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High Resolution Proton NMR Spectra of Guanosine-5'-Monophosphate-Mg^{2+} Interactions and Anticarcinogenic Effect of Magnesium

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Introduction

Experimental evidence [1, 2] is mounting in support of the hypothesis of an anticarcinogenic effect of magnesium. The role of magnesium deficiency in the development of cancers and other diseases has been reviewed [3]. It is however, known that only a narrow range of Mg^{2+} concentrations is effective to exercise control over DNA synthesis [4]. It has been also suggested that Mg^{2+} ions cause incorporation of the proper nucleotide in both DNA and RNA synthesis by natural selection of the nucleotide with or without the 2'-hydroxyl group. The Mg^{2+} ions are used to bind negatively charged phosphate groups in a compound helix. A double stranded helix may combine with another strand to form a triple helix or tetrahelix held together by salt bridges in which divalent cations such as Mg^{2+} hold together two phosphate groups [5, 6]. Recently, it has been shown by X-ray diffraction analysis that in a left-handed double helix Z–DNA two different conformations are observed for the phosphates in GpC sequences [7]. The phosphates are found to be rotated away from the conformation in which they are facing the helical grooves. This conformation is found only when hydrated magnesium ions are complexed to N7 of guanine and to the phosphate oxygen atoms through a water molecule coordinated with the magnesium [7].

In this report we will show high resolution proton NMR spectral evidence that the magnesium ions are bound to N7 of guanine and that the phosphate group is rotated most likely through hydrogen bond with water molecules coordinated to magnesium ions, in the simple mononucleotide (5'-GMP).

Experimental

Guanosine-5'-monophosphate disodium salt was purchased from Sigma Chemical Company. $MgCl_2 \cdot 6H_2O$ was certified Anachemia Chemicals Ltd. The D_2O (Merck, Sharp & Dohme, Canada Ltd.) solutions were prepared carefully by mixing weighed amounts of magnesium chloride with 5'-GMP. Fourier Transform Nuclear Magnetic Resonance Spectra were obtained with a Bruker High Resolution WH-400 MHz Proton NMR spectrometer. The chemical shifts are referenced relative to internal standard, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The pD of the solutions was measured both before and after recording the NMR spectra, with a Fisher Accumet 630 pH meter. The samples of 5'-GMP were lyophillized twice to reduce the HDO. A partial exchange of H8 was observed after lyophillization.

Results and Discussion

The structure of 5'-GMP and numbering of the atoms are as shown in Fig. 1. An evaluation of the ribose ring parameters and a determination of its conformational properties and those of the exocyclic carbinol and phosphate groups has been described [8, 9]. The interaction of Mg^{2+} ions with 5'-GMP changes the purine-sugar-phosphate parameters to some extent as is shown from high resolution NMR spectra (Fig. 2 and Tables I and II). The purine proton H8 shifted slightly to lower fields on interaction of the nucleotide with magnesium. This is evidence that Mg^{2+} ions are complexed to N7 of guanosine in agreement with the X-ray diffraction analysis [7] in which it was found that the magnesium ion was complexed to N7 of guanine in Z–DNA formed in a single crystal of a DNA fragment containing six base pairs. The interaction of magnesium ions with N7 also perturbs the C(1') protons of the sugar ring and shifts them to lower fields (see Table I). This again indicates that the purine base has taken a new

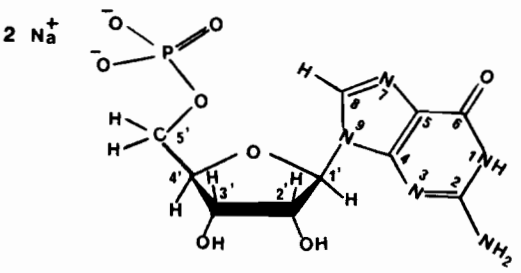

Fig. 1. The structure of Guanosine-5'-Monophosphate.

