Uniqueness of Guanosine among Nucleosides in Its Effect on RNA Folding and DNA Stability

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Tetrahymena thermophilia nuclear pre-rRNA selected guanosine uniquely as the cofactor that acts as the nucleophile in the self-splicing reaction.[1] For the optimal binding of guanosine at the active site of the RNA, the N1 imino and N2 exocyclic amino groups of the guanine base have been proposed to act as hydrogen-bonding (H-bonding) donors to the base pair G264-C311 of *Tetrahymena* group I intron.[2] From the observation of movement of the guide sequence during catalysis by *Tetrahymena* ribozyme, Wang *et al.*[3] demonstrated that large-scale conformational changes of RNA are required for RNA catalysis. And, previously, it was shown that the two ribose-hydroxyls of guanosine as well as N(1)H and NH_2(2) of guanine base have the potential to form two hydrogen bonds with a phosphate monoanion (see Figure 1),[4] and the stability of phosphate-guanine or phosphate-ribose association in DMSO (dimethyl sulfoxide) is much higher than that of the guanine-cytosine base pair.[5] These demonstrated data led us to the speculation that guanosine may play important roles in the hydrogen bonding interactions of guanosine with RNA phosphate groups nonspecifically, in addition to the well-known role as the exogeneous nucleophile by binding specifically to the active site in the *Tetrahymena* RNA catalysis, which has been shown to require large-scale conformational changes of RNA.[3] Thus, here, we were prompted to test the unique effect of guanosine among other various kinds of nucleosides on RNA folding and DNA stability.

Experimental Section

Calf thymus DNA (Type I, sodium salt, highly polymerized), RNA (Type III, from Bakers yeast), nucleosides, and other chemicals used in this work were purchased from Sigma Chem. Co. DNA concentrations are stated in terms of nucleotide phosphorus by using the extinction coefficient, ε_{260} = 6600 $M^{-1}cm^{-1}$. UV-VIS spectrophotometer (Kontron, Uvicon860) and spectropolarimeter (Jasco, J600) were used to measure absorbance and ellipticity, respectively. To obtain a net effect depending on variation of nucleoside concentrations, the same amount of the nucleoside was added to the reference cell too for UV absorption measurement. In the measurement of CD spectra and ellipticity at 260 nm, the nucleoside effect on the sample was corrected by subtracting the CD signal and ellipticity at 260 nm induced by nucleoside itself, respectively. All the experiments were performed at 25 °C.

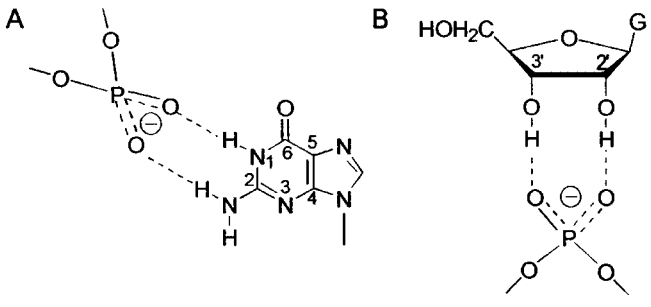

Figure 1. Proposed hydrogen-bonded chemical structures of guanosine with phosphate anion of phosphodiester bond.[4] (A) phsophate-guanine and (B) phosphate-ribose.

Results and Discussion

In order to investigate the uniqueness of guanosine among other nucleosides in its effect on RNA structure, the effects of guanosine and other nucleosides on the UV absorbance of the RNA at 260 nm were examined at neutral pH. Only guanosine in contrast to other nucleosides, e.g., adenosine and pyrimidine nucleosides, displayed a prominent hypochromic effect, indicating increased higher structural content by guanosine binding in aqueous buffer conditions as shown in Figure 2A. In view of the previous report that guanosine is the only nucleoside possessing two moieties, e.g., guanine base and two ribose hydroxyl groups, to form two hydrogen bonds with phosphate group of RNA chain, it seems reasonable to assume that the observed hypochromic effect brought forth only by guanosine is related to the unique H-bonding potential of guanosine.

