Natural Abundance ^{15}N CP MAS NMR as a Novel Tool for Investigating Metal Binding to Nucleotides in the Solid State

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Introduction

The involvement of metal ions and their biological significance in nucleic acid processes has been well documented.[1−5] Metal ions counter balance the negative charges of the phosphate groups of the nucleotides, and they also affect the structure of these derivatives in the solid state.[3,5] There are three potential metal binding groups on a nucleotide: phosphate, sugar, and nuclear base moiety.[3−5] The crucial role of a direct metal ion binding to N(7) of the purine residue has been emphasized by several investigators.[3,5] However, one impediment to the definitive evaluation of the importance of the N(7) binding is the lack of effective direct spectroscopic criteria for its assessment. There is an indirect evaluation of metal−N(7) binding from satellite bands due to the metal−proton coupling constant,[6] however, this is applicable only in solution.

Nitrogen-15 NMR has the potential to provide local information about inter- and intramolecular interactions to nitrogen sites. However, to date, no clear evidence has been given on the effect of metal binding to the ^{15}N nuclear shielding constants.[7] We report here the ^{15}N NMR spectra, in the solid state, of guanosine and inosine and the metal complexes formed between Na^+, Ba^{2+}, and Cd^{2+} and the mononucleotides guanosine-5'-monophosphate (5'-GMP^{2-}) and inosine-5'-monophosphate (5'-IMP^{2-}) (Figure 1), and we demonstrate, for the first time, the great sensitivity of ^{15}N shieldings to metal ion coordination.

Experimental Section

Materials. Na_2(5'-GMP)·H_2O and $CdCl_2$ were purchased from Aldrich Chemical and used without further purification. Na_2(5'-GMP)·$6H_2O$, Na_2(5'-IMP)·$7.6H_2O$, Guanosine·$0H_2O$, and Inosine·$0H_2O$ were purchased from Sigma Chemical and $BaCl_2$·$2H_2O$ from Fluka Chemical, and all were used without further purification.

Preparation of the Compounds. Synthesis of Cd(5'-GMP)·$5H_2O$. A microcrystalline precipitate was obtained by slow addition of an aqueous solution (10^{-2} M) of the disodium salt of guanosine 5'-monophosphate to an aqueous solution of cadmium chloride in an equimolar ratio at pH 6.3. Elemental Analysis; Anal. Calcd (found): C, 21.31 (21.44); H, 3.93 (3.69); N, 12.42 (12.93).

Synthesis of Ba(5'-GMP)·$5H_2O$. A microcrystalline precipitate was obtained by slow addition of an aqueous solution (10^{-2} M) of the disodium salt of guanosine 5'-monophosphate to an aqueous solution of barium chloride in an equimolar ratio at pH 7.0. Elemental Analysis; Anal. Calcd (found): C, 20.40 (20.34); H, 3.76 (3.59); N, 11.89 (12.03).

Guanosine·$2H_2O$. A microcrystalline precipitate was obtained by slowly cooling a saturated aqueous solution of guanosine·$0H_2O$ which was first heated at 58 °C.

^{15}N and ^{113}Cd CP MAS NMR Spectra in the Solid State. ^{15}N and ^{113}Cd CP MAS NMR spectra were recorded on a Brüker MSL-300 spectrometer. The ^{1}H, ^{15}N, and ^{113}Cd frequencies were 300.13, 30.45, and 66.54 MHz, respectively. Samples (250 to 400 mg) were spun, at ambient probe temperature, in 7 mm alumina or zirconia rotors in a Brüker double-bearing probe, at speeds of 4.3−5.0 kHz.

Elemental Analyses. The elemental analyses of all the complexes were carried out in the Department of Chemistry, University of Ioannina, using an EA-110B Carlo Erba Analyzer.

Results and Discussion

^{15}N CP MAS NMR spectra of commercial (anhydrated) and recrystallized (dihydrated) guanosine indicate that the ^{15}N shieldings depend on the degree of hydration. Assignments were based on comparison with previous ^{15}N NMR studies of some nucleosides and nucleotides in solution.[8,9] Two N(7) resonance lines at 229.9 and 231.2 ppm are observed for recrystallized guanosine·$2H_2O$ (Figure 2a, Table 1). X-ray structure determination of dihydrated guanosine indicates strong intermolecular hydrogen bonding at the N(7) site, with the N(7)−H[N(1)] bond distance ranging between 2.817 and 2.875 Å.[10]

The ^{15}N CP MAS NMR spectrum of the Na_2(5'-GMP)·$3H_2O$ complex shows the presence of a single resonance absorption for each nitrogen nucleus. The N(7) atom of the guanine moiety (237.6 ppm, Figure 2b) is deshielded compared to that of guanosine with no metal binding. The ^{15}N CP MAS NMR

