
The Molecular Structure of Indium-DTPA

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The x-ray structural characterization of $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7 \text{H}_2\text{O}$ is described. The structure is notable for two reasons. It is the first In^{3+} -complex with relevance to antibody attachment to be structurally characterized. Surprisingly, the complex has coordination number eight and is the first monomeric In^{3+} -complex with such a high coordination number. The potentially octadentate ligand binds through three amino groups and five deprotonated carboxylate groups to the metal ion. The ligand atoms surround the central indium atom in a slightly distorted Archimedean antiprismatic configuration. High resolution nuclear magnetic resonance studies in solution show that this high coordination number also pertains in aqueous medium. Consequently, one may conclude that octadentate ligands are optimal for the thermodynamic and kinetic stabilization of In^{3+} in living systems. It is also indicated that bifunctional diethylenetriaminepentaacetic acid (DTPA) is superior to amide bound (=sevendentate) DTPA in antibody labeling.

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Indium-111 diethylenetriaminepentaacetic acid²⁻ (^{111}In]DTPA) is a widely used compound in diagnostic nuclear medicine. It is the agent of choice for cerebrospinal fluid studies (CSF) and is indicated to determine the CSF dynamics and resorption or to diagnose cerebrospinal fluid leakage. Furthermore, this radiopharmaceutical is used for quantitative glomerular filtration rate (GFR) measurements (1). Moreover, the knowledge of its stability and molecular structure is of interest as the chelate is widely used to label monoclonal antibodies (MAbs) with metallic radionuclides (2-4).

DTPA is an octadentate ligand that can bind to a metal ion through the five deprotonated carboxylate groups and the three tertiary amino groups. The preferred conjugation of DTPA to a biomolecule is through the cyclic anhydride of the pentaacetic acid (cDTPA). This elegant method was developed by Hnatowich et al. (5) and cDTPA is the only bifunctional chelating agent commercially available. The method has a possible drawback as one of the In^{3+} binding carboxylate groups is used to form an amide bond with lysine side chains of the MAb, leaving the ligand heptadentate (Fig. 1B). This may lead to a decreased thermodynamic stability and consequent label instability in vivo as a result of transchelation to serum transferrin (6). For that reason, Esteban et al. synthesized 1-(p-isothiocyan-

atobenzyl) DTPA (SCN-Bz-DTPA), a bifunctional DTPA derivative that has the linking group inserted into the carbon backbone of the chelate (7). Thereby all eight coordination sites on DTPA are preserved (Fig. 1A). Indeed, authors report reduced liver uptake of ^{111}In in tumor-bearing mice and give as a possible explanation the higher stability of the In^{3+} complex, decreased in vivo transchelation to transferrin, and consequently decreased transferrin mediated liver uptake of ^{111}In (8). If this is true, then an important question must be asked: is the coordination number (CN) eight found in $\text{In}(\text{DTPA})^{2-}$ at all? It is important to note that no monomeric In^{3+} complexes with CN eight have been characterized so far. The preferred coordination number of In^{3+} is undoubtedly 6 (9,10) and only few seven-coordinate complexes have been characterized so far. Indium ethylenediaminetetraacetic acid (^{111}In]EDTA⁻) crystallizes in the presence of SO_3^{2-} as $\text{Na}_3\text{In}(\text{EDTA})(\text{SO}_3)$, a seven-coordinate capped trigonal prismatic complex (11). Moreover, the x-ray structures of the In^{3+} complexes of two triscarboxymethylated tetraazamacrocycles were solved. Both are seven-coordinate without coordination of solvent molecules indicating that heptadenticity of the ligands is sufficient for coordinative saturation of In^{3+} (12).

The importance of the compound $\text{In}(\text{DTPA})$ has led to some discussions concerning structure and stability of the compound and to speculations in the nuclear medicine literature (13) and radiopharmaceutical textbooks (14). Therefore, we decided to perform an x-ray crystal structure and report the results of it and of a