In order to reconfirm that the hypochromic effect of guanosine stems from the interaction with phosphate anions of phosphodiester bonds through H-bonding rather than from interactions with RNA bases through hydrophobic stacking interactions, test for the hypochromic effect for each nucleoside species was carried out in the presence of 100 mM NaCl and of 30% DMSO. In the test with 100 mM NaCl which should inhibit its H-bonding interactions with phosphate anions of phosphodiester bonds, guanosine as well as other nucleosides was unable to induce significant structural changes of RNA as shown in Figure 2B. On the other hand, only guanosine uniquely brings forth increased hypochromic effect (Figure 2C) in contrast with other nucleoside species tested when the test was performed in the presence of 30% DMSO, *i.e.*, under the condition that may disturb their hydrophobic interactions with RNA bases. The possibility of H-bonding and stacking interaction of guanosine with RNA

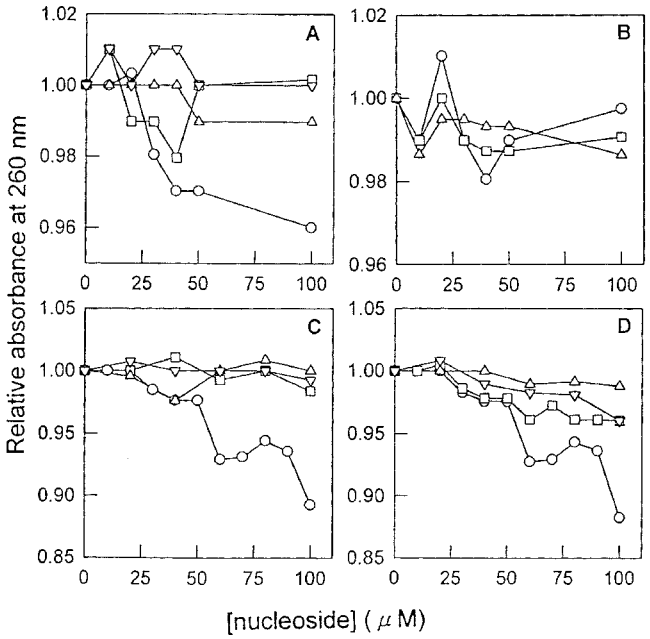

Figure 2. Nucleoside-dependent RNA folding. (A) UV absorbance of RNA (0.05 mg/ml) at 260 nm in 50 mM HEPES buffer (pH 7.5) was measured at 25 °C: guanosine (○), adenosine (□), uridine (△), and cytidine (▽). (B) Effect of NaCl. Absorbance was measured in the above buffer containing 100 mM NaCl: guanosine (○), uridine (□), and cytidine (△). (C) Effect of DMSO. Absorption was measured in the above buffer containing 30% (v/v) DMSO: guanosine (○), adenosine (□), uridine (△), and cytidine (▽). (D) Comparison of 2'-deoxyguanosine with guanosine: guanosine (○) and 2'-deoxyguanosine (▽) in the presence of 30% DMSO, and guanosine (□) and 2'-deoxyguanosine (△) without DMSO.

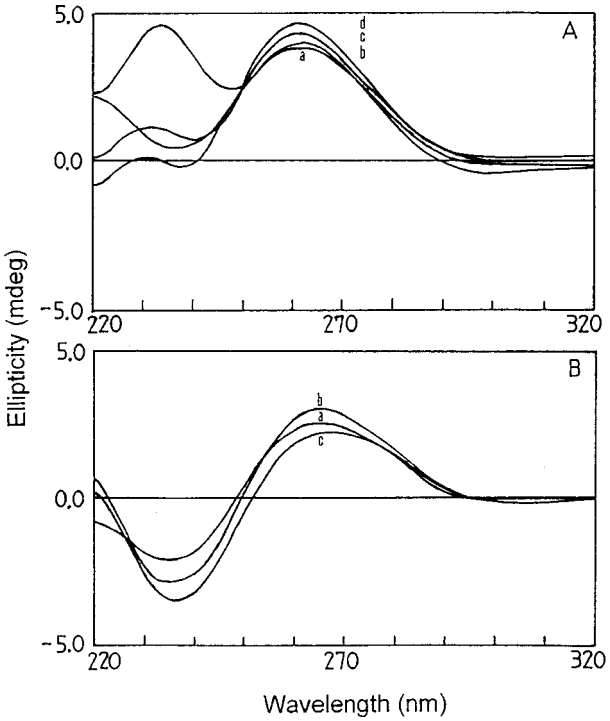

Figure 3. Guanosine effects on CD spectra of RNA and DNA . (A) CD spectra of RNA (0.05 mg/mL) were obtained in 50 mM HEPES buffer (pH 7.5) containing 30% DMSO. The concentrations of guanosine were zero (a), 40 μM (b), 80 μM (c), and 100 μM (d). (B) CD spectra of DNA (50 μM) were obtained in 50 mM HEPES buffer (pH 7.5). The concentrations of guanosine were zero (a), 50 μM (b), and 100 μM (c).

