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Environmental Effects on the Structure of Metal Ion–DOTA Complexes: An *ab Initio* Study of Radiopharmaceutical Metals

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Quantum chemical calculations were performed to study the differences between the important radiopharmaceutical metals yttrium (Y) and indium (In) bound by DOTA and modified DOTA molecules. Energies were calculated at the MP2/6-31+G(d)//HF/6-31G(d) levels, using effective core potentials on the Y and In ions. Although the minimum energy structures obtained are similar for both metal ion–DOTA complexes, changes in coordination and local environment significantly affect the geometries and energies of these complexes. Coordination by a single water molecule causes a change in the coordination number and a change in the position of the metal ion in In–DOTA, but Y–DOTA is hardly affected by water coordination. When one of the DOTA carboxylates is replaced by an amide, the resulting structures show a large variation between the Y and In ions. A six-residue model of the active site containing metal ion–DOTA showed that the Y–DOTA structure optimized to a structure similar to the crystal structure but that the water molecule in In–DOTA disrupts the salt bridge between Arg98B and a carboxylate side chain of DOTA. These observed differences could in part explain the differential binding constants for Y–DOTA and In–DOTA to the antibody 2D12.5.

Introduction

DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is a commonly used macrocycle to chelate metal ions in solution (Figure 1).[1] This metal chelator can octacoordinate a metal ion with its four ring nitrogens and four carboxylate side chains to create a tightly bound complex. The structures of many metal ion−DOTA complexes have been solved using X-ray crystallography and NMR.[2−6] These structural studies showed that metal ion−DOTA complexes exist primarily in two conformations termed antiprism and inverted antiprism geometries.[6] The lowest energy geometry for a DOTA complex is dependent on the metal ion coordinated. These complexes are highly stable, and metal loss by DOTA is minimal in solution, making them useful as magnetic resonance imaging probes when containing lanthanides.

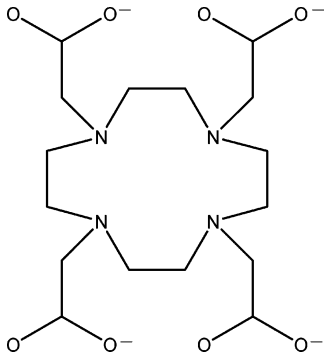

Figure 1. Molecular structure of 1,4,7,10-tetraazacyclododecane-1,4,7,-10-tetraacetic acid (DOTA).

Because of the great stability of these metal complexes, yttrium−DOTA-based molecules have been used as site-specific targeted therapeutic radiopharmaceuticals in attempts to deliver radionuclides to cancerous tumors.[7] Yttrium-90 is

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a common radionuclide (β-emitter) used for cancer therapy, but it is not easily imaged within the body because of its short-range emission. The biodistribution of yttrium-90 is commonly estimated in the body using the surrogate ion indium-111 (γ-emitter) because the ionic charges for these two metals are the same (+3), and the half-lives of these radionuclides are almost identical.[8] Although these ions share similarities, there are indications that the physical properties of indium-chelated DOTA may not be exactly the same as yttrium-chelated DOTA. Studies of a DOTA binding antibody showed that the uptake of ^{90}Y−DOTA was almost 3 orders of magnitude greater than that for ^{111}In−DOTA.[9] Recent HPLC measurements by Liu et al. have shown that the lipophilicities of these metal ions bound to a modified DOTA differ along with the solution equilibrium as determined by NMR.[10] Because of the critical importance in accurately knowing the biodistribution of radionuclides within the body and their localization within tissue,[11] a computational study was performed to better understand the similarities and differences between Y−DOTA and In−DOTA and their modified complexes.

Methods

All calculations were performed using the programs Gaussian 98 and Gaussian 03.[12,13] Full geometry optimizations of the metal ion−DOTA complexes were performed at the Hartree−Fock level of theory. The Hay−Wadt LANL2DZ effective core potential (ECP) was used for the yttrium and the indium ions (calculated in their +3 states), and the 6-31G(d) basis set was used for all other atoms.[14−16] This combination of methods was found to give structures in good agreement with experiment.[17,18] The energies of the complexes were obtained from MP2/6-31+G(d) single-point calculations using the optimized Hartree−Fock geometries (MP2/6-31+G(d)//HF/6-31G(d)). Harmonic frequency calculations were performed on all the optimized geometries. The zero-point vibrational energies (ZPE) from the frequency calculations were scaled by 0.893.[19] The structures were also optimized using density functional theory using a gradient-corrected hybrid exchange-correlation functional; results and methodological details are provided in the Supporting Information.