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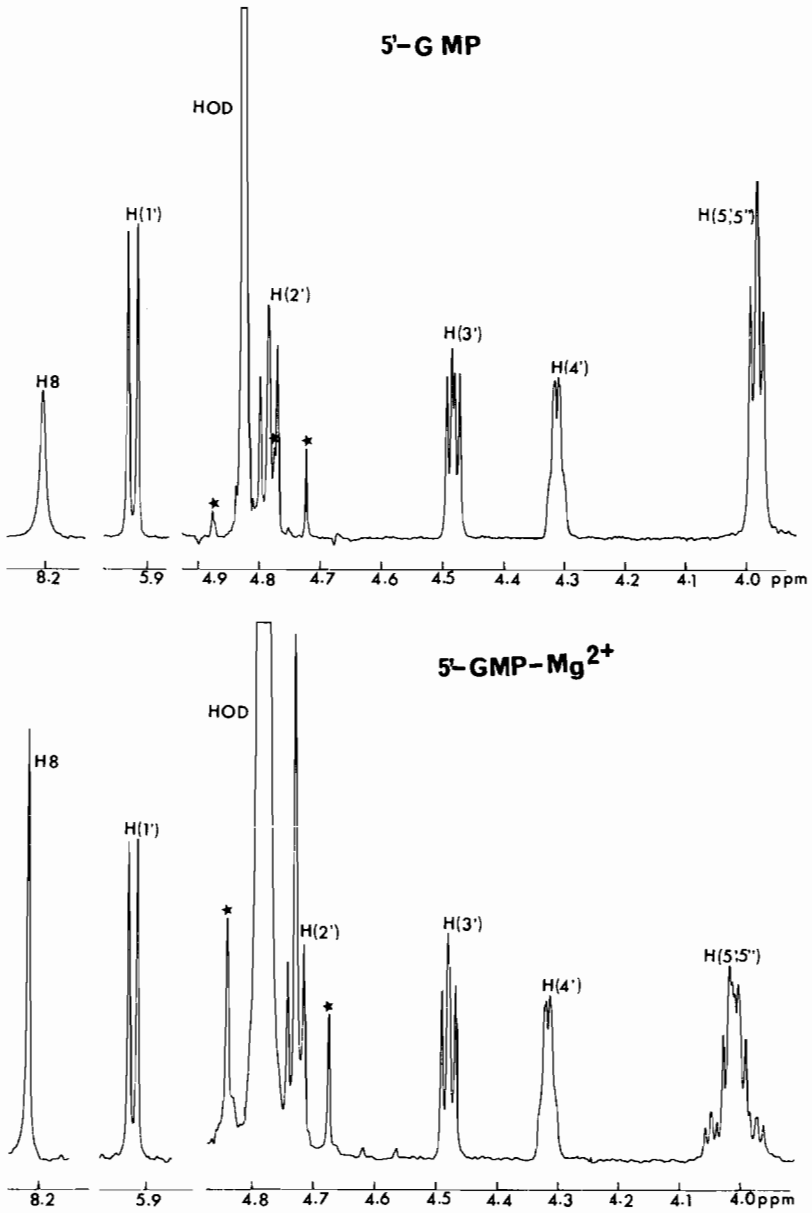

Fig. 2. 400-MHZ ^{1}H NMR spectra of Guanosine-5'-Monophosphate 20 m*M* in D_2O and Guanosine-5'-Monophosphate in the presence of 50 m*M* $MgCl_2 \cdot 6H_2O$. The side bands of HOD are indicated by an asterisk. The temperature was 20 ± 2 °C and the pD = 8.3 for 5'-GMP and for 5'-GMP–Mg^{2+} complex pD = 7.8.

orientation with respect to the sugar ring [8, 9]. The complexation of magnesium to N7 of guanine rotates the purine base around the glucosidic bond and perturbs slightly the syn-anti equilibrium. The proton H(2') on the contrary shifts to higher fields and in the case of r = 2.5 the shift is 0.054 ppm. This can be easily explained by a change of the S-type conformation to N-type, where the proton H(2') comes out of the purine plane and thus more shielded. The protons H(3') and H(4') are the least affected. Coupling constants are given in Table II and show that the rapid equilibrium between N-type [(3')-*endo*] and S-type [C(3')-*exo*] conformers N ⇄ S is also perturbed by the Mg^{2+}–N7 interaction. The S-type conformation is favored in ribonucleotides with the equilibrium lying somewhat more in favor of the S conformer *i.e.*, 70:30. The spin-coupling constants $J_{1',2'}$ and $J_{1',4'}$ for H(1', 2') and H(2', 3') protons have been altered (Table II). The data show that the $J_{1',2'}$ decreases and the $J_{3',4'}$ increases as the magnesium concentration in solutions increases. Both trends are in agreement with a slight