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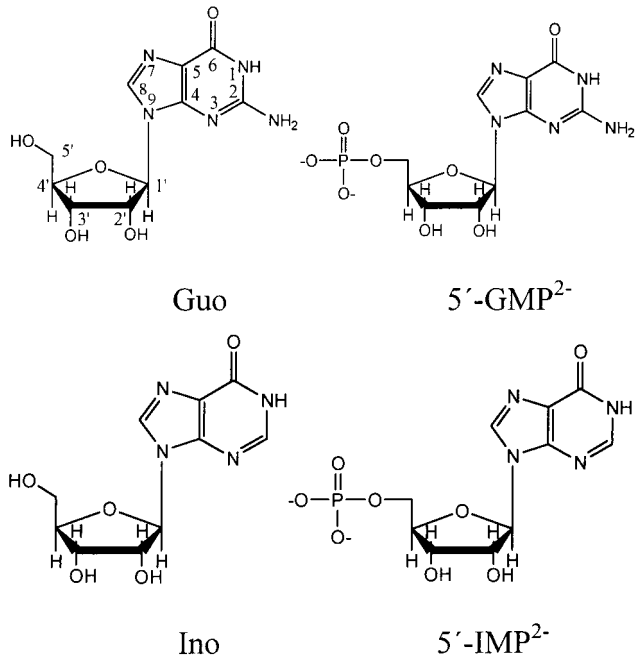

Figure 1. Schematic chemical structures of guanosine (Guo), guanosine-5'-monophosphate (5'-GMP^{2-}), inosine (Ino), and inosine-5'-monophosphate (5'-IMP^{2-}).

spectrum of the Na_2(5'-GMP)·$6H_2O$ complex shows splitting of the N(7), N(3), and $-NH_2$ resonances (Table 1), which indicates that changes in hydration influence the solid state structure of the complex. These results are in agreement with the X-ray structure of the strongly hydrated Na_2(5'-GMP)·$7H_2O$ salt,[11,12] which shows the presence of two independent nucleotides and four Na^+ ions in the asymmetric crystal unit (Table 2). The Na(1) ion, which is hydrated with four molecules of water, bridges between two *cis*-oriented 5'-GMP (A and B) molecules through N7(A) and N7(B) of the basis, with M−N(7) distances of 2.419 and 2.611 Å.

The ^{15}N CP MAS NMR spectrum of Na_2(5'-IMP)·$7.6H_2O$, on the other hand, shows no splitting of the resonances. The N(7) nucleus is slightly deshielded compared to the inosine molecule, which is isostructural to guanosine, with strong intermolecular hydrogen bonding between the N(7) and N(1) atoms of two hypoxanthines [N(7)−HN(1) = 2.8−2.88 Å].[10] The significant deshielding of the N(1) and N(3) nuclei of the inosine compound (~23.9 and 46.7 ppm, respectively), compared to that of guanosine, can be attributed to the effect of β-nitrogen substitution on the nitrogen chemical shifts.[9]

The ^{15}N CP MAS NMR spectrum of Ba(5'-GMP)·$5H_2O$ shows doublets for the N(9), N(3), and N(1) resonances, due to the presence of two inequivalent Ba(5'-GMP) molecules, and a single resonance absorption for N(7) due to accidental shielding equivalence. The X-ray structure of the Ba(5'-GMP)·$5H_2O$ complex[13] indicates the presence of two nucleotides and two Ba^{2+} ions per crystal unit in agreement with the NMR data in the solid. Ba(1) is highly hydrated, with seven molecules of water, and interacts with N7(A) and N7(B) of the basis, with M−N(7) distances of 2.85 and 3.08 Å, respectively (Table 2).

The ^{15}N CP MAS NMR spectrum of Cd(5'-GMP)·$5H_2O$ (Figure 2c) shows sharp single resonances for all the purine

