Phosphate-Guanosine Interactions

A MODEL FOR THE INVOLVEMENT OF GUANINE DERIVATIVES IN AUTOCATALYTIC REACTIONS OF RIBONUCLEIC ACIDS*  

(Received for publication, August 2, 1984)

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Proton magnetic resonance was used to study the interactions between nucleosides and phosphate monooanion in dimethyl sulfoxide. Ribose was able to form two mutually exclusive 1:1 complexes involving either OH₃⁺ and OH₅⁺ or OH₄⁻ and OH₂⁻ as hydrogen bond donor groups. Deoxyribose could form only one of these complexes. A specific interaction of phosphate with the base moiety of nucleosides was observed only with guanosine. A 1:1 complex was formed involving the N(1)H and NH₂(2) of guanine. Association constants for both the base and sugar complexes were determined to be in the range 50-60 M⁻¹ at 21 °C in dimethyl sulfoxide. This value is more than 1 order of magnitude higher than that measured for guanine-cytosine base pair formation under the same conditions. Water addition to dimethyl sulfoxide led to a decrease of all association constants but the guanine-phosphate "pair" remained more stable than the guanine-cytosine base pair.

It has recently been shown that some RNA molecules may carry out nucleolytic reactions. The RNA subunits of RNase P from Escherichia coli and Bacillus subtilis cleave a phosphodiester bond in their tRNA precursor substrates (1). The pre-rRNA from Tetrahymena thermophila performs below show that guanine is the only nucleic acid base which can form a 1:1 complex with a phosphate monooanion involving two hydrogen bonds. The ribose moiety of guanosine can also form additional 1:1 complexes involving two hydrogen bonds. Guanosine could therefore play an important role in the folding of RNA structures by interacting with phosphate groups of the RNA chain.

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EXPERIMENTAL PROCEDURES

Assignment of Sugar Protons of Guanosine—Double irradiations experiments showed that the assignment of the hydroxy protons of guanosine was not in agreement with previously published data (7). We therefore reassigned the protons of guanosine in dimethyl sulfoxide (20 mM) by using 2D-COSY (Fig. 1) and 2D-NOESY experiments. The J connectivities between individual protons were revealed by cross-peaks which appeared symmetrically with respect to the diagonal.

The H₂ and H₅ protons which are coupled to the H₃ proton and to the OH₂ proton were easily recognized in the range 3.48-3.66 ppm. Starting from this assignment and using the J connectivities all the protons were identified in a straightforward manner (Fig. 1). These assignments are in agreement with the data of the 2D-NOESY experiments where the cross-peaks arise from dipole-dipole or chemical-exchange-induced cross-relaxation during the mixing time tₑ as well as J-coupled coherences. Cross-peaks were also observed between the nearby protons H₆ and H₇ and between H$_{1'}$ and H$_{1''}$ (data not shown).

Selective Binding of Phosphate to Guanine—In nucleic acids the phosphates are in a diester form and bear one negative charge at neutral pH. We have used sodium diethylphosphate as a model of such phosphate groups. Each of the four bases of nucleic acids possesses several donor or acceptor sites for hydrogen bond formation. This is also true for ribose or

1 The abbreviations used are: eA, 9-ethyladenine; Guo, guanosine; dGuo, deoxyguanosine; eG, 9-ethylguanine; Me₂SO, dimethyl sulfoxide.
deoxyribose. In order to avoid competition for hydrogen bond formation between bases and sugar we used substituted bases (e9A, 1-cyclohexyluracil, e9G) where the sugar was replaced by a chemical group that was not able to form hydrogen bonds. Examination of different possibilities of hydrogen bond formation showed that among all four nucleic acid bases, only guanine was able to form a 1:1 complex involving two hydrogen bonds with the phosphate group. As a matter of fact, adding 20 mM sodium diethylphosphate to e9G induced downfield shifts of the N(1)H and NH2(2) protons of guanine (20 mM) by 0.237 and 0.129 ppm, respectively. The amino or imino protons of e9A, 1-cyclohexyluracil, or cytosine were shifted by less than 0.01 ppm under the same experimental conditions (Table I).

In order to demonstrate that both the N(1)H and the NH2(2) groups of guanine were involved simultaneously in hydrogen bond formation, a comparison was made between Guo and 1-methylguanosine. Addition of 20 mM Na diethylphosphate to a 20 mM solution of each of these compounds led to downfield shifts of the NH2 protons of 0.142 ppm for Guo and of 0.015 ppm for 1-methylguanosine (Table I). This result clearly demonstrated the role of the N(1)H proton in

**Fig. 1.** 300 MHz COSY spectrum (contour plot) of guanosine at 21 °C in Me2SO. The assignments are shown on Fig. 2.
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The fact that addition of 20 mM Na diethylphosphate to 20 mM inosine induced a downfield shift of the N(1)H proton by only 0.041 ppm (Table I) showed that the amino group of guanine was also involved in the binding of the phosphate group.

