NMR Spectroscopy of Gallium in Biology

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Synonyms

Quadrupolar NMR in biology; Group 13 elements NMR in biology

Definition

Even though no biological role is known for gallium, it is a useful element in NMR structural studies of biologically relevant systems such as metalloproteins and radiopharmaceutical agents with application in clinical diagnosis. Gallium has two isotopes suitable for NMR spectroscopy, ^{69}Ga and ^{71}Ga, being both quadrupolar nuclei (nuclear spin I > ½) of moderate frequency (relevant magnetic properties are summarized in Table 1). In spite of the lower natural abundance, ^{71}Ga is usually the most favorable isotope for direct NMR observations, due to higher receptivity and narrower line width than ^{69}Ga. The chemical shifts and line widths of the NMR signals originated by gallium may give structural information on the coordination environment of the trivalent metal ion.

^{27}Al (I = 5/2), ^{115}In (I = 9/2) and ^{205}Tl (I = 1/2), the group companion elements of gallium, are also adequate for NMR spectroscopy (André and Mäcke 2003).

Table 1 – NMR properties of gallium (adapted from Delpuech 1983)

	^{69}Ga	^{71}Ga
Nuclear spin, I	3/2	3/2
Isotopic abundance	60.4	39.6
NMR frequency [a]	24.00	30.50
Quadrupole moment Q (10^{-28} m^2)	0.178	0.112
Relative receptivity [b,c] R_x	0.042, 237	0.056, 319
Relative line width [d] W_x	5.94	2.35
Relative peak height [e] H_x	3.4	11.6

[a] In MHz for an induction of 2.348 T (^{1}H at 100 MHz).
[b] In relation to ^{1}H or ^{13}C (first and second values, respectively).
[c] Computed as the ratio R of the receptivities $\alpha_x\gamma_x\ I_x(I_x+1)$ at constant field of the mentioned isotope and of the reference nucleus.
[d] Computed as the ratio W_x of the values taken by the function $(2I + 3)Q^2/I^2(2I -1)$ for the mentioned isotope and for ^{27}Al nuclei.
[e] Computed as 100 times the ratio of the values taken by the function R_x/W_x for the mentioned isotope and for ^{27}Al nuclei.

Principles of quadrupolar NMR

Nuclei with nuclear spin I > ½ are called quadrupolar given that they have a non-zero nuclear quadrupole moment Q (a non-spherical charge distribution within the nucleus). The main relaxation pathway for quadrupolar nuclei is the quadrupolar relaxation mechanism. For isotropic molecular movements in liquids this mechanism is characterized by an electric interaction between the quadrupole moment and fluctuating electrical field gradients present at the site of the nucleus. This interaction is modulated by the molecular tumbling.

The decays of the longitudinal and transverse magnetizations of a quadrupolar nucleus consist of the sum of I (if I is integer) or I+1/2 (if I is half-integer) exponentials corresponding to single quantum transitions between the 2I + 1 allowed nuclear Zeeman energy levels. For example, in the case of gallium (I = 3/2), the observed magnetization is due to two components, I, the central transition (m_I = ½ → - ½), and II, the outer transitions (m_I = 3/2 → ½ and m_I = - ½ → -3/2). The relaxation of the individual components is highly dependent on the motion and frequency of the nucleus under study. For half-integer quadrupolar

nuclei three situations of molecular motion can be considered. (Aramini and Vogel 1998; Drakenberg et al. 1997)

i) Rapid isotropic motion (extreme narrowing conditions)

This situation occurs when the correlation τ_c time of the molecule to which the metal ion is bound is short (small molecules tumbling fast in solution) in comparison to the inverse Larmor frequency ($\omega_0\tau_c$ << 1). In such case, the longitudinal and transverse magnetizations will follow a single exponential decay with the same time constant. The general expression for the T_1 and T_2 relaxation times for any I > ½ nucleus is given by:

$$\frac{1}{T_1} = \frac{1}{T_2} = \pi\Delta\nu_{1/2} = \frac{3\pi^2}{10}\frac{(2I+3)}{I^2(I^2-1)}\chi^2\tau_c\left(1+\frac{\eta^2}{3}\right) \qquad (1)$$

The parameter χ, quadrupolar coupling constant, represents the magnitude of the interaction between the nuclear quadrupole moment (Q) and the electric field gradient at the nucleus, with q_{zz} as its biggest component.

$$\chi = \frac{e^2 Q q_{zz}}{h} \qquad (2)$$

e is the electron charge and h the Planck's constant. For complexed metal ions, the higher the symmetry of the coordination environment, the smaller the electric fie*l*d gradient at the nucleus due to the ligands, and consequently the lower the value of χ.