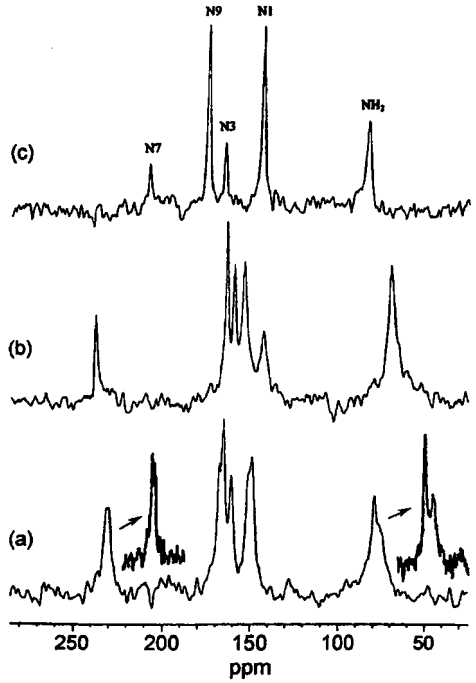

Figure 2. ^{15}N CP MAS NMR spectra of: (a) recrystallized guanosine·$2H_2O$ (400 mg), (b) Na_2(5'-GMP)·$3H_2O$ (400 mg), and (c) Cd(5'-GMP)·$5H_2O$ (300 mg) complexes recorded on a Brüker MSL-300 spectrometer. Recycle times were 2.4, 2.4, and 4 s for (a), (b), and (c), respectively, and the contact time was 2 ms. Number of scans were 25177, 33914, and 18724 for (a), (b), and (c), respectively. The ^{15}N chemical shifts were referenced to external liquid ammonia (25 °C, 0 ppm), were high-frequency positive, and were reproducible to ± 0.5 ppm. The inserts near the relevant peaks of (a) were obtained after resolution enhancement of the original spectrum.

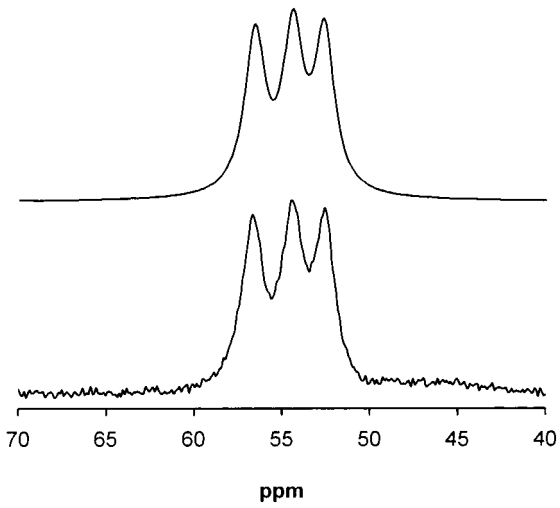

Figure 3. Bottom trace: experimental ^{113}Cd CP MAS NMR spectrum of the Cd(5'-GMP)·$5H_2O$ complex recorded on a Brüker MSL-300 spectrometer. Top trace: calculated ^{113}Cd NMR line shape for the ^{14}N−^{113}Cd spin pair using the following parameters: $\chi(^{14}N) = -2.4$ MHz, $\eta(^{14}N) = 0$, and $|^1J(^{14}N-^{113}Cd)| = 140$ Hz.

nitrogens, in agreement with the X-ray structure, which demonstrates the presence of one nucleotide and one metal ion per crystal unit.[14] The X-ray structure is typical for transition and heavy metal complexes with a 1:1 ratio of metal to guanosine-5'-monophosphate monomeric structure with the general formula [M(5'-GMP)$(H_2O)_5$]·nH_2O. The common features of this type of structure are the M−N(7) metal bonding and three intramo-

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Table 1. ^{15}N CPMAS NMR Chemical Shift (δ)[a] and Line width ($\Delta\nu_{1/2}$)[b] Data of Guanosine, Inosine, Guanosine-5'-Monophosphate and Inosine-5'-Monophosphate Complexes with Sodium, Barium and Cadmium Ions in the Solid State

compound	N7		N9		N3		N1		$-NH_2$	
	δ	$\Delta\nu_{1/2}$	δ	$\Delta\nu_{1/2}$	δ	$\Delta\nu_{1/2}$	δ	$\Delta\nu_{1/2}$	δ	$\Delta\nu_{1/2}$
guanosine·$0H_2O$	230.2	30	168.1	20	165.3	30	149.0	30	79.7	70
	231.5	30	166.0	30	160.9	20	151.6	30	75.7	~130
guanosine·$2H_2O$[c]	231.2	30	167.8	20	165.0	30	151.4	30	79.4	30
	229.9	30	165.5	20	160.8	20	148.6	30	75.5	~120
Na_2(5'-GMP)·$3H_2O$	237.6	30	163.9	20	159.7	30	154.0	30	70.6	60
Na_2(5'-GMP)·$6H_2O$	232.9	20	170.0[d]	20	166.9	10	150.5[d]	30	73.0	70
	235.7	20			160.9	20			70.4	70
Ba(5'-GMP)·$5H_2O$	239.2[d]	40	169.7	20	164.1	30	150.6	40	72.5	~200
			166.7	20	159.1	30	149.1	50	71.2	~200
Cd(5'-GMP)·$5H_2O$	208.0	30	175.2	20	165.4	30	144.0	20	83.6	50
inosine·$0H_2O$	236.2	30	174.6	30	207.6	30	172.9	40	–	
Na_2(5'-IMP)·$7.6H_2O$	237.7	20	176.9	50	207.4	10	171.6	20	–	

[a] The ^{15}N chemical shifts are given in ppm relative to external liquid ammonia (at 25 °C; 0 ppm). [b] The line widths are given in Hz. [c] Recrystallized guanosine. [d] Singlets due to accidental shielding equivalence.