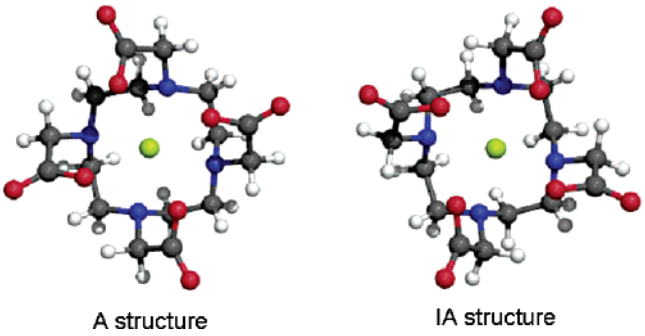

Figure 2. Molecular structure of the two calculated low energy conformations of DOTA-coordinating yttrium.

Constrained optimizations of metal ion−DOTA complexes with selected residues from the active site of the antibody 2D12.5 (PDB entry 1NC2) were also performed. The side chains of Tyr34A, Trp93A, Trp98A, Trp52B, and Arg98B, up to their Cα's, were included in the calculations along with metal ion−DOTA coordinated with a water molecule. The Cα's of each residue were changed to methyl groups, and their positions were fixed in space. Ser100B was also included in the calculation along with the amide and Cα of the following residue. The amide hydrogen bonds with one of the carboxylates of DOTA. The same level of theory as described above was also used for these optimizations. The coordinates for the structures shown in Figures 4, 6, and 7 are provided in the Supporting Information.

Results and Discussion

Metal Ion−DOTA Complexes. There are two minimum energy geometries apiece for the metal ion−DOTA complexes (Figure 2). The lowest energy conformer is the antiprism geometry (denoted A), and the higher energy conformer is the inverted antiprism geometry (denoted IA) for both yttrium− and indium−DOTA complexes. Both conformations have been characterized by solution NMR for

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Table 1. Selected Distances (Å) between Atoms in DOTA and Metal Ions

	Y−DOTA			In−DOTA		
	A structure Y−O	IA structure Y−O	crystal structure	A structure In−O	IA structure In−O	crystal structure[b] In−O
M−DOTA	2.27	2.27	NA	2.07	2.06	2.16
	2.27	2.27	NA	2.07	2.06	2.18
	2.27	2.27	NA	2.07	2.06	2.20
	2.27	2.27	NA	2.07	2.06	NA
	A structure Y−N	IA structure Y−N	crystal structure	A structure In−N	IA structure In−N	crystal structure[b] In−N
M−DOTA	2.73	2.76	NA	2.70	2.77	2.39
	2.73	2.76	NA	2.70	2.77	2.40
	2.73	2.76	NA	2.70	2.77	2.33
	2.73	2.76	NA	2.70	2.77	2.31
	A structure Y−O	IA structure Y−O	crystal structure[a] Y−O	A structure In−O	IA structure In−O	crystal structure
M−DOTA+ H_2O	2.33	2.32	2.33	2.08	2.06	NA
	2.32	2.33	2.32	2.08	2.06	NA
	2.28	2.27	2.33	2.04	2.02	NA
	2.28	2.27	2.33	2.04	2.02	NA
	2.52 (H_2O)	2.52 (H_2O)	2.42 (H_2O)	2.38 (H_2O)	2.33 (H_2O)	NA
	A structure Y−N	IA structure Y−N	crystal structure[a] Y−N	A structure In−N	IA structure In−N	crystal structure
M−DOTA+ H_2O	2.82	2.85	2.63	2.98	3.12	NA
	2.80	2.87	2.65	2.93	3.17	NA
	2.77	2.84	2.63	2.87	3.08	NA
	2.80	2.82	2.67	2.92	3.05	NA

[a] 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (ref 5). [b] 1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid (ref 2).