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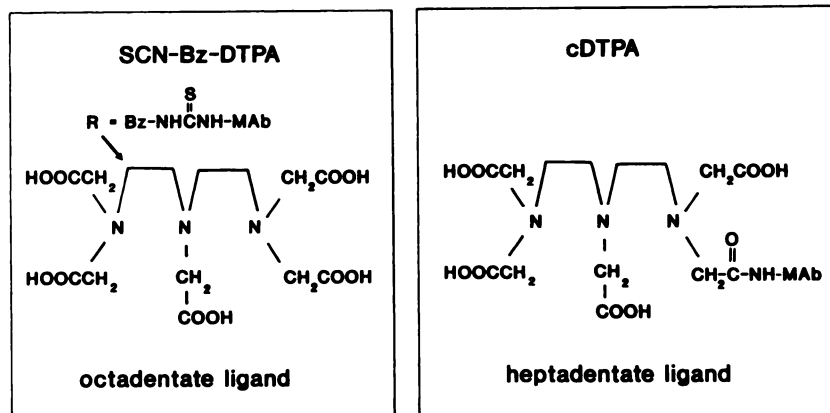


FIGURE 1
Left, right Structural formula of DTPA and modes of attachment to antibodies.

high resolution nuclear magnetic resonance (NMR) investigation in aqueous solution.

METHODS

Synthesis

Two hundred fifty milligrams (0.48 mmol) $\text{In}_2(\text{SO}_4)_3$ were added to a solution of 420 mg DTPA (1.07 mmol) in 10 ml H_2O and stirred 10–15 min. The pH was adjusted to 4–5 by the addition of NaOH. The complex was crystallized by the addition of ethanol. Suitable single crystals were grown from a water, ethanol, ether mixture. The complex crystallized as $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7\text{H}_2\text{O}$ and had a satisfactory elemental analysis.

NMR Spectra

Proton and carbon-13 (^{13}C) NMR spectra were recorded on a Varian VXR-400 spectrometer at 25°. Chemical shifts are relative either to TMS or sodium-3-trimethylsilyl-tetradecuterioproprionate. Solutions of the complex for NMR measurements (400 MHz, proton, ^{13}C) were made up in D_2O and the pD was adjusted with NaOD (40% NaOD in D_2O , >99.5%). The final pH was determined with a Radiometer PHM 64 pH meter equipped with a Metrohm pH electrode EA 125 and using the equation $\text{pD} = \text{pH} + 0.4$ (15). The proton NMR spectrum of DTPA D_2O (pD = 6.98) can be explained straightforwardly: (multiplicity, intensity, assignment): 3.098 ppm (t, 4H, 2 CH_2 , α -position to the central N), 3.318 ppm (s, 2H, $-\text{CH}_2-\text{COO}^-$), 3.432 (t, 4H, 2 CH_2 , β -position to the central N), 3.869 (s, 8H, 4- CH_2COO^-). The assignment of the peaks was done by using the multiplicities, relying on the integrated areas and the pD dependence of the chemical shifts. We used the known fact that protonation of the basic sites leads to a deshielding of the adjacent methylene groups and thus to a low field shift in the proton NMR spectrum (16). Complexation of DTPA to In^{3+} leads to a splitting of the peaks indicating the raising of the degeneracy of some of the protons.

Carbon-13 NMR (DTPA), (pD = 6.98) (intensity, assignment): 52.085 ppm (2C, α -position to the central N), 55.824 ppm (2C, α -position to the terminal N), 59.175 ppm (1C, $-\text{CH}_2-\text{COOH}$), 59.925 ppm (4C, $-\text{CH}_2\text{CO}$), 173.525 (NH- CH_2-CO), 181.322 (N- CH_2CO). The relative intensities of

the last two peaks are pD dependent. Upon complexation to In^{3+} the four equivalent carboxylate CH_2 -carbons of the free ligand are split into two signals. The CO_2^- carbons appear as three peaks with the ratio 2:2:1.

Carbon-13 NMR $\text{In}(\text{DTPA})^{2-}$ (pD=7.47): 57.762(2C), 58.167(2C), 59.632(1C), 64.018(2C), 64.715(2C), 179.026(1C), 179.252(2C), 179.744(2C).

