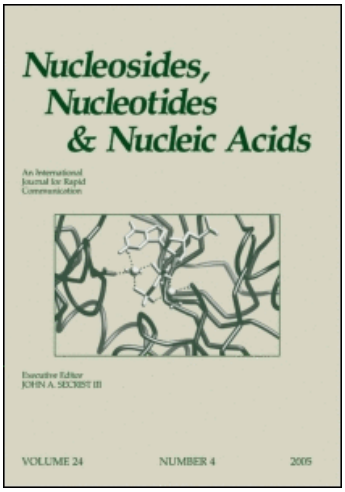

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What Are the Conformational Changes Induced by Hard and Soft Metal Ion Binding to 2'-Deoxyguanosine?

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WHAT ARE THE CONFORMATIONAL CHANGES INDUCED BY HARD AND SOFT METAL ION BINDING TO 2'-DEOXYGUANOSINE?

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ABSTRACT: The NMR study on the interactions of 2'-dG with Mg^{2+}, Zn^{2+} and Hg^{2+} ions in D_2O solution has shown that binding of softer metal ions to N7 shifts N $\rightleftarrows$ S pseudorotational equilibrium slightly towards N-type sugar conformations. There are no detectable changes for the conformational equilibria across C4'-C5' bond, whereas the population of the *syn* conformers is slightly increased.

INTRODUCTION

The interaction of divalent metal ions with nucleic acid constituents plays important role in defining structure and catalytic activity, as well as in the potential applications of metal ion complexes as drugs. Numerous studies have been concerned with defining metal binding sites on nucleosides and relative stabilities of their complexes.[1,2] We have directed our attention to the conformational changes of 2'-dG, which can be considered as the simplest model, where the J-coupling constants and nOe data can be obtained with high precision as a function of variable metal ion concentrations. Hard Mg^{2+} ions are expected to bind to O-donor atoms, whereas softer Zn^{2+} and Hg^{2+} are likely to prefer N-donor atoms.[1-3] We expect the binding of metal ions to 2'-dG to be manifested in a specific shift of North (≈ C3'-*endo*) $\rightleftarrows$ South (≈ C2'-*endo*) equilibrium, and in the changes of the other two rotational degrees of freedom, namely the conformational equilibria along C4'-C5' and glycosyl bonds (FIG. 1). The two-state N $\rightleftarrows$ S

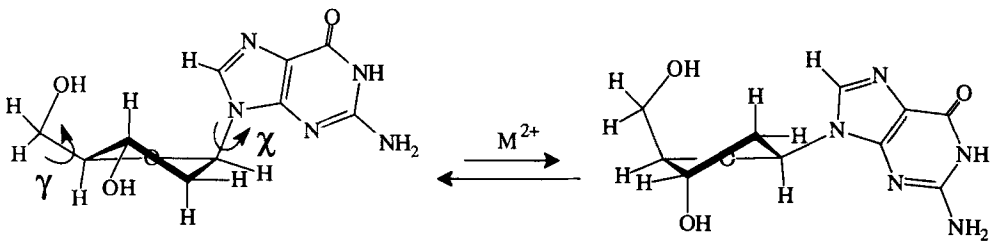

FIG. 1. The dynamic two-state N ⇄ S pseudorotational equilibrium of 2'-dG is driven by the anomeric effect of the nucleobase and [O4'-C4'-C3'-O3'H] *gauche* effect. The binding of metal ions is expected to change the electronic nature of guanine residue and therefore shift N ⇄ S pseudorotational equilibrium and conformational equilibria across C4'-C5' bond (γ) and glycosyl bond (χ) in a specific manner.

pseudorotational equilibrium is in solution controlled by the competing anomeric and *gauche* effects.[4] The protonation of 2'-dG results in the increased preference for N-type conformers by 16%, which has been attributed to the strengthening of the anomeric effect.[5] Softer metal ions are expected to bind to N7[3] which will change the electronic character of guanine in 2'-dG and should facilitate the interaction between lone-pair orbitals of O4' and orbitals of glycosyl bond (*i.e.* anomeric effect). The increase in the strength of anomeric effect will be observable through the changes in the bias of N ⇄ S pseudorotational equilibrium towards N-type conformers.