η is the asymmetry parameter of the electric field gradient at the position of the nucleus; its value varies between 0 and 1 and it gives the deviation of the electric field from axial symmetry, which mainly depends on the lack of spherical symmetry of the p electron density. Cubic, tetrahedral, octahedral or spherical symmetry have normally a zero field gradient (q = 0), which gives rise to sharp signals. Asymmetry in the ligand field produces an increase in the NMR line width (Delpuech 1983; Akitt 1987).

ii) Intermediate isotropic motion (near-extreme narrowing)

In this limit (important for low-frequency nuclei bound to relatively small proteins) $\omega_0\tau_c$ is no longer small compared to unity and the relaxation cannot be described exactly as monoexponential. In practice the relaxation will appear

exponential up to $\omega_0\tau_c \approx 1.5$, even though T_1 and T_2 are different and field dependent. The following equations apply to half-integer quadrupolar nuclei when $\omega_0\tau_c \leq 1.5$ (Aramini and Vogel 1998):

$$\frac{1}{T_1} = \frac{3\pi^2}{100}\chi^2 \frac{2I+3}{I^2(I^2-1)}\left[\frac{2\tau_c}{1+(\omega_0\tau_c)^2} + \frac{8\tau_c}{1+4(\omega_0\tau_c)^2}\right] \quad (3)$$

$$\frac{1}{T_2} = \frac{3\pi^2}{100}\chi^2 \frac{2I+3}{I^2(I^2-1)}\left[3\tau_C + \frac{5\tau_c}{1+(\omega_0\tau_c)^2} + \frac{2\tau_c}{1+4(\omega_0\tau_c)^2}\right] \quad (4)$$

iii) Slow isotropic motion (nonextreme narrowing conditions)

This is the case when $\omega_0\tau_c >> 1$. Under such conditions the relaxation of a half-integer nucleus is not exponential anymore and, as a result, the shape of the NMR signal is not Lorentzian. The concept of single relaxation does not apply and multiple time constants are necessary to describe the magnetization decay (Drakenberg et al. 1997). For half-integer quadrupolar nuclei in this situation (moderate to high frequency nuclei bound to large proteins) the central transition (m_I = ½ → - ½) can originate a relatively narrow signal, according to equation 5, while the peaks due to all outer components are broadened beyond detection.

$$\Delta\nu_{1/2}(m_I = {}^1/_2 \rightarrow -{}^1/_2) = k\left(\frac{\chi^2}{\nu_0{}^2\tau_c}\right)$$

$$I = \frac{3}{2},\ k = 2.0\text{x}10^{-2} \quad (5a)$$

$$I = \frac{5}{2}, k = 7.2\text{x}10^{-3} \quad (5b)$$

$$I = \frac{7}{2}, k = 4.5\text{x}10^{-3} \quad (5c)$$

Therefore the linewidth of this component decreases with increasing $\omega_0\tau_c$. Additionally, under these conditions one should theoretically observe only 40, 25.7 or 19.0% of the signal for I = 3/2, 5/2 and 7/2 nuclei, respectively (Aramini and Vogel 1998).

Another important feature of the signal due to the central transition in this slow motion limit is the field dependence of its chemical shift. Although this shift is always smaller than the width of the broader components in the resonance, it

will be significant compared to the central transition signal (Drakenberg et al. 1997).

Another noteworthy aspect is the fact that the intensity of the central transition signal of a half-integer quadrupolar nuclei in this motional situation is affected by the pulse angle (Drakenberg et al. 1997). An effective pulse length, t_p, of 90 ^{o} for this component is much shorter than for the same nucleus under extreme narrowing conditions, according to the equation:

$$t_p\left(m_I = {}^1/_2 \rightarrow -{}^1/_2\right) = \frac{t_p}{I + 1/2} \qquad (6)$$

This is valid when the radiofrequency pulse strength is much less than the quadrupole coupling constant (Aramini and Vogel 1998).

Coordination aspects of gallium

The +3 oxidation state of gallium is the most stable in aqueous solution. In the pH range of 3 – 7, Ga^{3+} can hydrolyse to insoluble trihydroxide if its concentration exceeds nanomolar level. Nevertheless this precipitation can be avoided in the presence of stabilising agents. At physiological pH, the solubility of gallium is high due to the almost exclusive formation of $[Ga(OH)_4]^-$.

The coordination chemistry of Ga^{3+} is quite similar to that of the high spin Fe^{3+} ion. To this contribute the same charge of both ions, similar ionic radii (62 pm for Ga^{3+} and 65 pm for Fe^{3+}) and the same major coordination number of six (Ga^{3+} chelates sometimes are four- and five-coordinated).

Ga^{3+} is classified as a hard Lewis acid, forming thermodynamically stable complexes with ligands that are hard Lewis bases. Thus ligands with oxygen and/or nitrogen donor atoms (like carboxylate, phosphonate, phenolate, hydroxamate and amine groups) constitute good chelating agents for this ion. Gallium(III) is suitable for complexation with polydentate ligands, both cyclic and open chain structures. The majority of ligands designed for Ga^{3+} are hexadentate although several chelates have been reported which are stable *in vivo* and have coordination numbers of four and five (André and Mäcke 2003).

