

# Molecular Structure and Dynamics of Aminopolycarboxylates and their Lanthanide Ion Complexes

Rune Fosshem<sup>a,b,\*</sup> and Svein G. Dahl<sup>a</sup>

<sup>a</sup>Institute of Medical Biology, University of Tromsø, P.O. Box 977, N-9001 Tromsø and <sup>b</sup>Nycomed AS, P.O. Box 4220 Torshov, N-0401 Oslo 4, Norway

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Molecular mechanics calculations and molecular dynamics simulations have been used to examine the molecular structures and dynamics of the four aminopolycarboxylate ligands, DOTA (1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid), DTPA (1,4,7-triazaheptane-1,1,4,7,7-pentaacetic acid), OTTA (1-oxa-4,7,10-triazacyclododecane-*N,N',N''*-triacetic acid), DO3A (1,4,7,10-tetraazacyclododecane-*N,N',N''*-triacetic acid), and their Gd(III) ion complexes. The results indicate that the *in vacuo* stability of the complexes is determined mainly by the number of liganding atoms and their charges, and by the increase in conformational energy of the ligand by complexation. The calculated reaction energies for complexation of DOTA, DTPA and DO3A with a Gd(III) ion are in reasonable agreement with the relative order of magnitude of experimental  $\log K$  values. Molecular dynamics simulations of the complexation reactions of DOTA and DTPA with a hydrated Gd(III) ion suggest cation coordination to one of the carboxylate groups as the first step in the formation of the complex.

Aminopolycarboxylates including DOTA (1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) and DTPA (1,4,7-triazaheptane-1,1,4,7,7-pentaacetic acid) form highly stable complexes with lanthanide ions.<sup>1</sup> Because gadolinium has a high magnetic moment, complexes with Gd(III) are used as contrast agents in magnetic resonance imaging techniques.<sup>1,2</sup> Since lanthanide ions are highly toxic in man,<sup>2</sup> the thermodynamic and kinetic stabilities of such complexes are important for their diagnostic properties and toxicity. The stabilities are dependent on stereochemical features of the ligands, since the ions show essentially no directional bonding preference.<sup>1</sup>

In order to gain further insight into the factors determining the stability of such complexes, molecular mechanics calculations and molecular dynamics simulations were performed on the aminopolycarboxylate derivatives shown in Fig. 1 and their Gd(III) complexes. Hydration effects may also be important for the stability of complex ions. Molecular mechanics calculations were therefore performed for the derivatives both *in vacuo* and with hydrated ligands and complexes, in order to examine solvation effects.

Complexation reactions of a hydrated Gd(III) ion with DOTA and DTPA were examined by molecular dynamics simulations, in order to examine the occurrence of structural changes when a lanthanide ion is encapsulated by such multidentate ligands.

\* To whom correspondence should be addressed.

## Methods

Molecular mechanics calculations and molecular dynamics simulations were performed with the AMBER 3.0 software package<sup>3</sup> on a VAX 8600/VMS system. Molecular graphics were performed with the MIDAS programs<sup>4</sup> on an Evans and Sutherland PS390 work-station with a MicroVax II/ Ultrix system as the host computer.

*Atomic point charges.* Atomic point charges,<sup>5</sup> which were used in the molecular mechanics calculations and molecular dynamics simulations, were obtained from *ab initio* calculations with the program QUEST 1.0,<sup>5</sup> which is based on the GAUSSIAN-80 program.<sup>6</sup> QUEST calculates quantum mechanical electrostatic potentials in a number of layers around the molecule, and atomic point charges by least-squares fitting to the electrostatic potentials. The *ab initio* calculations of electrostatic potentials and atomic charges were performed on a CRAY X-MP supercomputer using a STO-3G basis set. Owing to the size of the ligands, the quantum mechanical calculations were performed on two overlapping fragments of the ions. All calculations were performed on unprotonated ions.

*Geometry and molecular dynamics of free ligands.* Starting geometries for DOTA and DTPA were obtained from crystal structures of Na[EuDOTA]<sup>7</sup> and Ba[NdDTPA].<sup>8</sup> A starting geometry for DO3A (1,4,7,10-tetraazacyclododecane-*N,N',N''*-triacetic acid) was obtained by sub-