X-ray Crystallography

The structure of $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7\text{H}_2\text{O}$ was determined by x-ray crystallography. Crystal data: $\text{C}_{14}\text{H}_{32}\text{InN}_3\text{Na}_2\text{O}_{17}$, monoclinic, space group $\text{P}2_1/a$, $a=1408.0$ (1.5), $b=964.8$ (5), $c=1897.7$ (6) pm; $\beta=106.34$ (4)°, $V=2.4726 \cdot 10^9$ pm³; $Z=4$, crystal density: 1.813 (calc.), 1.83 (obs.), $F(000)=1376$, $T=293^\circ\text{C}$; $\text{MoK}\alpha$ ($\lambda=71.069$ pm) $\mu_{\text{max}}=26^\circ$; 5153 independent reflexions, 3846 ($F_o > 2\sigma[F_o]$) used in refinements, 334 variables, 11.51 observations per parameter; final $R_w=0.076$. Because a decay of unprotected crystals was observed and thought to be a loss of water molecules, a crystal had to be sealed in a glass capillary tube together with mother liquid. During the measurements on an Enraf Nonius CAD4 four circle diffractometer, an intensity loss of ~20% was observed with four standard reflexions monitored every 3,600 sec. Unit cell parameters were determined from 25 centered strong reflexions by the least-squares procedure. The raw data set was not corrected for crystal decay and polarization effects but not for absorption. Positional parameters of the metal ions were determined by a three-dimensional Patterson map (SHELXS-86, 17). The remaining atoms were localized by subsequent different Fourier maps and refined anisotropically with SHELXS-76 (17) by the full-matrix least-squares procedure to a ratio $\Delta/\sigma < 0.01$. Hydrogen atoms are in calculated positions with $U_{\text{iso}} = 0.07$. Scattering factors are given in SHELX-76 and in Cromer et al. (18). (Fractional coordinates are deposited in the Cambridge Data Base or are available from A.R.). Figure 2 shows an ORTEP plot of the $\text{In}(\text{DTPA})^{2-}$ anion and Figure 3 the coordinating atoms around the central In^{3+} cation. Selected bond lengths and angles are given in Table 1.

RESULTS

The solid-state structure of $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7\text{H}_2\text{O}$ was determined by x-ray crystallography. As the important

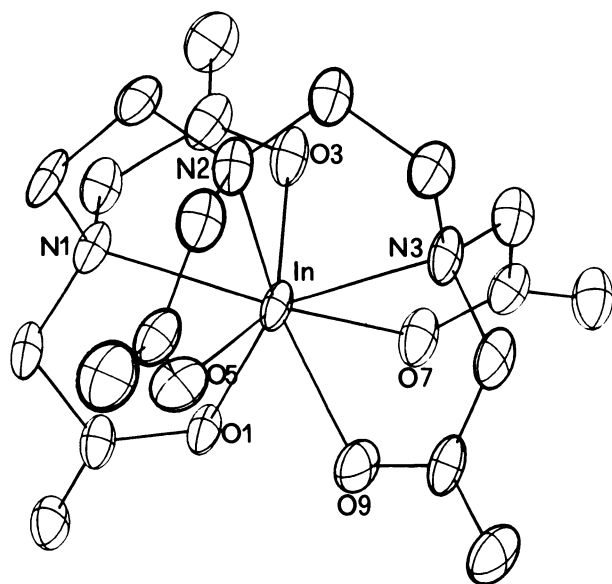


FIGURE 2
Ortep view of $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7 \text{H}_2\text{O}$.

and somewhat unexpected result the coordination number of In^{3+} in this complex is 8. The octadentate ligand DTPA binds through the three tertiary amino nitrogen donors and five deprotonated carboxylate oxygen atoms (Fig. 2) affording a twofold negative complex. The bond distances to the central metal ion are in the expected range. The bond lengths to the three nitrogen atoms differ only slightly averaging 240.1 pm, whereas the In—O bonds are rather diverse between 220.3 pm and 233.6 pm with an average of 224.9 pm. A comparison can only be made to few complexes as there is a lack of structurally characterized In^{3+} complexes with rele-

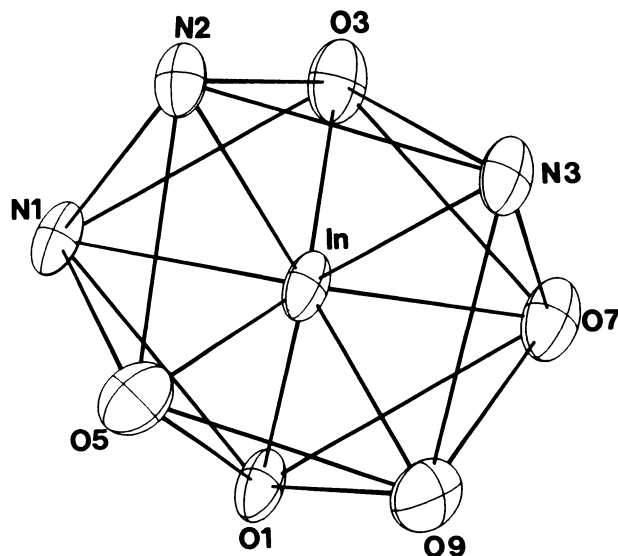


FIGURE 3
Ortep plot of the coordinating ligand atoms surrounding the central metal atom.