RESULTS AND DISCUSSION

(A) Binding sites

The affinity of Mg^{2+}, Zn^{2+} and Hg^{2+} ions for the specific electron rich binding sites on 2'-dG has been studied by following the changes in ^{1}H- and ^{15}N-NMR chemical shifts, as well as by proton spin-lattice relaxation time measurements. ^{1}H-NMR chemical shifts have been monitored as the concentration of the Mg^{2+}, Zn^{2+} and Hg^{2+} ions has been gradually increased by titration of 2'-dG with nitrate salts in aqueous solution (FIG. 2). Titration by Mg^{2+} ions resulted in no change of chemical shifts for H8 and sugar protons, which indicates the absence, or possibly a very weak interaction of Mg^{2+} with N7 or oxygen atoms of the sugar moiety. In the case of Zn^{2+}, the $\Delta\delta$ for H8 of 0.039 ppm has

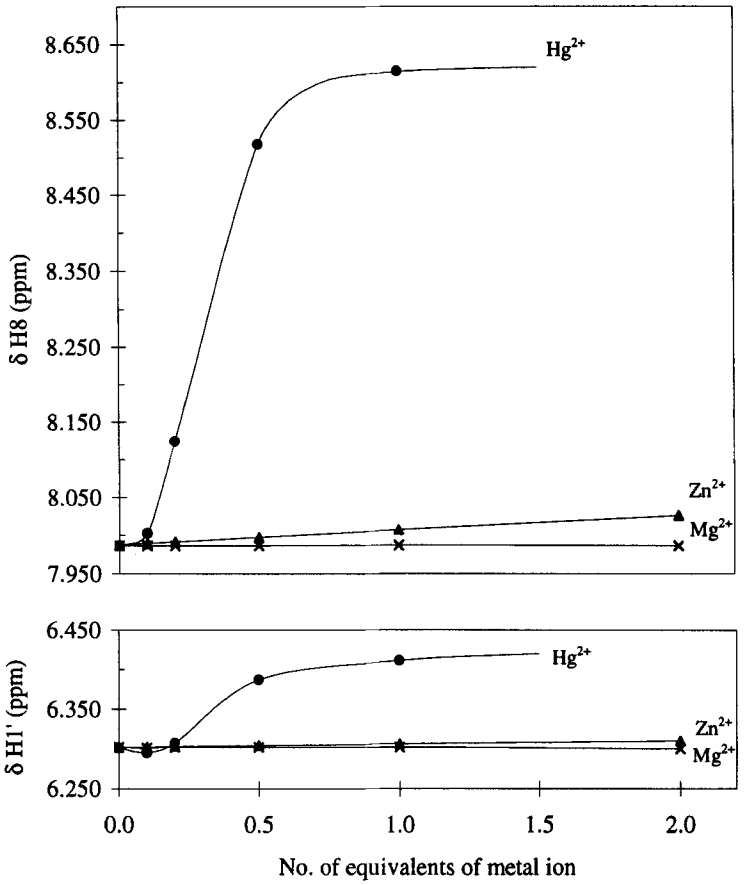

FIG. 2. Changes of ^{1}H-NMR chemical shifts as a function of increasing metal ion concentration in 10 mM D_2O solution of 2'-dG.

been observed (FIG. 2), which suggests binding to N7. The changes in chemical shifts of other proton resonances upon addition of two equivalents of Zn^{2+} have been smaller than 0.008 ppm. Titration of 2'-dG by one equivalent of Hg^{2+} induced a large downfield shift of H8 ($\Delta\delta = 0.629$ ppm) and of H1' ($\Delta\delta = 0.110$ ppm), which agrees with binding of Hg^{2+} to N7.[3] We note that, as the concentration of Hg^{2+} ions exceeded one molar equivalent relative to 2'-dG, broadening and the multiplication of signals occurred which can be explained with aggregation. After addition of two equivalents of Hg^{2+} two signals for H8 ($\delta = 8.559$ and 8.398 ppm) were observed, while other resonances were not resolved.

^{15}N-NMR has been used to get complementary and more straightforward information on the interactions of metal ions with guanine residue at 10 mM concentration of 2'-dG, at which all other studies were performed. The chemical shifts of N7 and N9 for 2'-dG were measured and determined to be -145.9 ppm and -207.7 ppm, respectively. The signals were assigned in accordance with the literature data.[6] Titration by two equivalents of Mg^{2+} ions induced no observable change in chemical shifts of N7 and N9 ($\Delta\delta < 0.5$ ppm). Upon addition of two equivalents of Zn^{2+} the signal for N7 moved upfield by 3.4 ppm, whereas the signal for N9 moved slightly downfield ($\Delta\delta$ = 0.5 ppm). Results obtained from ^{15}N-NMR spectra suggest binding of Zn^{2+} ions to N7. The softer Zn^{2+} and Hg^{2+} ions bind to N7, which is also the protonation site. It is noteworthy however, that $\Delta\delta$ of N7 upon protonation is 46 ppm,[6b] which means that the perturbation of the electronic character of guanine residue is much greater upon protonation than upon metal ion binding. The fact that N7 responds more significantly to protonation than to interaction with metal ions suggests that the shift of N $\rightleftarrows$ S pseudorotational equilibrium by the modulation of the strength of the anomeric effect will be an order of magnitude smaller in the case of the hydrated metal ion binding than it is for protonation/deprotonation (*vide infra*).