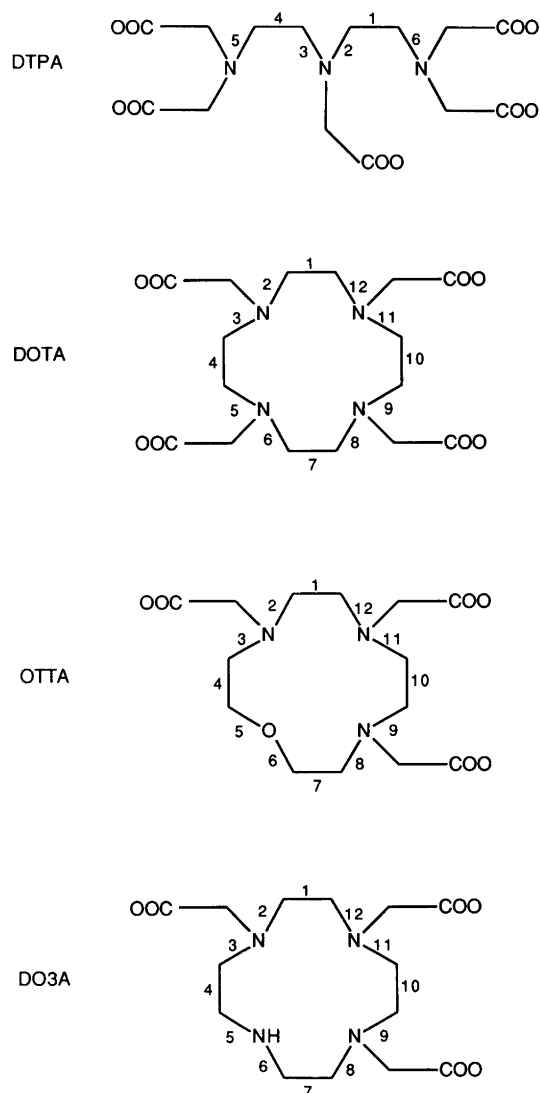


Fig. 1. Structural formulas of aminopolycarboxylate ligands with numbering of torsion angles.

stitution of one  $-\text{CH}_2\text{COO}$  group in DOTA with a hydrogen atom. From this DO3A structure the starting geometry of OTTA (1-oxa-4,7,10-triazacyclododecane- $N,N',N''$ -triacetic acid) was obtained by substitution of the  $-\text{NH}$  group with an oxygen atom.

The following calculations were performed on each of the four ligands. (1) The geometries were initially optimized by energy minimization without electrostatic interactions. (2) Based on these preliminary structures, atomic point charges were calculated. (3) Further geometry optimization was performed by energy minimization including electrostatic interactions. (4) The ligand structures calculated with electrostatic interactions were used as starting points for molecular dynamics simulations. All simulations were performed over 10 ps intervals at 300 K, after an initial 1 ps equilibration period starting at 0.1 K. The coordinates were stored 100 times during each simulation. (5) Among the coordinate sets obtained in each molecular

dynamics simulation the three structures of lowest potential energy were selected and used as starting points for geometry optimization by energy minimization.

In order to examine hydration effects, each of the four lowest-energy conformers of the ligands were submerged in a layer of water, approximately 8 Å thick. The energy of the solute and solvent was minimized using a water-oxygen charge  $Q_{\text{ow}} = -0.66$ . This value has previously been found to give reasonable water/chromophore hydrogen bonding structures and energies.<sup>9</sup> The energy minimizations in aqueous solution were performed with a constant dielectric function,  $\epsilon = 1.0$ . Energy minimizations and molecular dynamics simulations *in vacuo* were performed with a distance dependent dielectric,  $\epsilon = r_{ij}$ , where  $r_{ij}$  is the interatomic distance.

**Force-field parameters.** In order to perform molecular mechanics calculations on lanthanide ion complexes, parameters describing non-bonded interactions between ligand and cation had to be determined. Quantum mechanical calculations on lanthanide ion complexes is not feasible with the QUEST 1.0 software package. However, Eu(III) and Gd(III) have similar ionic radii, differing only by 0.01 Å. Therefore the experimentally determined coordination geometry of  $[\text{EuDOTA}]\cdot\text{H}_2\text{O}$  in crystals was used to determine the van der Waals radius ( $R_{\text{Gd}}^*$ ) and well-depth ( $e_{\text{Gd}}^*$ ) describing the Lennard-Jones potential of the Gd(III) cation, and to obtain a reasonable charge model for the complex.

The geometry of the GdDOTA complex was optimized by energy refinements and systematically changing the values of  $R_{\text{Gd}}^*$ ,  $e_{\text{Gd}}^*$  and ring nitrogen atom charge ( $Q_{\text{N}}$ ) between each minimization. The best fit to the  $[\text{EuDOTA}]\cdot\text{H}_2\text{O}$  crystal structure was obtained with  $R_{\text{Gd}}^* = 1.8$ ,  $e_{\text{Gd}}^* = 0.2$  and  $Q_{\text{N}} = -0.4$ . These parameters, which were used in the subsequent calculations, gave average distances of  $\text{Gd}-\text{O} = 2.404$  Å and  $\text{Gd}-\text{N} = 2.683$  Å, and a total bond strength<sup>10</sup> around the cation of  $S = 2.98$ . The corresponding values in the  $[\text{EuDOTA}]\cdot\text{H}_2\text{O}$  crystal structure were  $\text{Eu}-\text{O} = 2.390$  Å,  $\text{Eu}-\text{N} = 2.678$  Å and  $S = 3.14$ .

The derived atomic charge of nitrogen in complexes ( $Q_{\text{N}} = -0.4$ ) was slightly larger than the nitrogen charges calculated for free ligands, which ranged from  $-0.1$  to  $-0.3$ . The total charge of each ligand was therefore corrected by distributing the excess charge on the adjacent carbon atoms.

