13.4(–)	19.7(13.9)[b]	16.0[e]
La^{3+}	8.7(6.1)	14.3(10.2)[b]	13.5[f]
Ce^{3+}	9.5(5.7)	[c]	13.2[f]
Gd^{3+}	10.3(4.6)	[c]	14.4[f]
Y^{3+}	10.4(4.8)	[c]	[c]

[a]Present work (0.1 M NMe_4Cl, 25 °C). [b]From ref. 6 (1.0 M KNO_3, 25 °C). [c]Not determined. [d]From ref. 12 (0.1 M $NaNO_3$, 25 °C). [e]From ref. 13 (0.1 M KCl, 25 °C). [f]From ref. 4 (0.1 M NaCl, 25 °C).

relative order is also observed for the ligands, NOTP and NOTA. Protonated species appear to exist below pH 5–6 for lanthanide and Y–NOTPME complexes, the values of which vary with cation size (complexes with smaller cations are protonated at lower pH values, Table 1).

Zn^{2+} forms a considerably more stable complex with NOTPME than does Mg^{2+} or Ca^{2+}. This reflects the greater propensity of Zn^{2+} to form M^{2+}–N bonds than more ionic species, Mg^{2+} and Ca^{2+}. Both $Mg(NOTPME)^-$ and $Ca(NOTPME)^-$ are less stable than their respective NOTP and NOTA complexes [6, 12], which again reflects the more acidic nature of phosphonate ester side-chain ligands. However, NOTPME, like NOTP and NOTA, forms more stable complexes with the smaller Mg^{2+} ion than with Ca^{2+}, reflecting from the size selectivity of the triazacyclononane macrocycle in all three ligands. Similar size selectivity effects and side-chain acidity effects were observed for the lanthanide complexes of NOTPME, NOTP and NOTA (Table 1).

The ^{31}P NMR spectra of several 1:1 Ln(NOTPME) complexes (10 mM solutions) are shown in Fig. 3. The spectra of all paramagnetic complexes appear to have a common feature of one (usually dominant) highly shifted resonance (see the Nd complex for example) and three or four secondary less paramagnetically shifted resonances. The spectrum of Ce(NOTPME) has been examined in more detail as a function of concentration and pH. Those studies showed that the secondary resonances disappear upon dilution to 0.1 mM or when the solution pH is lowered to 4. This suggests that the single resonance present at low concentrations or at low pH is characteristic of a monomeric Ln(NOTPME) species. In the case of Lu(NOTPME) at pH 6.6, for example, we found that dilution to 0.1 mM did not totally remove the secondary peaks. The differences between the La(NOTPME) and Lu(NOTPME) spectra clearly demonstrate the greater propensity for the smaller lanthanide cation complexes to form these yet undefined 'oligomeric' species. ^{31}P NMR spectra of Y(NOTPME) also shows multiple resonances similar to those of Lu(NOTPME) while In(NOTPME) and Sc(NOTPME) show single resonances similar to that of La(NOTPME) (data not shown).

The ^{31}P NMR spectra of the Zn^{2+}, Ca^{2+} and Mg^{2+} NOTPME complexes show single resonances for each complex at all concentrations examined. These complexes show no tendency to form oligomeric species.

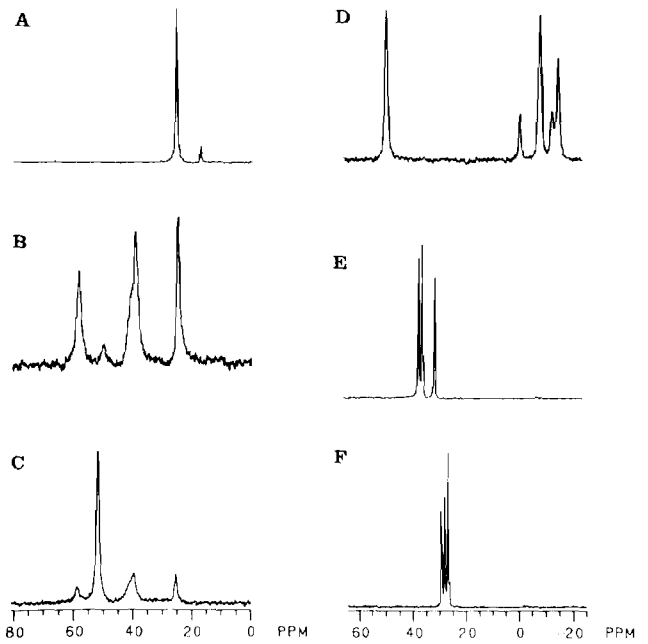

Fig. 3. ^{31}P NMR spectra of the Ln(NOTPME) complexes in aqueous solution. The lanthanide cation was (A) La^{3+}; (B) and (C) Ce^{3+}; (D) Nd^{3+}; (E) Sm^{3+}; (F) Lu^{3+}. The concentrations were 10 mM for all solutions and the pH was 7.4 for all solutions except (C) (pH 5.1).