The proton spin-lattice relaxation times (T_1) have been determined to examine if there are any local changes due to interactions of metal ions with specific atoms of 2'-dG (TABLE 1). In case of Mg^{2+} ions, the change of the relaxation times for H1' (-0.29 s) is twice as high, as it is for H8 (0.16 s) and H4' (-0.15 s), which could be interpreted by the reorientation around the glycosyl bond,[7] or possibly by the binding in the vicinity of O4'. The addition of two equivalents of Zn^{2+} ions resulted in the largest shortening of T_1 for H8 (-0.39 s) and H1' (-0.2 s), which supports the results from chemical shift analyses of N7 binding.

(B) N $\rightleftarrows$ S pseudorotational equilibrium

The analysis of solution conformation of 2'-deoxy-β-D-ribofuranosyl moiety in 2'-dG is based on five proton-proton coupling constants ($^3J_{1'2'}$, $^3J_{1'2''}$, $^3J_{2'3'}$, $^3J_{2''3'}$ and $^3J_{3'4'}$). The precise values for proton-proton coupling constants and chemical shifts from a seven spin system were obtained through the simulation procedure which is integrated into the

TABLE 1. Effect of metal ion interactions on T_1 relaxation times of 2'-dG.

Metal ion[a]	H8	H1'	H2'	H2"	H3'	H4'	H5'	H5"
none	2.36	2.95	0.75	0.76	2.15	2.55	0.73	0.71
Mg^{2+}	2.52	2.66	0.70	0.73	2.12	2.40	0.71	0.69
Zn^{2+}	1.97	2.75	0.72	0.76	2.14	2.49	0.72	0.70

[a]Two equivalents of metal ion added relative to 2'-dG. Error in T_1 values (in sec) is estimated to be smaller than ±0.07 sec.

Varian software package. The resulting vicinal coupling constants $^3J_{HH}$, which are presented in TABLE 2, have been interpreted in terms of a two-state equilibrium as is normally done for ribose or 2'-deoxyribose rings.[8] The increase in the metal ion concentration by titration of 2'-dG with Mg^{2+} and Zn^{2+} caused no observable changes in $^3J_{HH}$ coupling constants. In the case of Hg^{2+}, however we have observed changes in $^3J_{1'2'}$, $^3J_{2'3'}$ and $^3J_{2"3'}$ coupling constants which indicate that the N $\rightleftarrows$ S pseudorotational equilibrium is affected as the metal ion binds to N7. As the Hg^{2+} concentration was increased some aggregation was observed, which has been manifested in the broadening of the signals that prevented the extraction of individual $^3J_{HH}$ coupling constants at metal to nucleoside ratio higher than 1:5.

The analysis of $^3J_{HH}$ coupling constants consists of three standard translation steps. First step translates experimental proton-proton coupling constants to proton-proton torsion angles and is covered by the generalised Karplus-Altona equation.[8] Second step is the translation of proton-proton torsion angles into the corresponding endocyclic torsion angles and is formulated with the set of linear equations $\Phi_{HH} = A\nu_j + B$. Φ_{HH} is the torsion angle between two vicinal protons and ν_j is the corresponding endocyclic torsion angle.[8] Parameters A and B were determined from a large collection of X-ray data.[8] Third translational step of endocyclic torsion angles into the pseudorotational parameters is described by a simple cosine function $\nu_j = \Psi_m * \cos[P + (j-2) * 4\pi/5]$, where P is the phase angle of pseudorotation and Ψ_m is the maximum puckering amplitude. In the elaboration of the experimental $^3J_{HH}$ coupling constants at various metal ion concentrations we have used the computer program PSEUROT,[8c] which calculates the least-squares fit of the five