In order to derive hydration energies of coordinated water molecules in the complexes, the atomic charge ( $Q_{\text{ow}}$ ) and van der Waals radius ( $R_{\text{ow}}^*$ ) of water oxygen were determined from a system in which a Gd(III) ion coordinated to nine water molecules, was submerged in a layer of water, 5 Å thick. The total energy of the solvated ion and water molecules was minimized with various values of the atomic charge ( $Q_{\text{ow}}$ ) and van der Waals radius ( $R_{\text{ow}}^*$ ) of water oxygen, which were systematically changed between each minimization. The best fit to experimental values was obtained with  $Q_{\text{ow}} = -0.7$  and  $R_{\text{ow}}^* = 1.67$ , which gave a

Gd(III) ion–water interaction energy of  $-939 \text{ kcal mol}^{-1}$  and a mean cation–water distance of  $2.52 \text{ \AA}$  for the solvated Gd(III) ion. It has been reported that experimental total hydration energies were  $-874.7 \text{ kcal mol}^{-1}$  for Eu(III)<sup>11</sup> and  $-880.4 \text{ kcal mol}^{-1}$  for Gd(III),<sup>11</sup> and that the mean Eu–O distance is  $2.46 \text{ \AA}$  in a nona-aqua-coordinated Eu complex.<sup>12</sup>

*Geometry and energies of Gd(III) ion complexes.* Starting geometries for the four Gd complexes were obtained the same way as for the free ligands. Structures of GdDOTA and GdDTPA were obtained from crystal structures of Na[EuDOTA]<sup>7</sup> and Ba[NdDTPA].<sup>8</sup> A starting geometry for GdDO3A was obtained by substitution of one  $-\text{CH}_2\text{COO}$  group in the GdDOTA complex with a hydrogen atom. From this structure the starting geometry for GdOTTA was obtained by substitution of the  $-\text{NH}$  group with an oxygen atom.

The Gd(III) complexes with DTPA, DOTA, OTTA and DO3A were initially energy refined without coordinated water molecules. Around each complex was added a layer of water, approximately  $8 \text{ \AA}$  thick, and the energy of the solute–solvent system was minimized. The number of coordinated water molecules, obtained from the solvated structures after energy minimization, was 1 for GdDOTA and GdDTPA and 2 for GdDO3A and GdOTTA. The layers of water molecules were then removed from the solvated structures and energy minimizations performed on GdDOTA\*H<sub>2</sub>O, GdDTPA\*H<sub>2</sub>O, GdDO3A\*2H<sub>2</sub>O and GdOTTA\*2H<sub>2</sub>O complexes. As for the free ligands, a constant dielectric function ( $\epsilon = 1.0$ ) was used in the energy minimizations with a layer of water, otherwise a distance-dependent dielectric function was used.

The reaction energy of complexation *in vacuo* was calculated as in eqn. (1).  $E_{\text{ML}}$  is the total energy of the

$$E_{\text{R,g}} = E_{\text{ML}} - E_{\text{L}} \quad (1)$$

cation–ligand complex, and  $E_{\text{L}}$  is the energy of the lowest-energy conformer of the free ligand. Since eqn. (2) applies,

$$E_{\text{ML}} = E_{\text{I}} + E_{\text{LC}} \quad (2)$$

where  $E_{\text{I}}$  is the interaction energy between cation and ligand and  $E_{\text{LC}}$  is the energy of the ligand in the complex, and the constraint energy of the ligand in the complex may be defined as in eqn. (3), it follows that the reaction energy

$$E_{\text{D,L}} = E_{\text{LC}} - E_{\text{L}} \quad (3)$$

of complexation *in vacuo* also may be calculated as in eqn. (4).

$$E_{\text{R,g}} = E_{\text{I}} + E_{\text{D,L}} \quad (4)$$

The influence of two different hydration effects on complex stability was considered: (1) energy difference between hydration of carboxylate groups in free ligands and in complexes ( $E_{\text{H1}}$ ) and (2) hydration of the lanthanide ion in a complex by occupancy of vacant coordination sites ( $E_{\text{H2}}$ ). The hydration energy of the carboxylate groups was calculated as in eqn. (5), where  $E_{\text{H,ML}}$  and  $E_{\text{H,L}}$  are the

$$E_{\text{H1}} = E_{\text{H,ML}} - E_{\text{H,L}} + E_{\text{H,W}} \quad (5)$$

interaction energies between water molecules H-bonded to uncoordinated carboxylate groups in complexes (ML) and ligands (L). The water–water interaction energy was calculated as in eqn. (6), where  $n$  is the number of

$$E_{\text{H,W}} = -5.2 * n \quad (6)$$

carboxylate–water hydrogen bonds lost by complexation. The interaction energy of a water dimer ( $-5.2 \text{ kcal mol}^{-1}$ ) was calculated by molecular mechanics, using the AMBER force field.<sup>3</sup> Eqn. (6) is based on the assumption that each carboxylate–water hydrogen bond is exchanged with a water–water hydrogen bond.

The hydration energy of the lanthanide ion was calculated as in eqn. (7), where  $E_{\text{H,M}}$  is the mono- or

$$E_{\text{H2}} = E_{\text{H,M}} + E_{\text{H,W}} \quad (7)$$

di-hydration energy of the complexes in the molecular mechanics calculations. The Gd(III) ion loses one more water molecule by complexation with DTPA and DOTA than with OTTA and DO3A, such that two more water–water hydrogen bonds are formed by complexation with DTPA and DOTA than with OTTA and DO3A. Owing to this difference  $E_{\text{H,W}} = -10.4 \text{ kcal mol}^{-1}$  for DTPA and DOTA ( $n = 2$ ) and  $E_{\text{H,W}} = 0$  for DO3A and OTTA ( $n = 0$ ). Since only the relative energies are considered of interest in this context, hydration energies of the lanthanide ions were not included in the calculations.