lanthanides bound by DOTA, and the energy difference between the A and IA conformations can be reversed depending on the metal.[6,20] The metal ions are octacoordinated by one oxygen from each of the four carboxylate side chains and the four nitrogens in the DOTA ring. The difference in the A and IA structures is due to the orientation of the carboxylate side chains of DOTA. The change in side chain orientation affects the coordination of the metals. Although the metal ion−O distances are relatively unchanged when going from the A to the IA structure, the metal ion−N distances increase by 0.03 and 0.07 Å for yttrium and indium, respectively (Table 1). The energy difference between the A and IA structures are 3.66 and 5.60 kcal/mol for yttrium- and indium-bound DOTA, respectively. The overall structures of Y−DOTA and In−DOTA are similar, and the most significant difference is in the metal ion−oxygen distances. For both the A and IA conformations, the In−O bond (2.07 Å) is ∼0.2 Å shorter than the Y−O bond (2.27 Å) because of the smaller ionic radii of indium relative to yttrium (0.92 and 1.02 Å, respectively).[21] The bond lengths obtained for Y−O and Y−N are in good agreement with the crystal structure of DOTA coordinating a water molecule. The In−O bond is shorter, and the In−N bond is longer than expected from the crystal structure of indium coordinated by a DOTA molecule with three carboxylate side chains.[2]

Metal Ion−DOTA−H_2O Complexes. In the crystal structure of Y−DOTA and other lanthanide ions bound by DOTA, the metal ion also coordinates to a single water molecule.[3,22] NMR chemical shifts and XAFS measurements of metal ion−DOTA complexes also show a single water molecule coordinated to the metal ion in solution.[23,24] A recent crystal structure of the monoclonal antibody 2D12.5 bound with a modified Y−DOTA was shown to have the yttrium coordinated by a single water molecule. Because water coordination is a common feature of many metal ion−DOTA complexes, the calculated A and IA structures were optimized with a single water molecule coordinating the metal ion and placed above the carboxylate side chains to determine if a change in coordination affects the structures.

The calculated distance of the oxygen of water to yttrium was identical for the A and IA conformers (2.52 Å). These distances are in reasonable agreement with X-ray crystallography (2.42 Å).[5] For the Y−DOTA complexes, having the additional ligand has little effect on the geometry of the complex as compared with the complex without water. This result is consistent with the crystal structure. For both calculated conformers, there is an increase in the Y−O distances for the two carboxylates interacting with the hydrogens of the water, but the two other Y−O distances are almost identical to the non-water coordinating distances. The most significant change occurs in the Y−N distances. The Y−N distances increase by ∼0.07 and ∼0.09 Å for the

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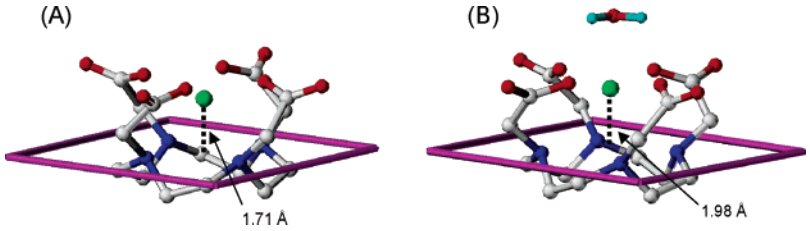

Figure 3. Picture showing the effect of water coordination on In−DOTA. (A) shows the A conformation, and (B) shows the same DOTA conformation with water coordinated to the indium. The box represents the plane formed by the nitrogens in the DOTA ring. The values are the indium-to-plane distances.

A and IA conformers (Table 1), respectively, relative to the Y−DOTA structures without a water. The change in the Y−N distance is due to repositioning of the yttrium within the ring structure of DOTA. In the complexes without a coordinating water, yttrium sits 1.71 and 1.78 Å above the plane formed by the nitrogens in DOTA in the A and IA conformers, respectively. When water coordinates the metal ion, the yttrium raises to 1.81 and 1.91 Å above the plane for the A and IA conformers, respectively (Figure 3).

