Role of the Ribose 2′-Hydroxyl Groups for the Stabilization of the Ordered Structures of Ribonucleic Acid

Sir:

A considerable amount of effort has recently been devoted to the elucidation of the structural role of the ribose 2′-hydroxyl groups in the ordered conformations of RNA. It has been suggested that the 2′-hydroxyl groups may stabilize the ordered structures of RNA by forming intramolecular hydrogen bonds to the nearby phosphate groups on the polymer backbone.[1,2] This hypothesis was based mainly on CD studies of dinucleotide model compounds. In particular, a substantially lower value for the enthalpy of destacking was determined for dApdA[3] as compared to ApA.[2] In the course of our work on the structural and functional role of the minor components of RNA we have recently demonstrated that methylation of the 2′-hydroxyl groups in poly A leads to a slight increase, rather than decrease, of the thermal stability of its double-stranded structure in acidic solution and has no significant effect on its single-stranded structure at neutrality.[4,5] Methylation of the backbone in poly A did not destabilize the double-stranded complex formed by poly A with poly U. It appeared unlikely, therefore, on the basis of our results that intramolecular hydrogen bonds involving the 2′-hydroxyl groups, if at all present, are of importance for the stabilization of the ordered structures of poly A. To further clarify this issue we have undertaken a comparative study of the structure of ApA and AmpAm in weakly alkaline solutions by nmr and CD spectroscopy.

AmpAm was prepared by the condensation of 5′-O-monomethoxytrityl-2′-O-methyladenosine and N-3′-O-diacetyl-2′-O-methyladenosine 5′-phosphate in the presence of DCC followed by removal of the protecting groups and purification on a DEAE-cellulose column. The protected nucleoside derivatives were synthesized essentially following standard procedures.[6-9] The dinucleotide was characterized by phosphate analysis and enzymatic digestion. Purified snake venom phosphodiesterase completely degraded AmpAm to equal amounts of 2′-O-methyladenosine and 2′-O-methyladenosine 3′-phosphate. Hydrolysis hypochromicity at 258 mμ under these conditions was 10% both for AmpAm and ApA.

The nmr spectroscopic characteristics of AmMP, AMP, and the two dinucleotides are summarized in Table I. The only significant effect of 2′-O-methylation on the spectrum of the mononucleotide is a slight shift of the signal of the anomeric proton to lower field ($\delta_{H\text{-}1',AmMP} - \delta_{H\text{-}1',AMP} = 0.08$ ppm). The coupling with H-2′, however, remains virtually unchanged. A major change of the sugar conformation due to methylation can therefore be excluded.[10] The chemical shift of the adenine H-8 proton is only very slightly influenced by 2′-O-methylation, indicating that AmMP preferentially assumes the *anti* conformation, as does AMP under the same conditions.[11,12] Very similar nmr spectra are also obtained for the methylated and unmethylated dinucleotide. As for the monomer the only significant effect of 2′-O-methylation is observed in the shielding of the anomeric protons. The difference between the chemical shifts of the anomeric protons of the 3′-bound units of ApA and AmpAm as well as of the anomeric protons of the 5′-bound units is 0.10 ppm. In contrast to the mononucleotide, an increase in the coupling constant $J_{H\text{-}1'\text{-}H\text{-}2'}$ is observed upon methylation both for the 3′-bound ($\Delta J = 1.0$ cps) and the 5′-bound ($\Delta J = 0.7$ cps) half of the molecule. This may reflect a larger dihedral angle between H-1′ and H-2′ in AmpAm as compared to ApA.[10] However, other interpretations may also be possible.[12] More importantly the lack of significant differences in the shielding of the base protons in AmpAm and ApA allows the conclusion that the orientation and the interactions of the adenine residues in both dinucleotides must at least be similar.