The reaction energy in aqueous environment,  $E_{\text{R,aq}}$  was calculated as in eqn. (8).

$$E_{\text{R,aq}} = E_{\text{R,g}} + E_{\text{H1}} + E_{\text{H2}} \quad (8)$$

*Molecular dynamics of complexation.* The complexation process of a hydrated Gd(III) ion with DOTA and DTPA was examined by molecular dynamics simulations. Starting models were obtained with the computer graphics system by placing a nona-aqua coordinated Gd(III) ion approximately  $10 \text{ \AA}$  from the center of the free ligand. From this model a 5 ps molecular dynamics simulation was started at  $0.1 \text{ K}$ . A final temperature of  $300 \text{ K}$  was reached after approximately  $0.1 \text{ ps}$ . The coordinates were stored at  $0.1 \text{ ps}$  intervals.

Table 1. Calculated conformations and relative energies of free ligands.

Ligand	Conf. No.	Torsion angle No. <sup>a</sup>												Relative energy/ kcal mol <sup>-1</sup>
		1	2	3	4	5	6	7	8	9	10	11	12	
DOTA	1	-g	-g	a	-g	-g	a	-g	-g	a	-g	-g	a	23.6
	2	-g	-g	a	-g	+g	-g	a	-g	a	-g	-g	a	5.1
	3	-g	-g	a	a	+g	+g	a	+g	+g	-g	-g	a	0.3
	4	-g	-g	a	a	+g	+g	a	+g	+g	-g	-g	a	0.0
OTTA	1	-g	-g	a	-g	-g	a	-g	-g	a	a	-g	a	11.6
	2	-g	-g	a	-g	+g	a	+g	-g	a	a	+g	+g	6.1
	3	-g	-g	a	-g	a	a	+g	-g	+g	a	+g	+g	4.3
	4	-g	-g	a	-g	a	a	+g	-g	+g	a	+g	+g	0.0
DO3A	1	-g	-g	a	-g	+g	a	-g	+g	+g	-g	-g	a	15.1
	2	-g	-g	+g	-g	a	a	+g	-g	+g	-g	-g	a	0.0
	3	-g	-g	a	-g	a	a	+g	-g	-g	-g	-g	a	0.2
	4	-g	-g	a	-g	a	a	+g	-g	a	-g	-g	a	0.2
DTPA	1	a	-g	a	a	a	a							5.5
	2	a	a	a	a	+g	a							3.0
	3	a	-g	a	a	+g	a							0.5
	4	a	-g	a	a	+g	a							0.0

<sup>a</sup>Torsion angles: *a* (*anti*): 120.0–240.0°, *-g* (*gauche*): -120.0–0.0°, *+g* (*gauche*): 0.0–120.0°. The numbering of torsion angles is shown in Fig. 1.

## Results and discussion

**Structure and energy of free ligands.** Calculated torsion angles of the backbone structure and relative energies of each ligand are given in Table 1. For each ligand, conformation 1 is the one obtained from the starting structure and conformations 2–4 are those obtained from molecular dynamics simulations and subsequent energy minimizations. Stereographic drawings of the lowest-energy conformation of each ligand are shown in Fig. 2.

The results given in Table 1 indicate that the 'square' conformation of the 12-membered rings, previously labeled [3333],<sup>13</sup> is not energetically favored in the free ligands. This conformation (1) of DOTA, which also was found in the Eu-complex,<sup>7</sup> is 23.6 kcal mol<sup>-1</sup> higher in energy than conformation 4 (Table 1). Our data indicate that the low-energy conformations of these ligands usually have carboxylate groups pointing to both sides of the macrocyclic ring plane.

All N–C–C–N torsion angles in the non-cyclic ligand DTPA are *anti* (Table 1) and the ligand has an extended conformation, which is apparently preferred owing to the bulky and negatively charged *N*-substituents. A similar situation is not found in the cyclic ligands, where conformational constraints are imposed by ring-closure. In the low-energy conformers of DOTA two of the N–C–C–N torsion angles (Nos. 1 and 10) are *gauche*, while the two other (Nos. 4 and 7) are *anti* (Table 1). Of the four OTTA conformers only one of the N–C–C–N torsion angles is *anti*, and none of the N–C–C–N torsion angles are *anti* in the calculated DO3A conformers (Table 1). All dihedral

angles involving unsubstituted N and O ring atoms are *gauche*. The fact that OTTA and DO3A only have three ring substituents whereas DOTA has four, may explain this conformational distinction. Furthermore, in DO3A the N-attached H atom of the macrocyclic ring points towards the ring center in all four conformers, presumably as a result of a favorable electrostatic interaction across the ring system.

**Structure and energy of Gd(III) complexes.** The ionic radius of Gd<sup>3+</sup> (1.02 Å) lies between those of Na<sup>+</sup> (0.95 Å) and K<sup>+</sup> (1.33 Å). The *R*\* values of Na<sup>+</sup> and K<sup>+</sup> used in the AMBER force field<sup>14</sup> are 1.6 and 2.0, respectively, and *R*\* values in the range 1.7–1.8 therefore seem reasonable for Eu(III) and Gd(III). The accuracy of the calculated parameters for the lanthanide ions does not permit distinction between the properties of the Gd(III) and Eu(III) ions.