The crystal structures of indium complexed with modified DOTAs do not have a water molecule coordinating the indium.[2,25] Although indium does not coordinate a water molecule, quantum chemical calculations can predict the effect of transiently bound waters that may not be observed in the crystal structure. The calculated oxygen of water to indium distances were 2.38 and 2.33 Å for the A and IA conformers, respectively. The In−DOTA geometries are more sensitive to coordination by a water molecule. When the In−DOTA complex is not coordinated with a water molecule, the indium is 1.71 and 1.83 Å above the plane formed by the nitrogens in DOTA in the A and IA conformers, respectively. When a water molecule coordinates to the indium, the metal ion raises within DOTA significantly more than in the Y−DOTA. In the A conformer, coordination of a water molecule causes the metal ion to rise to 1.98 Å above the plane. An even larger change occurs in the IA conformer with water coordination. Water coordination causes the indium to rise by almost an additional 0.5 Å above the plane (2.27 Å) relative to the non-water-coordinated structure. There is little change in the In−O distances (∼0.04 Å variation, see Table 1) for either conformer if water is absent or present in the complex. The change in position of indium because of water coordination within DOTA significantly weakens the In−N coordination. The In−N distances increase by ∼0.22 and ∼0.33 Å in the A and IA conformers, respectively. This change in structure is consistent with structural studies of indium showing that the preferred coordination number for this ion is 6 or 7.[26,27] The calculated change in position and coordination of indium in DOTA relative to yttrium is also in agreement with experimental observations that metal loss by a modified DOTA is more rapid for indium than yttrium.[28]

Metal Ion−DO3AM Complexes. Although DOTA is commonly used to chelate metal ions in chemical studies, modified versions of DOTA are used in most biological studies. It is common to have a molecule such as somatostatin analogues linked to DOTA either at one of the carboxylate side chains or to one of the ring carbons.[29,30] A recent experimental study investigated indium and yttrium coordinated by two DOTA analogues with a linker attached to one of the carboxylate arms, 1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecylacetylbenzylamine (DOTA−BA) and 1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecylacetyl-*R*-(+)- α-methylbenzylamine (DOTA−MBA). This study used NMR and HPLC measurements to show that the physicochemical properties of the complexes differ in solution depending on whether they coordinate In or Y.[10] The HPLC retention times for the metal ions bound by DOTA−BA and DOTA−MBA were significantly different between yttrium and indium even though the overall charges of the complexes were identical. The NMR studies showed that the Y-bound DOTA−BA and DOTA−MBA had only one major conformer in solution but that the In-bound complexes showed significant line broadening, implying multiple conformers. At elevated temperatures, the NMR spectrum of the Y-bound DOTA−BA and DOTA−MBA began to resemble the indium spectrum observed at lower temperatures.

The quantum mechanical calculations for metal ion coordination by a DOTA containing a single amide side chain (denoted DO3AM) showed that the lowest energy conformers (conformation A) are similar for indium and yttrium but are in equilibrium with different higher energy conformers (Figure 4). For Y−DO3AM, the lowest energy conformer is in equilibrium with conformer IA. Although the structure is similar to that for Y−DOTA, the amide carbonyl oxygen is not bound as strongly as a carboxylate, leading to an Y−O distance of 2.43 Å (conformer A) that is approximately 0.2 Å longer than the Y−O distance for a carboxylate oxygen (Table 2). The In−DO3AM lowest energy conformer (conformer A) is in equilibrium with a conformer (conformer B) that has the amide side chain completely extended away from indium, and an adjacent carboxylate side chain has rotated 71° relative to conformer A. This conformer is only slightly higher in energy (0.7 kcal/mol) relative to conformer

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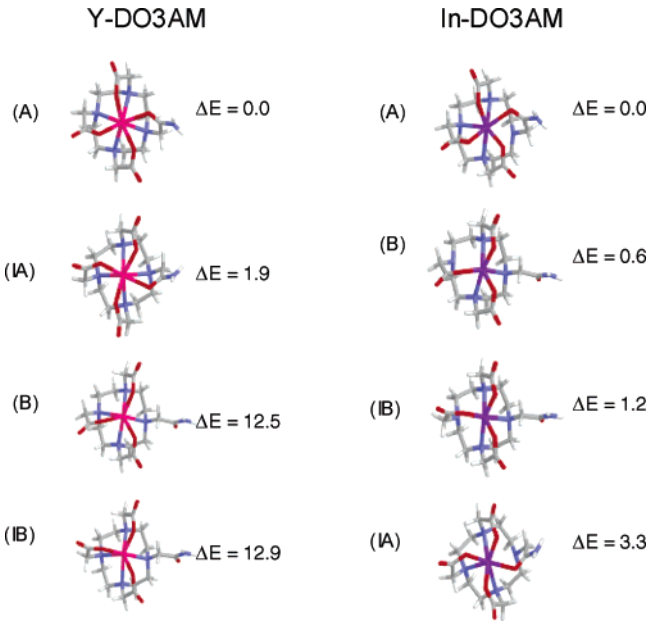

Figure 4. Optimized conformations for metal ion−DO3AM. The Δ*E* values (kcal/mole) are the energy difference relative to the A structure.