The CD spectra of both dinucleotides are shown in Figure 1. The following differences are noted in the spectrum of AmpAm as compared to ApA: (1) the rotational strength in particular of the positive band is

Table I. Nmr Spectroscopic Characteristics of AMP, AmMP, ApA, and AmpAm[a]

	H-8(5′)	H-8(3′)	H-2(5′)	H-2(3′)	H-1′(5′)	H-1′(3′)	2′-OCH$_3$(5′)	2′-OCH$_3$(3′)	$J_{H\text{-}1'\text{-}H\text{-}2'}$ H-1′ (5′)	H-1′ (3′)
AMP	9.08		8.74		6.58				5.3	
AmMP	9.11		8.74		6.66		3.93		5.5	
ApA	8.78	8.72	8.66	8.54	6.48	6.35			3.5	3.9
AmpAm	8.81	8.72	8.66	8.56	6.58	6.45	3.95	3.91	4.2	4.9

[a] Spectra were measured in D_2O on a Varian A-60A spectrometer equipped with a Varian-C-1024 time-averaging computer at a probe temperature of 37 ± 1°. Chemical shifts in parts per million are relative to TMS which was sealed in a capillary and immersed into the sample solution. The spectra were calibrated by generating side bands of TMS with an external oscillator (HP 200 CD) and the frequency was measured at a number of points. Line positions are accurate to ±0.017 ppm. The pD of the solutions was between 8 and 9 and was determined with a miniature glass electrode using the relation pD = pH + 0.4. The concentration of the solutions was 0.02 *M*. Coupling constants are in cycles per second.

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(3) Abbreviations: deoxyriboadenylyl-(3′→5′)-deoxyriboadenosine, dApdA; riboadenylyl-(3′→5′)-riboadenosine, ApA; 2′-O-methylriboadenylyl-(3′→5′)-2′-O-methylriboadenosine, AmpAm; 2′-O-methyladenosine 5′-phosphate, AmMP; adenosine 5′-phosphate, AMP; polyadenylic acid, poly A; polyuridylic acid, poly U.
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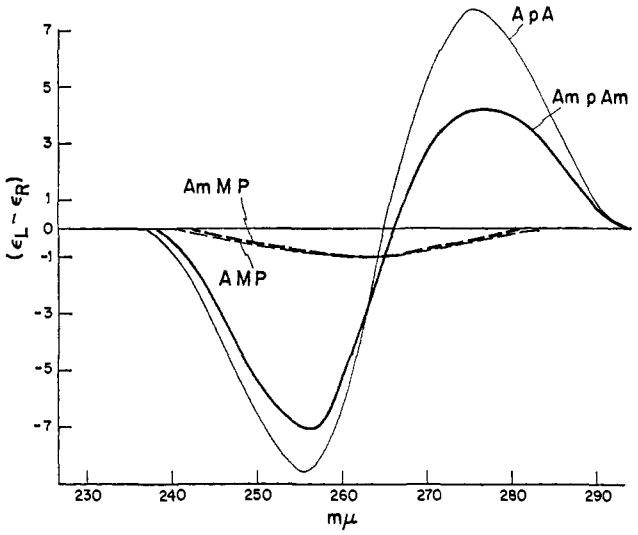

Figure 1. CD spectra of AMP, AmMP, ApA, and AmpAm in 4.7 *M* KF–0.01 *M* Tris buffer, pH 7.5, at 27°. A Cary 60 recording spectropolarimeter equipped with a 6001 CD-accessory and a JASCO Model ORD/UV/CD-5 were used. Differential dichroism absorption is given on a "per nucleotide residue" basis.

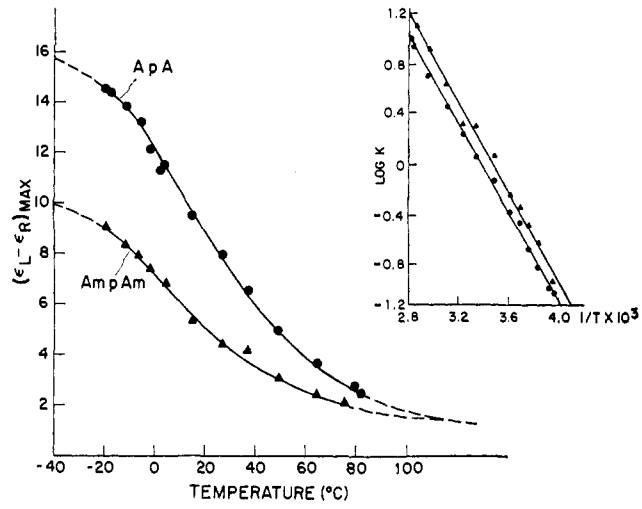

Figure 2. CD–temperature profiles of ApA and AmpAm in 4.7 *M* KF–0.01 *M* Tris buffer, pH 7.5. Differential dichroism absorption at the maximum of the positive band is plotted against temperature. Very similar melting curves were obtained in the region from 5 to 60° in 0.15 *M* NaCl. The inset gives a van't Hoff plot derived from these data on the basis of a two-state model. The high- and the low-temperature ends of the melting curves are approximated.