Energy minimizations with charges calculated from free ligands gave longer Gd–N distances and shorter Gd–O distances than in the EuDOTA crystal structure. The most likely reason is that polarization effects were not accounted for in the calculations of atomic charges of free ligands. This justifies the slightly higher atomic charge on nitrogen (*Q*<sub>N</sub> = -0.4) used for molecular mechanical calculations of the Gd(III) complexes.

Stereographic drawings of the four mono- and dihydrated complexes after energy refinement are shown in Fig. 3, and the energies are given in Table 2 together with the average cation–N and cation–O(carboxylate) distances. The coordination polyhedra of the four nine-

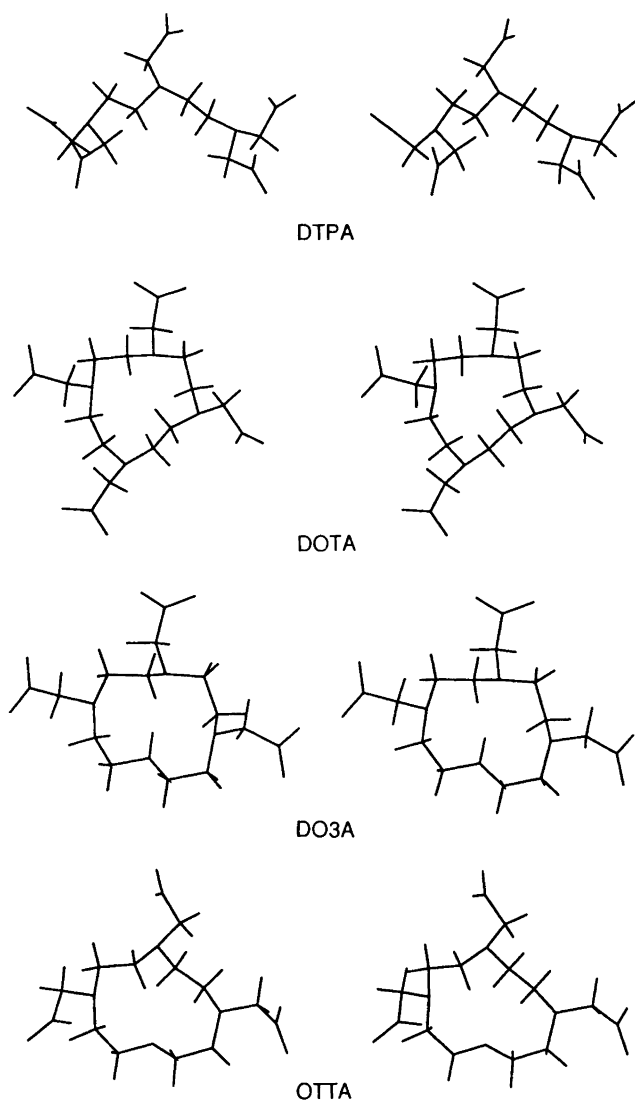


Fig. 2. Stereoscopic drawings of DOTA, DTPA, OTTA and DO3A free ligands.

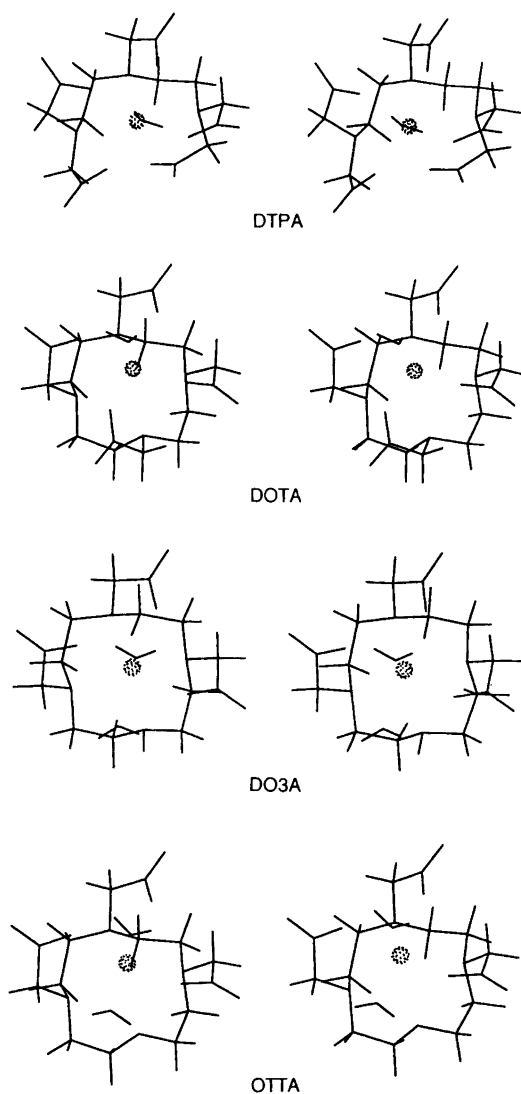


Fig. 3. Stereoscopic drawings of monohydrated DOTA and DTPA and dihydrated OTTA and DO3A Gd(III) complexes.

Table 2. Total energy, constraint energy of ligand by complexation, ligand-ion interaction energy, mono- and di-hydration energies of Gd(III) ion in complex, and interatomic distances in Gd(III) ion complexes *in vacuo*.