A, and the change in coordination results in a slight contraction in the In−O (carboxylates) distances. A second conformer with the amide side chain extended away from indium was obtained and denoted conformer IB. The difference between the B and IB structures in the carboxylate side chain opposite the amide is rotated (see Figure 4). Conformer IB for the In−DO3AM complex is also close in energy to conformer A. The IA conformer for In−DO3AM was the highest in energy. Interestingly, a recent crystal structure of In−DOTA−*p*-aminoanilide (DOTA−AA) is in the IA conformation.[25] The additional phenyl group in In−DOTA−AA may form interactions that make IA the most stable conformation in the solid state for this complex. The four calculated low energy conformers for the Y− and In−DO3AM complexes are shown in Figure 4 with their relative energies to the A structure. The four structures for In−DO3AM only differ by 3.3 kcal/mol at most from one another, and the structures with the amide side chain extended away from the indium differ by ∼1.0 kcal/mol from the A structure. Conversely, the B and IB structures for Y−DO3AM are over 12 kcal/mol higher in energy than conformer A, making them unlikely to be found in solution. These calculations are consistent with NMR results for Y− and In−DOTA−AA that show Y−DOTA−AA is octacoordinated in solution but In−DOTA−AA likely has the amide side chain dissociated from the metal ion.[25]

Coordination of a water molecule to the A and IA structures for these modified DOTA complexes had an effect on the structure similar to that in the DOTA complexes. A water molecule was able to coordinate to the metal ion for conformer B of Y−DO3AM and did not significantly change the overall structure of this complex. The change in carboxylate coordination allows the water molecule to come in closer contact with the yttrium (2.49 Å, Y−O distance) relative to that in Y−DOTA. Interestingly, a water molecule was not able to coordinate to indium in conformer B of In−DO3AM. The inability of water to coordinate to the indium in conformer B may be due to the metal ion being positioned more deeply within DO3AM. The In−N distances for the B structure are significantly shorter than those of either the A or IA structures, and indium is heptacoordinated (a preferred number for this ion).

DOTA−Antibody Complexes. Model System. Meares and co-workers have determined a crystal structure of the monoclonal antibody 2D12.5 binding a Y−DOTA analogue (Y−(*S*)HETD, in which the linker is attached to the ring).[31] The DOTA analogue binds on the surface of the antibody in a depression, and surprisingly, there are only a few strong interactions between the antibody and DOTA. The binding site is made up mainly of tryptophans that are able to form hydrogen bonds with the carboxylates of DOTA, and the methylene carbons of DOTA interact with the aromatic tryptophans (Figure 5). A single salt bridge is formed between Arg98B and one of the carboxylate side chains from DOTA. Interestingly, a single water molecule is coordinated with the yttrium and isolated from bulk solvent. This antibody is selective for Y−DOTA relative to In−DOTA. The measured binding constants showed that Y−DOTA is bound more than 100 times tighter than In−DOTA.[31,32] A better understanding of the differences of this system binding to Y−DOTA and In−DOTA could provide further insights into the differential behavior of these two molecules. Models for this system were constructed with metal ion−DOTA interacting with a single water molecule and methyl−guanidinium (to represent the arginine side chain) and optimized.

Two minimum structures were found for each metal ion−DOTA model (Figure 6). The higher energy structure for the Y−DOTA model resembles the conformation found in the active site of the antibody (Figure 6A). The guanidinium forms two interactions with the carboxylate (2.88 and 2.92 Å for the N−O distances), and the water oxygen is 2.56 Å from yttrium in the model system. The N−O distances are in agreement with the crystal structure although the Y−water oxygen distance is much shorter than that observed in the crystal structure (2.81 Å). Although the higher energy In−DOTA model is similar to the Y−DOTA model, there is only one interaction between the guanidinium and the carboxylate (2.92 Å, N−O distance). The second nitrogen of the guanidinium interacts with the oxygen of water (2.90 Å, N−O distance), causing the water to be no longer coordinated with the indium (3.43 Å, In−O distance). Although these structures resemble the conformation found in the crystal structure, a lower energy structure was found for these models. The low energy structure for both metal ion−DOTA complexes has the methyl−guanidinium coordinated to two carboxylates and positioned flat against DOTA (Figure 6B, D). This orientation also displaces the water molecule from the metal, leaving it no longer coordinated (4.11 and 4.22 Å, Y−O and In−O distances, respectively)

(31) Corneillie, T. M.; Fisher, A. J.; Meares, C. F. *J. Am. Chem. Soc.* **2003**, *125*, 15039−15048.
(32) Corneillie, T. M.; Whetsone, P. A.; Fisher, A. J.; Meares, C. F. *J. Am. Chem. Soc.* **2003**, *125*, 3436−3437.