Macrocyclic chelators, in particular triaza ligands, are very adequate for Ga^{3+} chelation due to their high conformational and size selectivities, allowing a good

fit of the relatively small cation in the macrocyclic cavity. Triaza macrocyclic ligands with different types of pendant arms (carboxylates, alkylphosphinates, methylenephosphonates) have proved to be suitable ligands regarding the stable complexes they form with the gallium ion. The complexes formed are usually octahedral or pseudo-octahedral, showing a C_3 symmetry axis, with the three nitrogen atoms occupying one facial plane and the oxygen atoms from the pendant arms occupying the other (N_3O_3 systems). For this reason the complexes are highly symmetrical at the coordination centre and the metal ion gives origin to sharp gallium NMR signals (André and Mäcke 2003 and references therein).

Protein studies

Proteins are often too big for complete structural determination in solution by current multidimensional NMR techniques (^{1}H NMR signals are intrinsically broad due to slow molecular tumbling). NMR spectroscopy of Group 13 elements, in particular of $^{71/69}$Ga, has been used to investigate directly the metals in their specific binding sites in transferrins and to reveal subtle inter-site differences. Human serum transferrin is the protein that transports Fe^{3+} ions and it is a member of a small group of monomeric non-heme proteins (MW *circa* 76-81 kDa), which includes lactoferrin, ovotransferrin and melanotransferrin. It has two binding sites for ferric ions (these are found in six-coordinate, distorted octahedral coordination geometry) which are identified as C-terminal and N-terminal sites. Two tyrosines, one histidine, and one aspartic acid constitute four ligating groups to the metal ion. It requires a synergistic anion for the formation of stable metal complexes (*in vivo* the CO_3^{2-} as a bidentate ion serves this purpose by coordinating directly to the metal in the fifth and sixth coordination positions). Since serum transferrin is normally only about 30% saturated with iron, it retains a relatively high capacity for binding other metal ions.

Vogel and Aramini demonstrated the feasibility of using NMR quadrupolar metal nuclei to probe the metal ion binding sites in large proteins based on the detection of the central transition (m_I =1/2→ m_I =-1/2) of a half-integer quadrupolar nucleus (I = *n/2, n=3, 5, 7*), which is facilitated by increasing nuclear resonance frequency and protein size ($\omega_0\tau_c$ >> 1). These authors showed that important information about the metal ion binding site, namely the

symmetry of the site (i.e., χ, the quadrupole coupling constant) and the motion of the bound metal ion (i.e, τ_c, the rotational correlation time) may be extracted from the magnetic field dependence of the chemical shift and the line width of the signal due to the bound metal ion (Aramini and Vogel 1993; Germann et al. 1994; Aramini et al. 1994)

Vogel and Aramini investigated the binding of Al^{3+} to ovotransferrin and its half molecules in the presence of ^{13}C-enriched carbonate and oxalate using ^{27}Al and ^{13}C spectroscopy (Aramini and Vogel 1993). They pointed out that the detectability of the central transition of quadrupolar nuclei bound to large proteins depends considerably on a number of factors: (i) the strength of the external magnetic field (ω_0); (ii) the dimensions and motion of the macromolecule (τ_c, temperature, solution viscosity), (iii) the intrinsic quadrupole moment of the specific nucleus (Q), and (iv) the nature of the electric field gradient at the metal ion binding site.

$^{69/67}Ga$ NMR studies with ovotransferrin (oTf) have shown that the metal ion interacts preferentially with the N-site of the intact protein, as previously found for the Al^{3+} binding to oTf when carbonate serves as the synergistic anion (Aramini and Vogel 1993). In the presence of oxalate, oTf exhibits no site preference for Ga^{3+}. The isotropic chemical shifts of the oTf-bound $^{71/69}Ga$ NMR signals fall well within the range of Ga^{3+} bound to six oxygen-containing ligands (+40 to +80 ppm) (Germann et al. 1994; Aramini et al. 1994). Using the observed chemical shifts and line width of the protein-bound ^{71}Ga signals at two magnetic fields, the quadrupole coupling constants (χ) for the the ^{71}Ga nucleus were calculated as well as the rotational correlation time (τ_c) of the bound metal.

Complexes with relevance for nuclear medicine

^{67}Ga (γ, $t_{1/2}$ 3.25 days), ^{68}Ga (β^+, $t_{1/2}$ 68 min) are radionuclides that find a wide scope of applications in diagnostic radiopharmaceuticals due to their emitting properties and to their suitable half lives. In particular ^{68}Ga is nowadays a very much sought after radionuclide for positron emission tomography (PET).