TABLE 1
Selected Structural Parameters for $\text{Na}_2\text{In}(\text{DTPA}) \cdot 7 \text{H}_2\text{O}$

Bond lengths (pm)			
In—N(1)	240.1(7)	In—O(3)	221.7(8)
In—N(2)	241.4(8)	In—O(5)	224.9(8)
In—N(3)	238.8(8)	In—O(7)	233.6(7)
In—O(1)	220.3(6)	In—O(9)	223.8(7)

Angles (deg)			
N(1)—In—N(2)	75.2(3)	N(1)—In—N(3)	145.6(3)
N(1)—In—O(1)	71.7(3)	N(1)—In—O(3)	72.2(3)
N(1)—In—O(5)	78.8(3)	N(1)—In—O(7)	125.6(3)
N(1)—In—O(9)	135.9(3)	N(2)—In—N(3)	73.5(3)
N(2)—In—O(1)	143.7(3)	N(2)—In—O(3)	79.6(3)
N(2)—In—O(5)	71.4(3)	N(2)—In—O(7)	135.9(3)
N(2)—In—O(9)	122.6(3)	N(3)—In—O(1)	142.0(3)
N(3)—In—O(3)	88.4(3)	N(3)—In—O(5)	104.1(3)
N(3)—In—O(7)	71.8(3)	N(3)—In—O(9)	74.8(3)
O(1)—In—O(3)	103.7(3)	O(1)—In—O(5)	87.7(3)
O(1)—In—O(7)	77.5(3)	O(1)—In—O(9)	75.0(3)
O(3)—In—O(5)	143.2(3)	O(3)—In—O(7)	73.1(3)
O(3)—In—O(9)	144.9(3)	O(5)—In—O(7)	143.6(3)
O(5)—In—O(9)	71.7(3)	O(7)—In—O(9)	72.4(3)

vance to binding to MABs. As mentioned (11) $\text{In}(\text{EDTA})(\text{SO}_3)^{3-}$ was studied by x-ray crystallography. The two In—N bond distances average 234.5 pm and the four In—O bonds 221.3 pm whereas the In—N bonds average 242 pm and 236 pm in $\text{In}(14\text{-aneN}_4\text{triac})$ and $\text{In}(12\text{-aneN}_4\text{triac})$, respectively (12). The In—O bonds average 220 pm and 218 pm, respectively, in these macrocyclic complexes. Figure 3 shows the coordinating ligand atoms around the central In^{3+} . The eight coordinating ligand atoms surround the central indium in a distorted Archimedean antiprismatic configuration (Fig. 3). The two planes are composed of N(1)O(3)O(7)O(1) (plane 1) and N(2)N(3)O(5)O(9) (plane 2). Deviations from the best planes are reasonable, 14 pm for plane 1 and 15.4 pm for plane 2. The two planes are almost parallel, the dihedral angle between them being only 5.2°. In^{3+} resides 122.2 pm above plane 1 and 126.9 pm below plane 2. As can be seen from Table 1, the In—O(9) bond is clearly longer than the average of the four remaining In—O bonds (222.6 pm) indicating that this bond may be much weaker than the others. Possibly, this can be a crystal packing effect or can be caused by steric crowding. Because the interpretation of structural data obtained from the solid state must be done with caution and the solution structure of a metal complex may differ from the one obtained from a single crystal structure, we undertook a NMR study to ascertain the octa-coordination in solution. Carbon-13 NMR spectroscopy should be especially useful for this purpose. The data are given in Table 2, along with the chemical shifts of metal-DTPA and EDTA complexes of known coordination numbers. Most important to the question of coordinated or free CO_2^- group are the $^{13}\text{CO}_2^-$ resonances. As can be seen