Table 2. Selected Distances (Å) between Atoms in DO3AM and the Metal Ions

	A structure Y−O	IA structure Y−O	B structure Y−O	IB structure Y−O	crystal structure Y−O[a]
Y−DO3AM	2.23	2.26	2.20	2.20	2.24
	2.21	2.22	2.19	2.19	2.25
	2.26	2.23	2.20	2.19	2.28
	2.426 (amide)	2.450 (amide)	5.464 (amide)	5.467 (amide)	2.318 (amide)

	A structure Y−N	IA structure Y−N	B structure Y−N	IB structure Y−N	crystal structure Y−N
Y−DO3AM	2.69	2.71	2.69	2.62	2.39
	2.62	2.63	2.73	2.71	2.41
	2.64	2.70	2.59	2.69	2.43
	2.86 (amide)	2.82 (amide)	2.69 (amide)	2.69 (amide)	2.44 (amide)

	A structure Y−O	IA structure Y−O	B structure Y−O	crystal structure Y−O[b]
Y−DO3AM+ H_2O	2.24	2.27	2.21	2.35
	2.25	2.25	2.20	2.27
	2.30	2.28	2.25	2.26
	2.44 (amide)	2.45 (amide)	5.50 (amide)	2.34 (hydroxyl)
	2.49 (H_2O)	2.52 (H_2O)	2.49 (H_2O)	2.51 (H_2O)

	A structure Y−N	IA structure Y−N	B structure Y−N	crystal structure Y−N
Y−DO3AM+ H_2O	2.75	2.80	2.64	2.63
	2.69	2.70	2.87	2.66
	2.72	2.78	2.83	2.58
	2.93 (amide)	2.89 (amide)	2.73 (amide)	2.61 (hydroxyl)

	A structure In−O	IA structure In−O	B structure In−O	IB structure In−O	crystal structure In−O[c]
In−DO3AM	2.01	2.03	1.99	2.02	2.22
	2.05	2.05	1.99	1.99	2.27
	2.04	2.01	2.02	2.00	2.28
	2.38 (amide)	2.40 (amide)	5.28 (amide)	5.28 (amide)	2.31 (amide)

	A structure In−N	IA structure In−N	B structure In−N	IB structure In−N	crystal structure In−N
In−DO3AM	2.60	2.64	2.55	2.43	2.37
	2.48	2.49	2.85	2.77	2.41
	2.53	2.62	2.39	2.54	2.42
	2.91 (amide)	2.88 (amide)	2.49 (amide)	2.49 (amide)	2.52 (amide)

	A structure In−O	IA structure In−O	B structure In−O
In−DO3AM+ H_2O	2.03	2.02	1.99
	2.04	2.04	2.00
	2.05	2.02	2.04
	2.25 (amide)	2.23 (amide)	5.32 (amide)
	2.46 (H_2O)	2.42 (H_2O)	4.98 (H_2O)

	A structure In−N	IA structure In−N	B structure In−N
In−DO3AM+ H_2O	2.76	3.01	2.52
	2.63	2.64	2.80
	2.72	2.86	2.40
	3.07 (amide)	3.17 (amide)	2.47 (amide)

[a] DOTA−D-PheNH_2 (ref 29). [b] 10-(2-Hydroxypropyl)-1,4,7,10-tetraazacyclodecane 1,4,7-triacetic acid (ref 4). [c] 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*p*-aminoanilide) (ref 25).

but instead interacting with the methyl−guanidinium. Although the structures obtained for Y−DOTA and In−DOTA from *ab initio* calculations have similar conformations, the energy barriers separating the low energy and high energy structures differ significantly. For Y−DOTA, there is a 3.29 kcal/mol difference in energy between the structures. A larger barrier of 10.19 kcal/mol between the In−DOTA structures was obtained. One difference in the structures is that In−DOTA only forms one interaction with the methyl−guanidinium, and the other guanidinium interaction is with the water molecule. Both oxygens of the carboxylate from Y−DOTA form interactions with the methyl−guanidinium.