bases can not be ruled out from the above results, however, the differential effects of NaCl and DMSO on guanosine binding may reflect that such hydrophobic and stacking interactions may not be the main source of the observed hypochromic effect of guanosine. Next, the hypochromic effect of 2'-deoxyguanosine was compared with guanosine to prove whether the two ribose hydroxyl groups are involved in the hypochromic effect. Interestingly, 2'-deoxyguanosine, in contrast with guanosine, showed a negligible hypochromic effect even in 30% DMSO buffer as shown in Figure 2D. Therefore, it can be inferred from the lack of the hypochromic effect of 2'-deoxyguanosine that the two ribose hydroxyl groups of guanosine are involved in the hypochromic effect, and this H-bonding potential of guanosine possessing the two moieties to form two H-bonding pairs is responsible for in the unique effect of guanosine on RNA structure. Further, in order to demonstrate the actual increase of secondary structure content of RNA by guanosine binding, CD (circular dichroic) spectroscopic titration was performed in 30% DMSO buffer. As expected from the hypochromic effect, addition of guanosine led to gradual increase of ellipticity at 260 nm, indicating increased secondary structure content of RNA.

In addition, if the hypochromic effect of guanosine on RNA is mediated by H-bonding interaction with phosphate groups of RNA chain, guanosine can also influence DNA strucure. As shown in Figure 3B, guanosine caused ellipticity increase of the positive and negative bands of calf thymus DNA when the ratio of [guanosine]/[phosphate] was 1. Whereas further increase of guanosine concentration led to decrease of the positive band accompanying with a red shift

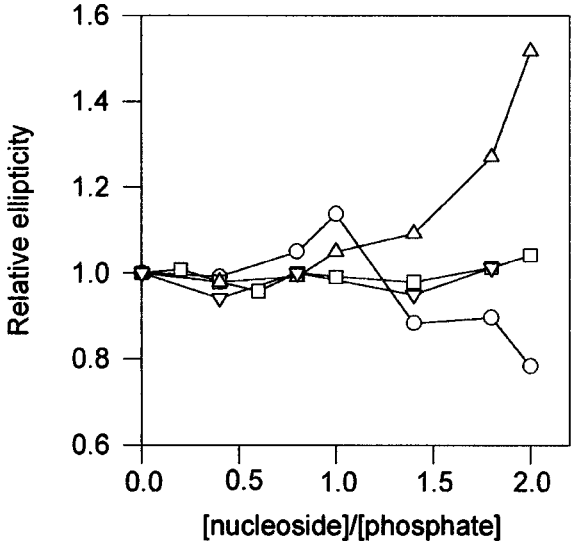

Figure 4. Effect of guanosine on the ellipticity of DNA. Ellipticity of DNA (50 μM) at 260 nm was measured in 20 mM HEPES buffer (pH 7.5): guanosine (○), adenosine (□), thymidine (▽), and cytidine (△).

of node, similar to the spectral changes of denatured DNA upon addition of 8 M urea (data not shown). Figure 4 shows relative ellipticity changes at 260 nm depending on the ratio of [nucleoside]/[phosphate], which also demonstrate the unique effect of guanosine on double-stranded DNA as expected from the hypochromic effect on RNA. The ellipticity increase brought forth by the increase of cytidine concentration may be due to the ellipticity of cytidine induced by DNA. Thus, the consistent unique effect both on RNA hypochromicity and DNA stabilization, observable only with guanosine, may support the possibility of H-bonding interaction of guanosine with phosphate anions of phosphodiester linkages of nucleic acid chain. On the contrary, destabilization of DNA might be induced by hydrophobic interaction with DNA bases.[6]

Guanosine was unique among nucleosides in enhancing RNA double-strandedness, monitored by hypochromicity at 260 nm and CD spectra, and causing stabilization of DNA (at [G]/[P]<1) and destabilization of DNA at high guanosine concentration (at [G]/[P]>1) probed by CD spectral changes and ellipticity at 260 nm. The hypochromic effect of guanosine on RNA was inhibited by high salt concentration, whereas was enhanced by DMSO. 2'-Deoxyguanosine, in contrast with guanosine, showed a negligible hypochromic effect. In view of the previous demonstration that guanine and ribose moiety of guanosine molecules can form a 1 : 1 complex with a phosphate monoanion through two hydrogen bonds, respectively, as verified by proton magnetic resonance in DMSO,[4] we infer from our present UV (hypochromicity) and CD (ellipticity) spectroscopic data that H-bonding interactions of the guanosine molecules with phosphate anions of phosphodiester linkages of nucleic acids may bring forth the effect of the observed RNA hypochromicity and DNA stability via structural effects. Thus, guanosine molecules may exert their regulatory influences on RNA structure[7] and dynamics,[3] especially involved in the *Tetrahymena* RNA catalysis, not only by specific interaction at the active site, but also by nonspecific extensive interactions with phosphate groups of RNA chain, which may induce global conformational changes of RNA required for RNA catalysis.

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