TABLE I. Chemical shifts of Guanosine-5'-Monophosphate in D_2O solutions with Mg^{2+} ions.[a]

$[Mg^{2+}]$	r	H8	(H1')	H(2')
0	0	8.202	5.920	4.783
10	0.5	8.208	5.919	4.763
20	1	8.213	5.918	4.744
50	2.5	8.218	5.921	4.729
100	5	8.240	5.935	4.725
200	10	8.253	5.950	4.730

[a]Proton chemical shifts measured from DSS as internal reference in 20 m*M* D_2O solutions at 20 ± 2 °C to an accuracy of ±0.002 ppm. Mg^{2+} ions were added as $MgCl_2 \cdot 6H_2O$. PD = pH meter reading + 0.40 [11], The pD of the 5'-GMP solutions was 8.3 and for the solutions in the presence of Mg^{2+} ions it varied between 8.3 and 7.6. r = ratio (Mg/5'-GMP).

increase of the N-type conformer at the expenses of S-type conformer *i.e.*, the new N ⇄ S equilibrium is 59:41.

The proton NMR spectra of the exocyclic carbinol H(5',5'') do clearly show that the phosphate conformation about the C(5')–O(5') bond is also affected by the presence of magnesium ions (See Fig. 2). The Mg^{2+} ions perturb the rotamer populations of *gauche-gauche, gauche-trans* and *trans-gauche* by increasing slightly the gg rotamers which are overwhelmingly preferred in all 5'-ribo and deoxyribonucleotides [9]. The spectra show a narrowing of the H(5',5'') proton resonances coupled with phosphorus and that the phosphate group rotates around the C(5')–O(5') sugar–phosphate bond when the magnesium ion is complexed to N7. The rotation is rather slow as the estimates of the coupling constants show. This suggests that 5'-GMP molecules in solution in the presence of high concentration of Mg^{2+} ions exist in a mixture of conformations gg, gt and tg with the exact proportions depending on the concentration of Mg^{2+} ions and temperature [10]. ^{31}P nuclear magnetic resonance studies and temperature effects on these systems will be reported elsewhere [10].

The effect of magnesium on the conformation about the glucosidic bond is interesting and it is shown here that this effect increases with the Mg^{2+} concentration in solution (Table I). The 5'-GMP molecules in the presence of magnesium stabilize an N ⇄ S equilibrium which favors N-type [C(3')-*endo*] conformation as in A-DNA, with the plane of the base moving towards the sugar oxygen.

The phosphate group (PO_3^{2-}) has rotated and the oxygens on the phosphate come closer to the H8 position. The H(5')–C(5')–O(5') dihedral angle has rotated and the phosphate oxygens may form a hydrogen bond with a water molecule coordinated

TABLE II. Spin-Coupling Constants of Guanosine-5'-Monophosphate in D_2O solutions and in the presence of Mg^{2+} ions.[a]

$[Mg^{2+}]$	r	$J_{1',2'}$	$J_{3',4'}$
0	0	6.1	3.4
10	0.5	6.1	3.4
20	1	5.8	3.7
50	2.5	5.8	3.7
100	5	5.5	3.7
200	10	5.5	3.9

[a]5'-GMP was 20 m*M*. The accuracy of the spin-coupling constants is ±0.1 Hz.

to the magnesium ion which is also complexed to N7 of guanine [7].

These experiments show for the first time quantitatively the effect of magnesium ions on nucleotides in solution, as it perturbs the conformation about the glucosidic bond and the rotamer population of conformers about the C(5')–O(5') bond. It is suggested from these results that magnesium may stabilize a particular conformation of DNA and thus counteracts countless oncos and mutagens.

Acknowledgement

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