DOTA−Active Site Residues. Although it is informative to use a model system to investigate the strong interaction between metal ion−DOTA complexes and arginine, a larger system is needed to better understand how changes in the

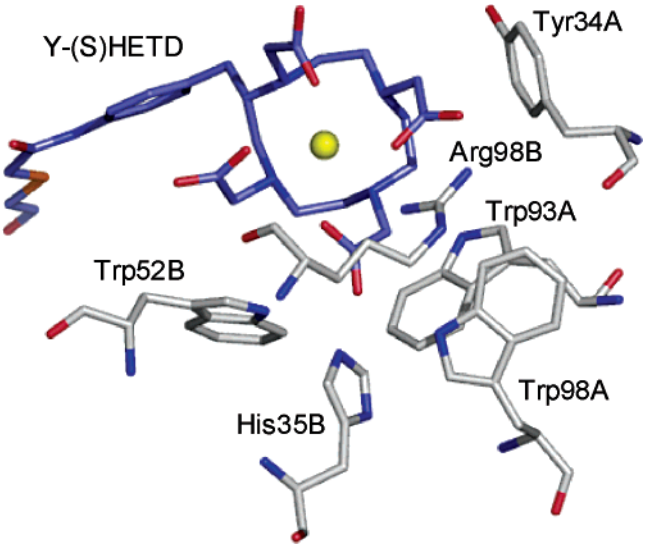

Figure 5. Picture of Y−(*S*)HETD (modified DOTA compound) in the active site of the 2D12.5 monoclonal antibody (1NC2, this structure was reported in ref 31).

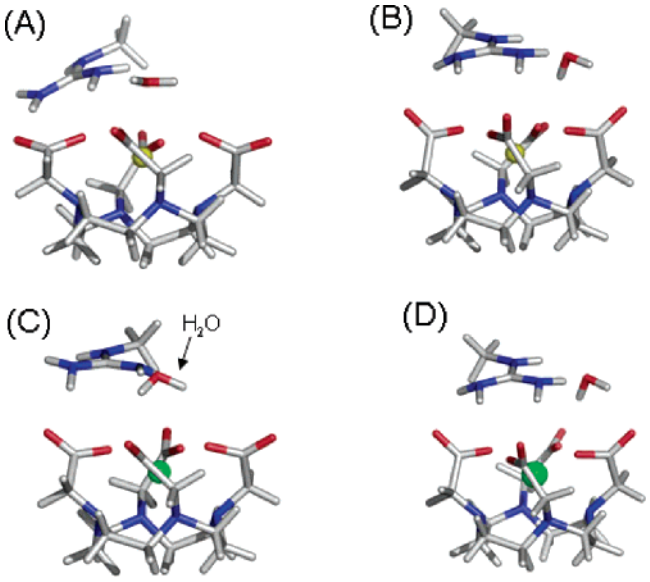

Figure 6. Minimum energy structures of Y− and In−DOTA interacting with water and methyl−guandinium. (A) and (C) show conformations similar to the crystal structure for Y− and In−DOTA, respectively. (B) and (D) show the minimum energy structures for the model systems of Y− and In−DOTA, respectively.

metal ions might affect the binding within the active site of the antibody. The model used to represent active site binding has six residues in direct contact with metal ion−DOTA and a single water molecule coordinated to the metal ion.

Constrained optimization using the crystal structure coordinates of Y−DOTA (changed from Y−(*S*)HETD) in the active site showed only modest changes from the starting structure. After optimization, the water is more closely coordinated to the yttrium (see Figure 7A). In the crystal structure, the oxygen of water is 2.81 Å from the yttrium, and after optimization, the distance shortens to 2.55 Å. The antibody forms six strong interactions with Y−DOTA, and each carboxylate side chain of DOTA forms at least one interaction with the antibody. Arg98B forms a salt bridge with a carboxylate side chain of DOTA. The guanidinium