Complex (Coord. number)	Energy / kcal mol <sup>-1</sup>				Average distance / Å	
	Total	Ligand constraint, $E_{D,L}$	Inter- action, $E_i$	Hydration, $E_{H,M}$	Gd-O	Gd-N
GdDOTA (8)	-412.6	93.8			2.384	2.636
GdDOTA·H <sub>2</sub> O (9)	-429.6	91.7	-540.8	-19.2	2.404	2.683
GdDTPA (8)	-411.7	122.1			2.378	2.703
GdDTPA·H <sub>2</sub> O (9)	-428.6	120.1	-596.8	-19.5	2.408	2.766
GdOTTA (7)	-355.1	59.4			2.365	2.594
GdOTTA·2H <sub>2</sub> O (9)	-399.7	57.7	-432.8	-26.5, -23.7	2.394	2.657
GdDO3A (7)	-360.0	66.2			2.368	2.600
GdDO3A·2H <sub>2</sub> O (9)	-403.3	63.5	-435.7	-26.3, -22.9	2.398	2.662

**Table 3.** Difference in hydration energy of carboxylate groups in complex and free ligand ( $E_{\text{H1}}$ ), total hydration energy of lanthanide ion in complex, corrected for water–water interactions ( $E_{\text{H2}}$ ), and reaction energies of complex formation *in vacuo* ( $E_{\text{R,g}}$ ) and in aqueous solution ( $E_{\text{R,aq}}$ ). All energies are in kcal mol<sup>-1</sup>.

Complex	$E_{\text{R,g}}$	$E_{\text{H1}}$	$E_{\text{H2}}$	$E_{\text{R,aq}}$
GdDOTA	-449.1	95.7	-29.6	-383.0
GdDTPA	-476.7	146.1	-29.9	-360.5
GdOTTA	-372.2	69.5	-50.2	-352.9
GdDO3A	-375.1	57.3	-49.2	-367.0

coordinate complexes are close to monocapped square antiprisms in GdDOTA, GdDO3A and GdOTTA, and close to a tricapped trigonal prism in GdDTPA. This is in agreement with crystal structural data for DOTA<sup>7</sup> and DTPA.<sup>8</sup> The caps are formed by N1, N3 and O(water) in GdDTPA and by O(water) in the other compounds.

Comparison of the unhydrated complexes with their mono- and di-hydrated counterparts reveals that water coordination renders the ligands slightly less tightly coordinated, and that the average Gd–O(carboxylate) and Gd–N distances are similar for complexes with the same coordination number (Table 2). The distances in the 9-coordinate complexes are in good agreement with those in GdDTPA, determined by X-ray crystallography:<sup>15</sup> average Gd–O(carboxylate) distance 2.404 Å, average Gd–N distance 2.640 Å.

The calculated hydration energies decrease with increasing denticity of the ligands. The hydration energies are larger in DO3A and OTTA than in DOTA and DTPA (Table 2), which may be relevant to the water relaxivity of these paramagnetic complexes. Gain in relaxivity obtained by increase in the number of coordinated water molecules may be partly impaired by a slower exchange rate.

**Complex stability.** Reaction energies of complexation *in vacuo* and in aqueous solution are given in Table 3. The complex stabilities *in vacuo* decrease in the order GdDTPA > GdDOTA >> GdDO3A > GdOTTA. This stability sequence is caused by the relative magnitudes of the constraint energies of ligands in complexes ( $E_{\text{D,L}}$ ) and the cation–ligand interaction energies ( $E_{\text{I}}$ ) (Table 2). The constraint energy of DTPA was larger than that of DOTA, presumably because the five carboxylate groups in DTPA causes a larger ligand–ligand repulsion energy than the four carboxylate groups in DOTA. However, the cation–ligand interaction energy ( $E_{\text{I}}$ ) is larger between Gd(III) and DTPA than between Gd(III) and DOTA (Table 2), which could indicate that GdDTPA is more stable than GdDOTA *in vacuo*. Although the constraint energies by complexation of DO3A and OTTA are relatively small, the loss of cation–ligand interaction energy resulting from lower denticity (Table 2) obviously makes these two complexes less stable *in vacuo* than the other two.

In aqueous solution the oxygen atoms of free ligands and complexes act as acceptors in hydrogen bonds to water molecules. The average interaction energy per hydrogen bond was slightly higher for the ligands (–10.8 kcal mol<sup>-1</sup>) than in the complexes (–10.3 kcal mol<sup>-1</sup>). Both these values are in relatively good agreement with the previously reported interaction energy (–10.5 kcal mol<sup>-1</sup> per hydrogen bond) between a formate ion and a water molecule, calculated by *ab initio* methods.<sup>16</sup>

The binding constants of lanthanide ion–aminopolycarboxylate complexes have been examined by spectrophotometric and other experimental techniques. The reported log *K* values at 298 K and derived free energies (in parentheses) were the following: EuDOTA 28 (–38.2 kcal mol<sup>-1</sup>),<sup>17</sup> GdDOTA 25 (–34.1 kcal mol<sup>-1</sup>)<sup>18</sup> and 24.6 (–33.5 kcal mol<sup>-1</sup>),<sup>19</sup> GdDTPA 22 (–30.0 kcal mol<sup>-1</sup>)<sup>18</sup> and 21.6 (–29.4 kcal mol<sup>-1</sup>),<sup>19</sup> DO3A 20.2 (–27.5 kcal mol<sup>-1</sup>).<sup>19</sup> The reaction energies in aqueous solution ( $E_{\text{R,aq}}$ , Table 3) indicate that GdDOTA is the most stable of the four complexes, and that GdDTPA and GdDO3A are less stable and have somewhat similar stabilities. This is in relatively good agreement with reported log *K* values and the derived free energies, although these indicate that GdDTPA is slightly more stable than GdDO3A. It should be noted, however, that the relationship between free energies and computed reaction energies ( $E_{\text{r,aq}}$ ) does not take into account possible differences in reaction entropies between the acyclic ligand DTPA and the cyclic ones, and between complexes involving one or two water molecules.