pseudorotational parameters defining the two state N ⇄ S equilibrium (P_N, Ψ_m^N, P_S, Ψ_m^S and x_S) to the set of experimental $^3J_{HH}$. As $^3J_{HH}$ coupling constants indicate a strong bias of N ⇄ S conformational equilibrium in 2'-dG towards S, the pseudorotation parameters of the minor N-type component were constrained (P_N = 19°, Ψ_m^N = 36°)[8] to obtain the parameters of the major S-type component. The resulting values for P_S and Ψ_m^S were fixed in further optimizations of the minor N-type conformer, which after convergence greatly improved the agreement between the experimental and the calculated $^3J_{HH}$. In the absence of metal ions, the overall r.m.s. error dropped from 0.46 Hz to 0.21 Hz, and the maximum discrepancy between the experimental and the calculated coupling constants (ΔJ^{max}) dropped from 0.4 Hz to 0.3 Hz. This iterative procedure was repeated few times for each metal ion data set of experimental $^3J_{HH}$ to assure the convergence towards the global minima (r.m.s. error < 0.23 Hz, ΔJ^{max} < 0.3 Hz) which are given in TABLE 2.

We feel that two important conclusions can be drawn from data in TABLE 2. First, the geometry of the N- and S-type conformers does not change significantly with the addition of any of the three metal ions to the solution of 2'-dG. Secondly, the population of the N-type conformers increases only slightly as the concentration of Mg^{2+} and Zn^{2+} is increased up to two equivalents. Upon addition of 0.2 equivalents of Hg^{2+} ions, however, the population of the N-type conformers has increased by 4%.

(C) Conformational equilibrium across C4'-C5'

The conformational equilibrium between three staggered conformers (*gauche*-plus, γ = 53° ⇄ *gauche*-trans, γ = 180° ⇄ *gauche*-minus, γ = -70°)[9] has been assumed. The experimental $^3J_{4'5'}$ and $^3J_{4'5''}$ are weighted time-averaged values which are related to the limiting coupling constants and populations of the three rotamers with the following equations: $^3J_{4'5'} = 1.63\ x_{\gamma+} + 2.86\ x_{\gamma t} + 10.39\ x_{\gamma-}$ and $^3J_{4'5''} = 1.83\ x_{\gamma+} + 10.77\ x_{\gamma t} + 2.71\ x_{\gamma-}$. Note that the above limiting coupling constants have been calculated with the use of six-parameter Karplus equation.[8] Our analyses of the experimental $^3J_{4'5'}$ and $^3J_{4'5''}$ (see footnote of TABLE 2) has shown that 2'-dG is characterised by 51.4% γ^+, 30.3% γ^t and 18.2% γ^-. We have observed no change in $^3J_{4'5'}$ and $^3J_{4'5''}$ coupling constants regardless of the type and the concentration of metal ion. The fact that the conformational equilibrium across C4'-C5' is not affected by increasing the concentration of metal ions suggests that

TABLE 2. The comparative effect of metal ions on the N ⇄ S equilibrium of 2'-dG.

Metal ion	No. equiv.	$^3J_{1'2'}$	$^3J_{1'2''}$	$^3J_{2'3'}$	$^3J_{2''3'}$	$^3J_{3'4'}$	P_N	Ψ_m^N	P_S	Ψ_m^S	%S[a]
none	0.0	7.4	6.4	6.2	3.6	3.2	-22°	34°	160°	39°	66.2
Mg^{2+}	2.0	7.4	6.4	6.2	3.6	3.3	-20°	34°	161°	39°	65.9
Zn^{2+}	2.0	7.3	6.4	6.3	3.6	3.2	-22°	34°	160°	39°	65.4
Hg^{2+}	0.1	7.2	6.4	6.4	3.7	3.1	-27°	33°	159°	40°	63.9
	0.2	7.0	6.4	6.5	3.8	3.2	-27°	33°	159°	40°	62.1

[a] Complete set of $^3J_{HH}$ values (in Hz, ±0.1 Hz) at five different metal ion concentrations for Mg^{2+}, Zn^{2+}, and at two metal concentrations for Hg^{2+} has been used to calculate the pseudorotational parameters of sugar moiety of 2'-dG. δ(10 mM in D_2O, pD = 7.5, 298K): 7.99 (H8), 6.30 (H1'), 4.63 (H3'), 4.13 (H4'), 3.83 (H5'), 3.76 (H5"), 2.79 (H2'), 2.51 (H2") ppm. $^3J_{4'5'}$ = 3.6 Hz, $^3J_{4'5''}$ = 4.7 Hz, $^2J_{2'2''}$ = -14.1 Hz and $^2J_{5'5''}$ = -12.5 Hz.

metal ions do not bind in the vicinity of O5'. It is noteworthy however, that ^{13}C-NMR study on guanosine has shown Δδ for C5' of 0.33 ppm, 0.48 ppm and 0.80 ppm for $Ba(NO_3)_2$, $Zn(NO_3)_2$ and $HgCl_2$, respectively.[3]