Discussion

The complex forming characteristics of NOTPME are strongly influenced by the very high value of the first protonation constant as well as by the conformational and size requirements of the triaza macrocyclic ring. The fact that Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, La^{3+}, Ce^{3+}, Gd^{3+} and Y^{3+} complexes of NOTPME could be studied by the usual potentiometric methods indicates that the complexes are formed relatively quickly in solution. As summarized in Table 1, Zn^{2+} forms a more stable complex with NOTPME than does either Mg^{2+} or Ca^{2+}. This same trend was observed for the triazacyclononane triacetate chelate, NOTA, with these same ions; reported log K values for $Zn(NOTA)^-$, $Mg(NOTA)^-$ and $Ca(NOTA)^-$ are 18.3, 9.69 and 8.92, respectively (Table 1) [12, 13]. Thus, one might conclude that the carboxylate chelate forms more stable complexes with all three metal ions largely due to the greater basicity of the carboxylate oxygen ligands over the phosphonate oxygen ligands. The parent phosphonate, NOTP, shows the same trend in its metal complexing ability with Zn^{2+}, Mg^{2+} and Ca^{2+} but in this case the stability constants of the resulting complexes (with the exception of Ca^{2+}) are several orders of magnitude higher than the corresponding values with NOTPME or NOTA. The reported log K values for $Zn(NOTP)^{4-}$, $Mg(NOTP)^{4-}$ and $Ca(NOTP)^{4-}$ are 24.9, 11.0 and 6.4, respectively (Table 1) [6]. Since the nitrogens and the phosphonate oxygens in NOTP are considerably more basic than those in NOTPME, the resulting stability

constants with these metal ions are all larger. The trend $Zn^{2+} \gg Mg^{2+} > Ca^{2+}$, however, is preserved.

NOTPME forms considerably less stable complexes with the trivalent lanthanides than the ligands, NOTP (methylenephosphonate arms) or NOTA (acetate arms). The lanthanide–NOTPME complexes tend to form multiple species in solution at concentrations above 0.1 mM that are in slow exchange on the ^{31}P NMR time scale. Since lanthanide–NOTP complexes have previously been shown to aggregate in solution [7], we tentatively ascribe the observations reported here as reflecting multiple oligomeric forms of the neutral Ln(NOTPME) complexes, perhaps due to the weakly coordinated phosphonate monoester pendant arms forming bridging dimers, trimers, etc. Such formation of aggregates has also been proposed in order to explain the relaxivity behavior of a 1 mM Gd(NOTPME) solution [14]. This does not appear to happen with the monoprotonated lanthanide–NOTPME complexes (charged), the divalent cation complexes of NOTPME (charged), or with the Sc^{3+} and In^{3+} complexes of NOTPME (neutral). Therefore, aggregation of these complexes, as judged by ^{31}P NMR, seems to predominate with Y^{3+} and the smaller Ln^{3+} cation complexes.

Acknowledgements

We thank the Meadows Foundation and the Robert A. Welch Foundation (At-584) for financial support.

References

1 D. C. Buster, M. M. C. A. Castro, C. F. G. C. Geraldes, C. R. Malloy, A. D. Sherry and T. C. Siemers, *Magn. Reson. Med., 15* (1990) 25.
2 (a) V. M. Runge, D. Y. Gelblum, M. L. Pacetti, F. Carloan and G. Heard, *Radiology, 177* (1990) 393; (b) J.-C. Bousquet, S. Saini, D. D. Stark, P. F. Hahn, M. Nigam, J. Wittenberg and J. T. Ferrucci, *Radiology, 166* (1988) 693.
3 R. Ramasamy, I. Lazar, E. Brücher, A. D. Sherry and C. R. Malloy, *FEBS Lett., 280* (1991) 121.
4 W. P. Cacheris, S. K. Nickle and A. D. Sherry, *Inorg. Chem., 26* (1987) 958.
5 E. Brücher and A. D. Sherry, *Inorg. Chem., 29* (1990) 1555.
6 M. I. Kabachnik, T. Ya. Medved, Yu. M. Polikarpov, B. K. Shcherbakov, F. I. Bel'skii, E. I. Matrosov and M. P. Pasechnik, *Izv. Akad. Nauk SSSR, Ser. Khim.,* (1984) 769.
7 C. F. G. C. Geraldes, R. D. Brown III, W. P. Cacheris, S. H. Koenig, A. D. Sherry and M. Spiller, *Magn. Reson. Med., 9* (1989) 94.
8 C. F. G. C. Geraldes, A. D. Sherry and W. P. Cacheris, *Inorg. Chem., 28* (1989) 336.
9 S. Cortes, E. Brücher, C. F. G. C. Geraldes and A. D. Sherry, *Inorg. Chem., 29* (1990) 5.
10 H. M. Irving, M. C. Miles and L. D. Petit, *Anal. Chim. Acta, 38* (1967) 475.
11 M. S. Caceci and W. P. Cacheris, *Byte, 5* (1984) 340.
12 A. Bevilacqua, R. T. Gelb, W. B. Hobard and L. F. Zompa, *Inorg. Chem., 26* (1987) 2699.
13 A. Hama and S. Takamoto, *Nippon Kagakin Kaishi,* (1975) 1182.
14 C. F. G. C. Geraldes, R. D. Brown III, E. Brücher, S. H. Koenig, A. D. Sherry and M. Spiller, *Magn. Reson. Med.,* in press.