Table 2. Selected X-ray Crystallographic Data of Metal Ion (Na^+, Ba^{2+}, Cd^{2+}) − Guanosine-5'-Monophosphate (5'-GMP) Complexes

metal ion	nucleotide	no of M. I.[a]	no of nucl.[b]	coord. numb.	coordinated atoms	M−N7 (Å)	M−O(W) (Å)	M−O(P) (Å)	M−O(2') (Å)	M−O(3') (Å)	M displ (Å)[c]	ref.
Na^+	5'-GMP	4	2 (A and B)	6	**Na(1)**: 4W, N7(A), N7(B)	2.419, 2.611	2.365−2.495					
				6	**Na(2)**: only water O atoms, 6W		2.403−2.758					11
				6	**Na(3)**: only water O atoms, 6W		2.341−2.618					
				6	**Na(4)**: 4W, O(2′)B, O(3′)B		2.271−2.828		2.471	2.314		
Na^+	5'-GMP	4	2 (A and B)	6	**Na(1)**: 4W, N7(A), N7(B)	2.415, 2.613	2.346−2.491				0.94(A), 0.67(B)	
				6	**Na(2)**: only water O atoms, 6W		2.382−2.746					12
				6	**Na(3)**: only water O atoms, 6W		2.328−2.611					
				6	**Na(4)**: 4W, O(2′)B, O(3′)B		2.281−2.803		2.492	2.305		
Ba^{2+}	5'-GMP	2	2 (A and B)	9	**Ba(1)**: 7W (2 water O atoms bind also with Ba(2)), N7(A), N7(B)	2.85, 3.08	2.69−3.08					13
				9	**Ba(2)**: 3W, O (phosph.A), O(3′)A, O(2′)B, O(3′)B		2.78−2.97	2.84	3.01	2.79, 2.77		
Cd^{2+}	5'-GMP	1	1	6	**Cd(1)**: five (5) water O atoms and N7 of the guanine ring	2.37	2.24−2.34				0.02	14

[a] Number of metal ions per unit cell. [b] Number of nucleotides per unit cell. [c] Metal deviation from the mean plane of the guanine ring.

lecular hydrogen bonds, one between a water molecule and O(6) and two between water molecules and phosphate oxygens. The N(7) resonance absorption is strongly shielded by −29.6 and −31.9 ppm compared to that of the Na^+ and Ba^{2+} complexes, respectively. This demonstrates significantly different binding modes to N(7), although X-ray structural data have been interpreted in terms of direct metal ion−N(7) interaction for Na^+, Ba^{2+}, and Cd^{2+} complexes.[11−14]

Unambiguous demonstration of a direct Cd−N coordination bond is provided by the ^{113}Cd CP MAS NMR spectrum, which indicates the presence of an asymmetric 1:1:1 triplet due to (^{113}Cd, ^{14}N) indirect and residual dipolar spin−spin interactions[15,16] (Figure 3). Indirect spin−spin coupling results in three equally spaced lines. The perturbation, however, of the ^{14}N spin states due to the quadrupolar interaction shifts the lines from their unperturbed (symmetric) positions. Detailed line shape analysis was carried out using an automated fitting procedure similar to that presented in ref 17. Assuming that the direction of the nitrogen lone pair coincides with the *z*-axis of the ^{14}N electric field gradient tensor, and using Cd − N crystallographic distance of 2.37 Å, the line shape analysis results in ^{14}N quadrupolar coupling constant of −2.4 MHz, asymmetry parameter of 0, and one-bond (^{113}Cd,^{14}N) spin−spin coupling of 140 Hz.

In conclusion, in the M-GMP complexes of the sodium and barium ions the bond length of M−N(7) and orientation of the N(7) lone pair results in very weak, if any, metal ion-N(7) binding. This is also the case for the Na(1) ion of the Na_2(5'-GMP)·$7H_2O$ complex with a M−N(7) distance of 2.419 Å, which is comparable to that of the Cd complex (M−N(7) distance of 2.37 Å). This is due to the fact that the Na(1) ion strongly deviates from the least-squares planes of the A and B purine rings (0.94 Å (22.90°) and 0.67 Å (14.86°), respectively); this is contrary to the Cd complex in which the Cd ion lies 0.01 Å off the mean plane through the guanine ring (Table 2).

The results reported here show that ^{15}N NMR shieldings are useful probes of metal coordination to mononucleotides, and further demonstrate that the sizes of the chemical shift changes could be correlated, at least to a first approximation, with the strength and directionality of metal to nitrogen coordination. This approach should be particularly valuable in studies of site-specifically ^{15}N labeled DNA and RNA fragments in the solid state.

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