**Binding of Phosphate to Ribose**—Examination of CPK models showed that a phosphate diester was able to form two mutually exclusive 1:1 complexes with ribose involving OH2 and OH5 for one complex and OH3 and OH5 for the other one. Adding 20 mM sodium diethylphosphate to a solution of 20 mM purine riboside (where only the ribose OH groups could act as H-bond donors) led to downfield shifts of the OH3, OH5, and OH5 protons of 0.120, 0.180, and 0.096 ppm, respectively. A similar result was obtained with 1-methylguanosine (Table I). In each case, the downfield shift of the OH3 proton which is participating in both 1:1 complexes was about the sum of the downfield shifts observed for OH3 and OH5. A similar result was observed with Guo even though the changes in chemical shifts were smaller due to the competition between ribose and base hydrogen-bonding sites.

In order to demonstrate that the OH5 group of ribose was involved together with the OH3 group in one of the ribose complexes a comparison was made between Guo and dGuo. Addition of 20 mM Na diethylphosphate to a 20 mM solution of each of these compounds led to downfield shifts of the OH5 proton of 0.132 ppm for Guo and 0.081 ppm for dGuo and to downfield shifts of the OH5 proton of 0.067 ppm for Guo and 0.069 ppm for dGuo (Fig. 2 and Table I). As expected, the formation of two complexes each involving OH5 led to a much larger downfield shift of this proton resonance in the ribose-containing molecule.

A similar result was obtained by comparing the deoxyribose and the ribose derivatives of pyrimidines. Addition of 50 mM Na diethylphosphate to a 50 mM solution of uridine induced downfield shifts of the OH2, OH3, and OH5 protons of 0.186, 0.458, and 0.297 ppm, respectively, while downfield shifts of 0.223 and 0.246 ppm were obtained for the OH3 and OH5 protons, respectively, when a 50 mM solution of thymidine was used instead of uridine. Again formation of two complexes in the case of uridine led to a much larger downfield shift of the OH5 proton which is involved in both complexes.

**Binding Parameters in Dimethyl Sulfoxide**—The large downfield shifts of the N(1)H and NH2(2) resonances of
TABLE I

Change in chemical shifts of the proton resonances of nucleic acid derivatives (20 mM) in the presence of 20 mM sodium diethylphosphate.

In each row the upper figure is the proton chemical shift of the free nucleic acid derivative and the lower figure is the downfield shift observed upon adding sodium diethylphosphate. Measurements were carried out at 21 °C in dimethyl sulfoxide.

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<th>N(1)H</th>
<th>NH₂</th>
<th>OH₁</th>
<th>OH₂</th>
<th>OH₃</th>
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<td>Inosine</td>
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The value of δ₁ - δ₀ for OH₂ and OH₃ was computed by using the same value of K (51.1 M⁻¹) determined from the changes in chemical shifts of the OH₃ resonance.

Effect of Water on Guanine-Phosphate Interaction in Dimethyl Sulfoxide—Guanosine was not soluble enough in water to allow us to study its binding to phosphate ions. In water, guanine in the presence of diethylphosphate were used to determine the binding parameters of eG with sodium diethylphosphate. Increasing amounts of diethylphosphate were added to a 50 mM solution of eG in Me₂SO until the ratio of eG and phosphate concentrations reached a value of 5. The association constant (K) for complex formation was determined from a least-squares analysis of changes in chemical shifts (8). By using the N(1)H resonance, the best fit was obtained with K = 59.4 ± 6.5 M⁻¹ and δ₁ = δ₀ = 1.114 ± 0.023 ppm, and by using the NH₂(2) resonance the best fit was obtained with K = 59.0 ± 7.1 M⁻¹ and δ₁ - δ₀ = 0.586 ± 0.013 ppm (Table II) (δ₁ and δ₀ represent the chemical shifts of the complexed and free base, respectively).

The same analysis was carried out for the binding of phosphate to the ribose group by using the titration curve of uridine protons (50 mM) by diethylphosphate. Unfortunately the three OH resonances partially overlapped one another or overlapped the Hᵥ resonance for several phosphate concentrations. Only the OH₂ resonance could be used to determine the association constant. The best fit was obtained with K = 51.1 ± 9.2 M⁻¹ and δ₁ - δ₀ = 0.848 ± 0.033 ppm (Table II).