The calculated complexation energies *in vacuo* and in aqueous solution indicate that solvation effects are important for the stability of the complexes. The removal of hydrogen-bonded water molecules by complexation of DTPA, disfavors complexation of this ligand compared with those with fewer carboxylate groups ( $E_{\text{H1}}$ , Table 3). It appears, therefore, that although a carboxylate is considered a stronger ligand-binding group *in vacuo* than an amine, this difference is partly counteracted by hydration effects in aqueous solution. Decreasing the desolvation energy of the liganding groups by shielding them from water molecules prior to complexation might therefore be a way of increasing complex stability.

Our results indicate that stability of such complexes would increase if the ligand–cation interaction energy ( $E_{\text{I}}$ ) could be increased and the ligand constraint energy ( $E_{\text{D,L}}$ ) reduced. A way of increasing the ligand–cation interaction energy would be to use strongly liganding groups. It is possible that the ligand constraint energy might be reduced by constraining the free ligand, by covalent bonds, to a conformation which resembles that in the complex.

**Complexation mechanisms.** Figs. 4 and 5 show the starting structures and those observed after 0.5, 1.0 and 5.0 ps of molecular dynamics simulations of the complexation of DOTA and DTPA with a hydrated Gd(III) ion. In both cases the cation is captured by the ligand, resulting in a gradual loss of the coordinated water molecules. The

structures observed at the end of both simulations had one coordinated water molecule and were similar to those obtained by energy minimization. A common feature of the molecular dynamics simulations was that complexation started with coordination of the cation to one of the carboxylate groups, resulting in loss of two or three coordinated water molecules. At this stage of the complexation reaction the Gd(III) ion is symmetrically coordinated to the carboxylate oxygens, which lie in the COO plane in DTPA and are somewhat displaced from this plane in DOTA. Such symmetrical cation-carboxylate coordination

geometries are often found in crystal structures,<sup>20</sup> which indicates that they are energetically favorable. If complexation of aminopolycarboxylates is usually mediated by capture of the cation by the carboxylate groups, then the number and mutual orientation of these groups may be of importance for the kinetics of complex formation. As shown in Fig. 4, one water molecule fits closely into the cavity formed by the DOTA ligand and the incoming cation. In DTPA there are no such steric interactions between the ligand and the leaving water molecules, probably due to the high flexibility of the ligand.