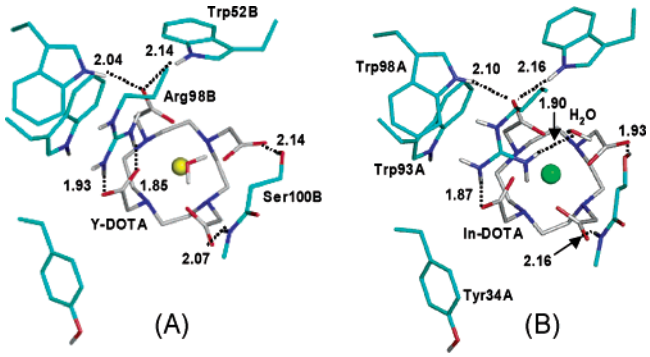

Figure 7. Constrained optimized structures of Y−DOTA (A) and In−DOTA (B) in active site model of the 2D12.5 antibody. Only polar hydrogens are shown for ease of viewing. Numerical values are in angstroms.

group interacts with both oxygens of the carboxylate giving hydrogen (guanidinium) to oxygen (DOTA) distances of 1.85 and 1.93 Å. Trp 98A and Trp 52B form hydrogen bonds with the same carboxylate oxygen of another side chain of DOTA, 2.04 and 2.14 Å (both are HE1 to carboxylate oxygen distances), respectively. The hydroxyl hydrogen of Ser100B forms a hydrogen bond with another carboxylate side chain (2.14 Å) of DOTA, and the backbone amide hydrogen of Tyr101B interacts with the final carboxylate (2.07 Å). The structure of Y−DOTA in the active site is similar to the other calculated yttrium structures. The Y−O distances range from 2.27 to 2.32 Å, and the Y−N distances range from 2.69 to 2.75 Å.

The optimized structure of In−DOTA in the active site starting from the crystal structure showed changes larger than Y−DOTA (see Figure 7B). The water molecule does not coordinate to indium in the optimized structure. The preference of indium not to coordinate water disrupts the interaction between Arg98B and DOTA. The guanidinium of Arg98B forms a single ionic interaction with a carboxylate oxygen (1.87 Å, H−O distance), and the water molecule breaks the other ionic interaction (2.71 Å, H−O distance) to form an interaction with the guanidinium group (1.90 Å, hydrogen to water oxygen distance). The hydrogens of the water molecule are also able to form interactions with the oxygen of a carboxylate side chain of DOTA (2.20 Å) and with the hydroxyl oxygen of Ser100B (1.90 Å). Trp98A and Trp52B hydrogen bond with a carboxylate side chain, 2.10 and 2.16 Å (HE1 to carboxylate oxygen distance), respectively. The hydroxyl hydrogen of Ser100B forms a hydrogen bond with another carboxylate oxygen (1.93 Å), and the backbone amide hydrogen of Tyr101B hydrogen bonds with the final carboxylate side chain (2.16 Å). Within the In−DOTA molecule, the In−O distances range from 2.08 to 2.13 Å, and the In−N distances range from 2.54 to 2.57 Å. The In−N distances for the In−DOTA complex in the active site are much closer to those observed in crystal structures of indium bound by modified DOTA molecules. The shortening of the In−N distance appears to be related to the interaction with the guanidinium because similar changes also occur in the In−DOTA interacting with the methyl−guanidinium. Although this is not the definitive answer to the observed difference in the binding constants of Y−DOTA and In−

DOTA to the antibody 2D12.5, because the binding energies were not determined, these calculations do illustrate the sensitivity of indium to its local environment and how it can differ from yttrium.

Conclusions

These *ab initio* calculations provide insight into the differences between yttrium− and indium−DOTA complexes. Y−DOTA and In−DOTA share similar structures, but as the coordination and ligands change, In−DOTA is more affected than Y−DOTA. Calculations with even a minimal model for the active site of a DOTA binding antibody show that there can be conformational differences between Y−DOTA and In−DOTA. Experiments by Liu and co-workers have shown that in certain cases ^{90}Y−DOTA and ^{111}In−DOTA conjugated complexes are biologically equivalent in tumor uptake and tissue distribution even though these molecules were physically different.[33] Although these molecules can be biologically equivalent in certain cases, for other functions, this may not be true, such as in recognition of In−DOTA and Y−DOTA complexes by the 2D15.2 antibody (binds 100 times tighter to Y−DOTA). This study has shown that, depending on its environment, In−DOTA may not always be structurally similar to Y−DOTA and care should be taken when interpreting results from one metal ion to another.

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Supporting Information Available: Cartesian coordinates for various metal ion−DOTA complexes and selected optimized distances between atoms in DOTA and metal ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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