Association of the phosphate group with ribose leads to the formation of two exclusive 1:1 complexes C₁ and C₂. Let us designate the two binding constants by K₁ and K₂, the free phosphate concentration by P (P₀ for total concentration), and the free ribose concentration by R (R₀ for total concentration). We can write the following:

C₁ = K₁ × R × P 
C₂ = K₂ × R × P 
R = R₀ - C₁ - C₂

P = P₀ - C₁ - C₂

The observed chemical shifts δ are then given by Equation 5:

δ = δ₀ = (δ₁ - δ₀)K₁ + (δ₂ - δ₀)K₂ 
K₁ + K₂

Equation 6 can be compared with Equation 7 which gives the variation of chemical shift for a single 1:1 complex.

δ₂ = (δ₁ - δ₀)K₁ + (δ₂ - δ₀)K₂ 
K₁ + K₂

The computed constant K is therefore the sum of the individual constants K₁ and K₂ independently of the proton resonance under investigation, and the computed variation of chemical shift is

δ₂ = (δ₁ - δ₀)K₁ + (δ₂ - δ₀)K₂ 
K₁ + K₂

Equation 8 can be applied to each of the OH₂, OH₃, and OHᵥ protons. We were not able to compute the individual association constants K₁ and K₂. However, the values of the downfield shifts of the OH₂ and OHᵥ protons are very similar (see Table I). Therefore, we can assume that the two constants are very close to each other and are of the order of 20-30 M⁻¹. In the case of uridine the sum of the two association constants (K = K₁ + K₂) was calculated to be ≈51 M⁻¹ from the changes in chemical shift of the OH₂ proton (Table II). Using the same value of K we computed downfield shifts of 0.344 ppm for the OH₂ and OHᵥ protons, respectively. Using the variation of chemical shift of thymidine in the presence of 50 mM sodium diethylphosphate and δ₁ - δ₀ calculated for the OH₂ proton of uridine, we computed an association constant of 25 M⁻¹ for the deoxyribose complex which is in good agreement with the above assumptions of approximately identical association constants for the two ribose complexes.
of N(1)H could not be detected any more. The downfield shifts of the other protons was 70–80% of those observed in pure Me₂SO. For water concentrations higher than 2 M, the hydroxyl protons of ribose were broadened in the presence of phosphate ions and it was not possible to measure the value of their chemical shifts. By assuming that the downfield shift of the NH proton of the phosphate-guanine complex did not depend on water concentration, an association constant of 4.6 M⁻¹ was computed for the binding of guanosine to phosphate monoanion in the presence of 9 M water.

**DISCUSSION**

The results presented above demonstrate that phosphate monoanions strongly bind to the guanine ring and do not bind strongly to any other base. The results obtained with different guanine derivatives show that both NH₁(2) and N(1)H groups of guanine are involved in this interaction. A 1:1 complex is formed which involves a pair of hydrogen bonds with these two groups as shown on Fig. 3. Guanine is the only base that is able to form these two hydrogen bonds with phosphate. None of the other bases has two donor groups in the correct location. This explains the highly selective interaction of phosphate ions with guanine.

The NMR results also demonstrate that phosphate ions bind strongly to ribose in Me₂SO. Two different 1:1 complexes are formed involving either the OH₃ and OH₅ groups or the OH₅ and OH₂ groups. Only one of these complexes is formed in the case of deoxyribose.

The stability of phosphate-guanine or phosphate-ribose association in Me₂SO is much higher than that of the guanine-cytosine base pair for which an association constant of 3.6 M⁻¹ was previously determined in Me₂SO (9). Corresponding values are 59 M⁻¹ for the guanine-phosphate complex and approximately 20–30 M⁻¹ for each of the ribose-phosphate complexes (Table II). Addition of water reduces all association constants. However, as already demonstrated in the case of acetate-guanine interactions (4, 10), the phosphate-guanine “pair” remains more stable than the cytosine-guanine base pair.

The auto-splicing of pre-rRNA from T. thermophila requires guanosine as a cofactor in vitro (2, 3). Both OH₂ and NH₂ groups of the ribose moiety as well as the N(1)H and NH₁(2) groups of the base are required to obtain the highest rate of the splicing reaction (3). In contrast the OH₅ group does not appear to be involved in the reaction. The results presented above show that guanosine could play a unique role among nucleosides due to its specific interaction with the phosphate groups of phosphodiester linkages. Phosphate-guanine and phosphate-ribose interactions might contribute to the formation of a specific secondary structure for a RNA-guanosine complex. Such a secondary structure could be a prerequisite for the nucleolytic and/or ligation reactions which take place in these complexes. The interaction of a phosphate monoanion with the OH₂ and OH₅ ribose groups might also facilitate the nucleophilic attack at the phosphate of the 5' splice site of the pre-rRNA. The splicing reaction of messenger RNA precursors might also involve guanine-phosphate interactions. This might explain why guanine is always present at both the 5' and the 3' ends of introns (11).

**REFERENCES**