(D) Syn ⇄ anti equilibrium of the nucleobase

1D ^{1}H nuclear Overhauser enhancement spectroscopy (nOe) has been used to get the semiquantitative information about conformational equilibrium across glycosyl bond in 2'-dG. The populations of both conformers can be estimated by saturating H8 and measuring the nOe effects at H1' for *syn,* and the sum of nOe effects at H2' and H3' for *anti* conformers.[10] Unfortunately, the H3' signal is overlapping with the signal of residual HOD for 2'-dG in the absence of metal ions and in the presence of two equivalents of Mg^{2+}. The shift of the *syn* ⇄ *anti* equilibrium of 2'-dG by metal ion interactions has been calculated from the slopes of straight lines in calibration graphs.[10] The population of *syn* conformers of 2'-dG in the absence of metal ions was found to be 45%. Upon addition of two equivalents of Mg^{2+} the population of *syn* conformers has been raised to 58%. Titration by two equivalents of Zn^{2+} resulted in the population of ca. 50% of *syn* conformers (52% *syn* from H8 to H1' nOe enhancement, and alternatively 49% *syn* from H8 to Σ(H2'+H3') nOe enhancements). Although the calculation error was estimated to be ≈10%, our data shows that binding of divalent metal ions to 2'-dG causes a slight shift

of *syn* ⇄ *anti* equilibrium towards *syn*, which is in good agreement with the trends of qualitative interpretation of T_1 data (TABLE 1).

The present findings may be summarised as follows: Titration by Mg^{2+}, Zn^{2+} and Hg^{2+} ions produced negligible conformational changes across C4-C5' bond, while *syn* ⇄ *anti* equilibrium is shifted slightly towards *syn*. The binding of the hydrated Zn^{2+} and Hg^{2+} ions to N7 shifts N ⇄ S pseudorotational equilibrium of 2'-dG only slightly towards N-type conformers. The perturbation of the electronic character of guanine residue by outer sphere binding of aquo-metal ions is small, which is consistent with low $\Delta\delta$(N7) values. The minor increase in the population of the N-type conformers by interaction of Hg^{2+} to N7 can be interpreted by strengthening of the anomeric effect due to the variation in the π-electron rich imidazole delocalization in guanine residue.

EXPERIMENTAL SECTION

NMR spectra were recorded at 299.942 MHz on Varian Unity Plus 300 and at 600.113 MHz on Varian Inova 600 NMR spectrometers at the National NMR Center of Slovenia. D_2O (99.96% deuterium) was used as a solvent. Trimetylsilyl propionic acid was used as internal standard for ^{1}H-NMR (δ = 0 ppm) spectra, and MeCN (δ = -135.8 ppm) as external reference for ^{15}N-NMR spectra. The sample temperature (298K) was controlled to approximately ±0.5 K. Sample concentration was 10 mM. Metal ion concentration was gradually raised by simple titration of 2'-dG by nitrate salts (0.1, 0.2, 0.5, 1.0 and 2.0 molar equivalents of metal ions relative to nucleoside). 1D ^{1}H measurements were performed under identical spectral and processing conditions: 9 ppm sweep width, 32K time domain, zero filling to 128K, and slight apodization to give resolution enhancement. Spectra were simulated with a standard computer simulation algorithm, which is integrated into Varian software package (VNMR rev. 5.3B), in order to obtain accurate J-coupling data (error ±0.1 Hz) and chemical shifts. 1D difference nOe experiments were run with 5s irradiation time with saturation of individual lines within the multiplet and internal subtraction of on- and off-resonance spectra. T_1 relaxation times for protons were measured with inversion recovery method with repetition time longer that five times T_1 values. ^{15}N-NMR chemical shifts were obtained with 2D HMBC experiments using gradients for coherence selection; 4096 (ω2) x 128 (ω1) data points,

320 scans per FID, 16 dummy scans, appropriate delays were calculated according to $^{1}J_{NH}$ = 90 Hz and $^{n}J_{NH}$ = 4 Hz, 6 kHz (ω2) x 15 kHz (ω1) spectral width was transformed after multiplication with a sine square filter in both ω2 and ω1 to give 8Kx1K matrix.

The conformational analysis of sugar moiety has been performed by the computer program PSEUROT[8c] with the use of λ electronegativities for the substituents along H-C-C-H fragments and the six parameter set[8a] from 1994 for the generalised Karplus-type equation.

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