TABLE 2
Carbon-13 NMR Data for the CO₂ Group in Metal-DTPA and EDTA Complexes

Compound	CN [†]	pD	Chemical shifts [†] of CO ₂ ⁻ carbon ppm	Assignment
In(DTPA) ²⁻	8	3.02 4.71 7.47 9.3	179.020; 178.976; 178.956 179.736; 179.312; 179.084 179.74; 179.25; 179.026 179.78; 179.34; 179.11	5 COO ⁻ -In ³⁺ Intensity: 2:2:1
Ga(DTPA) ²⁻	6	3.1 8.4	172.942 178.021; 177.771; 177.180 181.25 178.729; 178.68; 178.623	2 free COOH 3 COO ⁻ -Ga ³⁺ 2 free COO ⁻ 3 COO ⁻ -Ga ³⁺
Ga(EDTA) ⁻	6	8.67	178.715	4 COO ⁻ -Ga ³⁺
Y(DTPA) ²⁻	8	8.65	184.346; 183.363; 183.216	5 COO ⁻ -Y ³⁺

[†] Coordination number.
[†] Sodium-3-trimethylsilyl-tetradeteriopropionate as internal standard.

from the ¹³C NMR spectrum in Figure 4 the five CO₂⁻ groups exhibit three signals around 179 ppm with the approximate intensity of 2:2:1. From the data in Table 2 it is clear that the chemical shifts are essentially independent on pD between pD 3.02 and 9.3 and differ only slightly (max. 0.6 ppm) indicating that within this pD-range all five carboxylate groups are coordinated. This is further corroborated by the ¹³C NMR data of Ga(DTPA)²⁻ and Y(DTPA)²⁻. Whereas Ga³⁺ forms octahedral complexes with coordination number six, the larger Y³⁺ is known to have coordination number 8 (19,20). Accordingly, Ga(DTPA)²⁻ exhibits a triplet ~178.7 ppm corresponding to three coordinated CO₂⁻ groups with an approximate intensity of 1:1:1 and a broader band centered ~181.25 ppm indicative of free carboxylate groups. Upon protonation at pD≈3 this last peak shifts to 172.9 ppm. Eight-coordinate Y(DTPA)²⁻ on the other hand shows three peaks within ~1 ppm

with the approximate intensity 2:1:2, indicating that all five CO₂⁻ groups are coordinated to the metal ion.

CONCLUSION

The single-crystal x-ray structure of Na₂In(DTPA)·7H₂O showed the complex to have coordination number 8 indicating that octadentate ligands are ideal and superior to ligands with smaller denticity for an application of ¹¹¹In in living systems. High resolution ¹³C NMR spectra showed that this high coordination number also pertains in aqueous solution. Although the additional carboxylate group of the SCN-Bz-DTPA chelate may not add much thermodynamic stability to the complex it is most likely that it retards dissociation resulting from the prevention of nucleophilic attack of the hydroxyl ion at the metal center. This will be

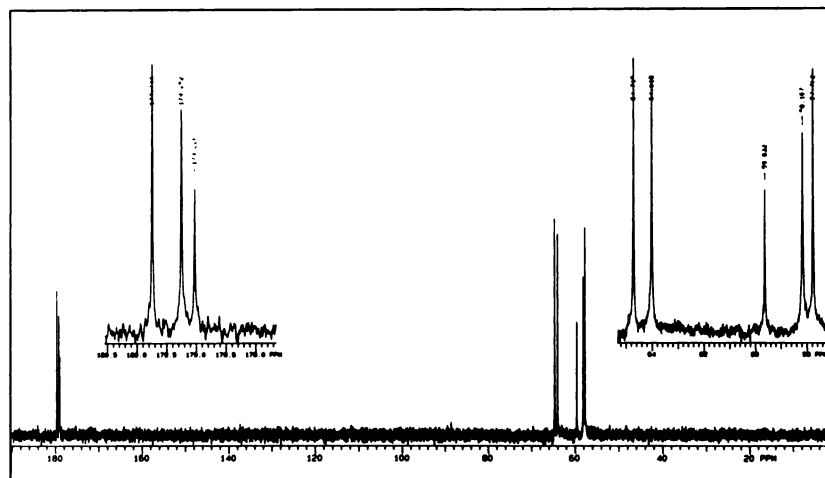


FIGURE 4
Carbon-13 NMR spectrum in aqueous solution at pD = 7.47.

assessed by a kinetic study involving a pH-dependence of the dissociation rate.

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