Metal radionuclides have to be chelated with suitable ligands that form kinetically and thermodynamically stable complexes *in vivo* or, otherwise the radiometal can be donated to endogenous high-affinity binding sites such as

those located on the serum transferrin which displays two iron binding sites with high affinity for this metal ion (Maecke and André 2007).

^{69}Ga NMR spectroscopy has shown that the complex Ga(NODASA) has a remarkable stability with respect to acid-catalysed dissociation, similarly to what Parker *et al* have found for Ga(NOTA) (André and Mäcke 2003 and references therein). The complexes in the solid state are approximately octahedral, and ^{1}H NMR studies have suggested that these structures are maintained in solution. The pair of C_3–symmetric "facial" N_3 and O_3 donors lead to a minimal electric field gradient at the metallic centre in the "*x-y*" plane and consequently to the observation of narrow ^{69}Ga resonances (Table 2).

The coordination chemistry of gallium with α–aminoalkylphosphinic acid ligands based on triazacyclononane (L1, L2 and L3 in Table 2) was investigated by Parker *et al* and compared to that of the analogous α–aminocarboxylate ligands (André and Mäcke 2003 and references therein). The ^{71}Ga NMR signals of the phosphinate complexes appear at lower frequencies (between +130 and +140 ppm, Table 2) than the signal of Ga^{3+} in a tris(amino)tris(carboxylate) environment. Moreover, it was found that increasing the size of the alkyl group on the phosphorus atom increased the ^{71}Ga line width (L3 in Table 2). As in each of these cases the electric field gradient about the quadrupolar nuclei is very similar, the line width change was attributed to a change in molecular tumbling (τ_c).

When the oxygen donors were from phenolates, in tripodal aminophenolate ligand complexes (TAMS, TACS and TAPS), Caravan *et al* have found that the chemical shift range of ^{71}Ga moves to even lower frequencies (+18 to +57 ppm) but still lie in the range that is expected for octahedral Ga^{3+} complexes. (André and Mäcke and references therein). Orvig *et al* demonstrated the formation of highly symmetrical bicapped bisligand complexes of gallium (and of aluminum and indium) with the N_4O_3 tripodal phosphonic ligand H_3ppma, which give very narrow signals in the ^{27}Al NMR spectra (and in the ^{71}Ga and ^{115}In spectra) as a result of a S_6 symmetry (Table 2) (André and Mäcke 2003 and references therein).

Table 2 – NMR chemical shifts and line widths of some complexes of ^{71}Ga(III) (The data presented are from several authors in various publications and, if not otherwise stated, are summarized in André and Mäcke 2003)

Ligand	δ (ppm)	$\nu_{1/2}$ (Hz)
H_2O (1:6)	0	53
NOTA	+171	210 320 (^{69}Ga)
NOTAC6 [a]	+165.5	528.5
NOTAC8 [a]	+165.8	621.8
NODASA	+165 (^{69}Ga)	1000
NOTP	+110	434
NOTMA	+149	
L1	+132	560
L2	+139	200
L3	+130	1220
TTHA-$(BuA)_2$	+134	35000
H_3ppma (1:2)	-62.3	50
TAMS	+34	3400
TACS	+18	1000
TAPS	+57	1230

[a] (de Sá et al. 2010)
NOTA: 1,4,7-triazacyclononane-1,4,7-triacetic acid
NOTAC6: 1,4,7-triazacyclononane-1-hexanoic acid-4,7-diacetic acid
NOTAC8: 1,4,7-triazacyclononane-1-octanoic acid-4,7-diacetic acid
NODASA: 1,4,7-triazacyclononane-1-succinic acid-4,7-diacetic acid
NOTP: 1,4,7-triazacyclononane-1,4,7-tris-(methylenephosphonic acid)
L1: 1,4,7-triazacyclononane-1,4,7-triyltrimethylenetris(phenylphosphinic acid)
L2: 1,4,7-triazacyclononane-1,4,7-triyltrimethylenetris(methylphosphinic acid)
L3: 1,4,7-triazacyclononane-1,4,7-triyltrimethylenetris(benzylphosphinic acid)
TTHA-$(BuA)_2$: triethylenetetramine-N,N',N'',N'''-tetraacetic acid-N,N'''-bis(butylamide)
H_3ppma: tris(4-phenylphosphinato)-3-methyl-3-azabutyl)amine
TAMS: 1,1,1-tris(((2-hydroxy-5-sulfobenzyl)amino)methyl)ethane
TACS: *cis,cis*-1,3,5-tris((2-hydroxy-5-sulfobenzyl)amino)cyclohexane
TAPS: 1,2,3-tris((2-hydroxy-5-sulfobenzyl)amino)propane

Cross-References

Gallium uptake by transferrin; Inhibition of the proteosome activity by gallium(III) complexes; Therapeutic effects of gallium

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