considerably lower for AmpAm; (2) the spectrum is less conservative; (3) the crossing point is shifted to slightly longer wavelength.[13] The spectral differences indicate that a slight change in the orientation of the bases has occurred in ApA upon 2′-O-methylation which has not been readily discernible from the nmr spectra. CD temperature profiles of the two dinucleotides are shown in Figure 2. Both profiles have sigmoidal shape. A somewhat lower T_m is obtained for AmpAm (approximately 15°) than for ApA (approximately 21°) from the curves. A van't Hoff plot derived from the melting data on the basis of a two-state model is given in the inset of Figure 2. Rather close values are obtained for the thermodynamic parameters $\Delta H°$, $\Delta S°$, and $\Delta F°$ (at 0°) for the methylated and unmethylated dinucleotide.[14]

The following general conclusions can be reached from our results. Although a small change in the three-dimensional structure and the stability of the ordered conformation of ApA does occur upon methylation of the 2′-hydroxyl groups, the change is not as substantial as for the case where the 2′-hydroxyl groups are replaced by hydrogens as in dApdA.[2] It appears, therefore, that an important requirement for the increased stability of oligo- and polyribonucleotides relative to their deoxyribonucleotide counterparts is the presence of an oxygen atom in the 2′ position of the sugar. The hydrogen bond donor capabilities of unsubstituted 2′-hydroxyl groups, however, do not seem to be essential. Based on the results obtained with arabinosyl dinucleotides[15,16] it is most likely that the stereochemistry of carbon 2 of the sugar is furthermore of importance. It is not possible at present to decide if the 2′-oxygen atoms exert their stabilizing effect on RNA conformation due to their properties as hydrogen bond acceptors, van der Waals interactions, or both.

(13) Since 2′-O-methylation has introduced changes both in the shape and the intensity of the CD bands, the spectral differences between ApA and ApmAm cannot be adequately interpreted on the basis of simple exciton theory.

(14) Identical values are obtained for the enthalpies of the disordering process for both dinucleotides ($\Delta H°$ = 8.1 kcal/mol), while $\Delta S°$ is slightly higher for AmpAm (28 eu/mol) than for ApA (27 eu/mol). Correspondingly, $\Delta F°$ at 0° is lower for AmpAm (0.3 kcal/mole) than for ApA (0.6 kcal/mole). The larger value for $\Delta S°$ and the lower stability of the ordered structure of AmpAm may be due to a larger amount of rotational freedom gained in the disordering process of AmpAm than of ApA. Model building (Corey–Pauling–Koltum models) indicates that introduction of the bulky methyl substituents may diminish the flexibility of the backbone predominantly in the ordered conformation of AmpAm. A change in solvation resulting from the replacement of the polar hydroxyls by essentially nonpolar methoxy groups may also be of importance. While this interpretation seems plausible, the following should be kept in mind: for the determination of the T_m's and the derivation of the thermodynamic data the high- and the low-temperature branches of the melting curves have to be approximated. This may give rise to considerable error, especially for the low-temperature ends of the curves. Furthermore, the thermodynamic treatment is based on the two-state model which is not clearly established.

Acknowledgment. We have profited in the course of this work from discussions with Walter Kauzmann. This research was supported by Grant GM-14090 from the National Institutes of Health, by Grant GB-4781 from the National Science Foundation, and by a grant from Hoffman-La Roche Inc., Nutley, N. J.

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Received June 4, 1969

The Mechanism of α-Ketoglutarate Oxidation in Coupled Enzymatic Oxygenations[1]

Sir:

A group of hydroxylases requires α-ketoglutarate as a cofactor.[2–6] For γ-butyrobetaine hydroxylase[7] and

(1) This work was supported by grants from the Swedish Medical Research Council (13 X-585) and Alfred Österlunds Stiftelse.