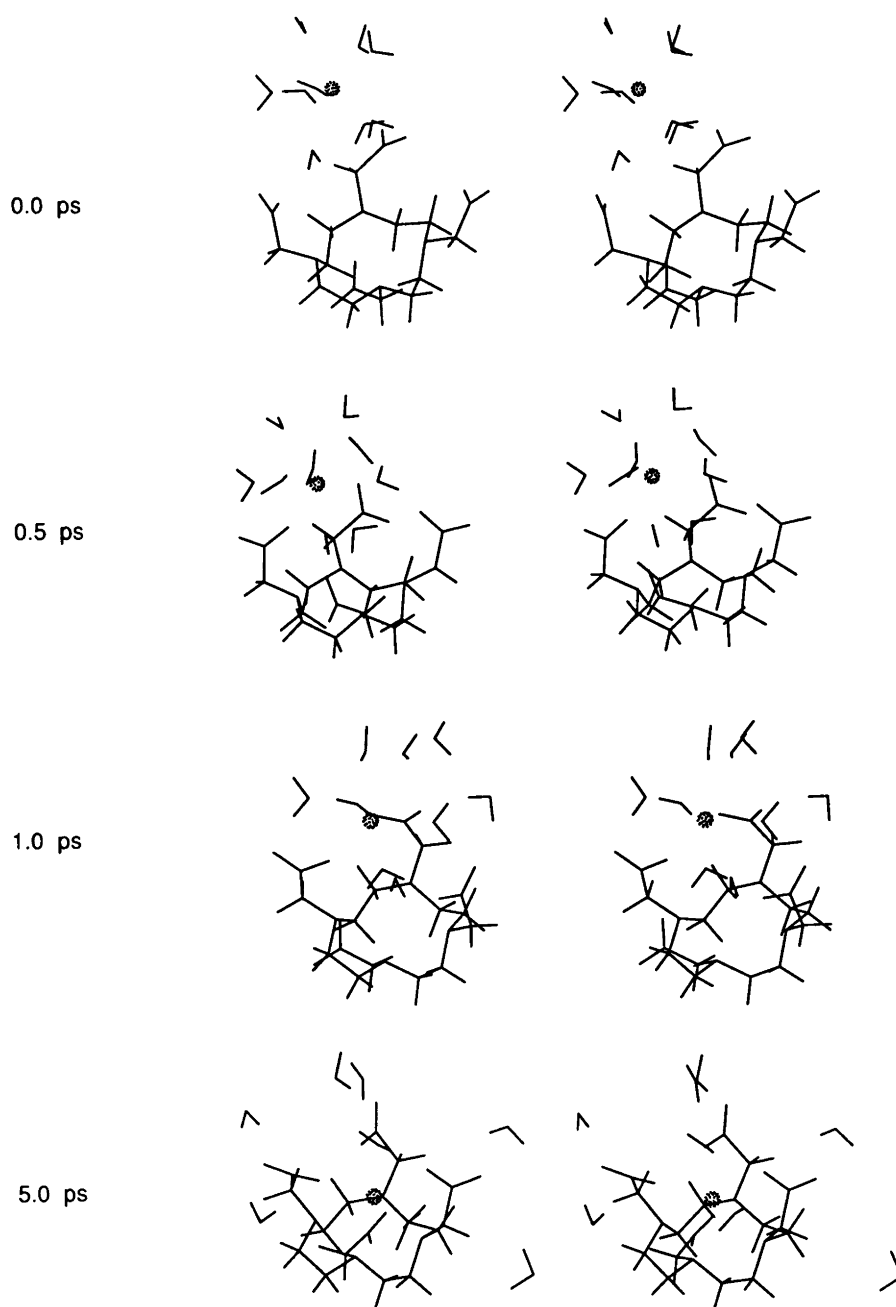


Fig. 4. Stereoscopic drawings of four structures obtained during a molecular dynamics simulation of the complexation of a nonhydrated Gd(III) ion with DOTA.

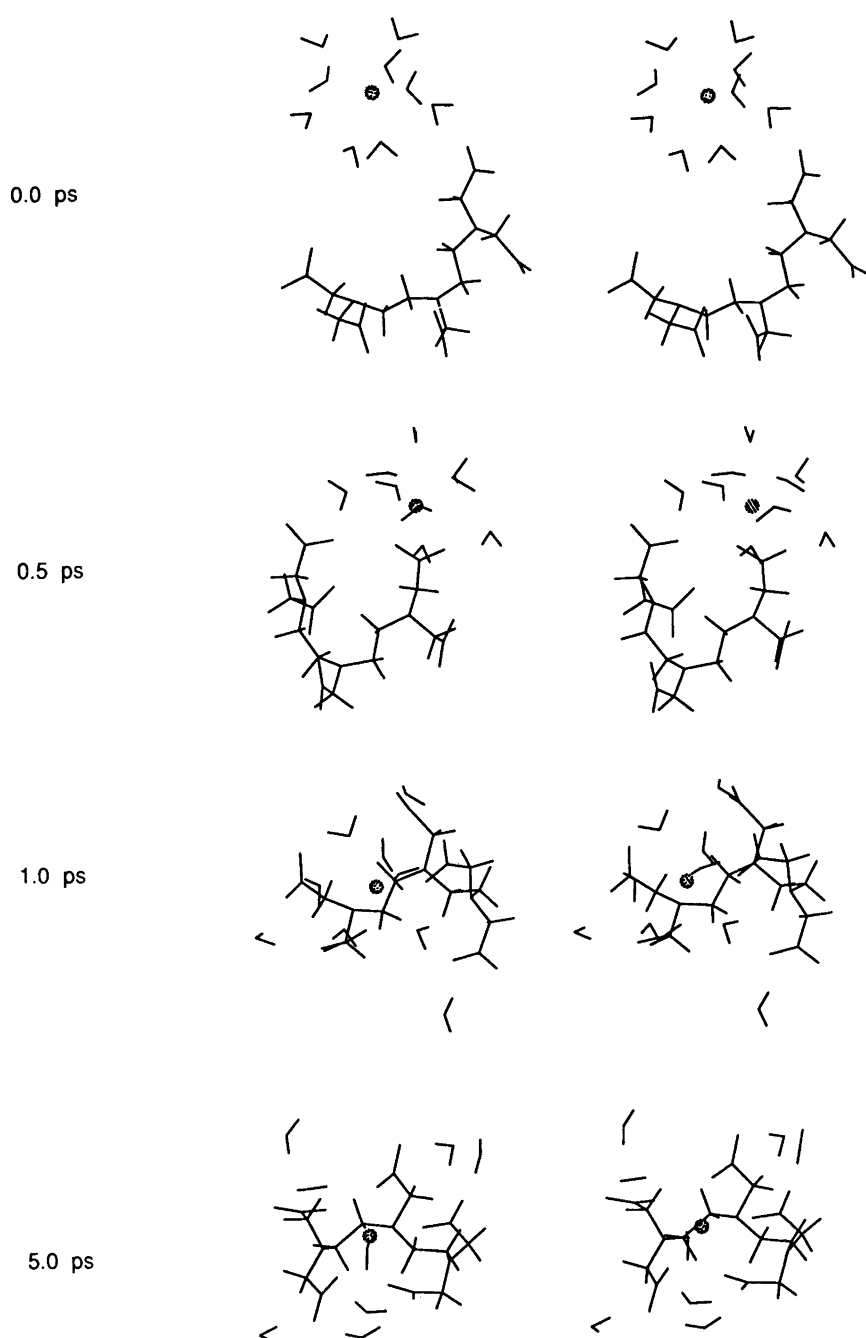


Fig. 5. Stereoscopic drawings of four structures from a molecular dynamics simulation of the complexation of a nonahydrated Gd(III) ion with DTPA.

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