Conformational Study of Ribonucleotides, 2′-Deoxyribonucleotides, and Arabinonucleotides by Carbon-13 Nuclear Magnetic Resonance

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Synopsis

The geminal and vicinal ^{13}C-^{31}P coupling constants have been monitored, as a function of pH, for a series of uracil and cytosine 3′- and 5′-nucleotides with a ribose, arabinose, or 2′-deoxyribose sugar. Data were also obtained for two 3′,5′-diphosphates in the ribose and arabinose series. The geminal J(C5′-P5′) and J(C3′-P3′) couplings show only a small dependence on the ionization state of the phosphate, decreasing by < 0.5 Hz in the pH 5–7 range. For the ribose and arabinose 3′-nucleotides, the vicinal J(C4′-P3′) increase (up to 1.5 Hz) on secondary phosphate ionization in the pH 5–7 range, whereas their J(C2′-P3′) couplings decrease (up to 1.5 Hz) over the same pH range. In contrast for the 2′-deoxyribose molecules, both couplings decrease (~0.5 Hz) on phosphate ionization. The titration curves provide information about the influence of the sugar on the conformation about the C3′—O3′ bond. Some conformational trends could be rationalized by consideration of the sugar-pucker-dependent contact interactions between the 3′-phosphate and the substituents on the furanose ring.

INTRODUCTION

An important feature of polynucleotides is the conformation about the C3′—O3′ bond, which defines the orientation of the 3′-phosphate relative to the sugar moiety (Fig. 1). The conformational behavior of this bond can be studied by x-ray diffraction methods,[1,2] theoretical calculations,[3–8] and nuclear magnetic resonance (nmr).[9–14] The properties of the C2′ substituents undoubtedly influence the torsional motion about C3′—O3′ and contribute to the stereochemical differences between RNA and DNA. Several groups[9–15] have used ^{13}C-^{31}P coupling constants for the conformational analysis of the C3′—O3′ and the C5′—O5′ bonds of nucleotides. To obtain information about the influence of the 2′-hydroxyl group, we have monitored the ^{13}C chemical shifts and ^{13}C-^{31}P coupling constants as a function of pH in aqueous solution for a series of 3′- and 5′-ribonucleotides, 2′-deoxyribonucleotides, and arabinonucleotides with a uracil (U) or cytosine (C) base (Fig. 1). The molecules studied were (a) the ribose series: (3′UMP, 5′UMP, 3′CMP, 5′CMP); (b) the 2′-deoxyribose series: (3′dUMP, 5′dUMP, 3′dCMP, 5′dCMP); and (c) the arabinose series: (3′aUMP,

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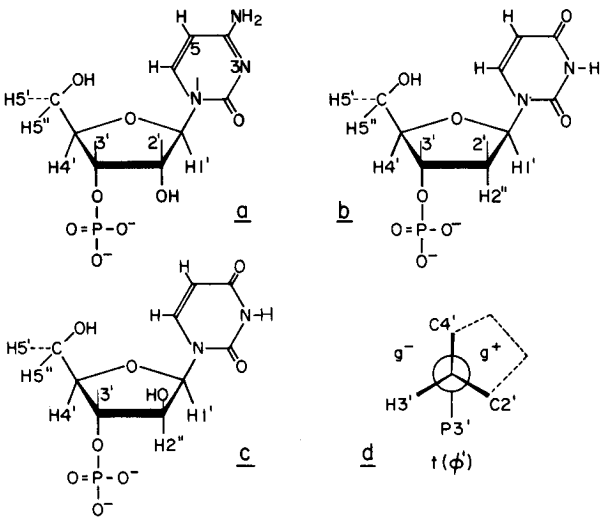

Fig. 1. Structures of (a) cytidine-3′-monophosphate, (b) 2′-deoxyuridine-3′-monophosphate, and (c) arabinouridine-3′-monophosphate. (d) Newman projection viewed along the C3′—O3′(ϕ') bond of a 3′-monophosphate in the $t(\phi')$ domain ($\phi' = 180°$ shown). g^- and g^+ show the location of P3′ in the $g^-(\phi')$ domain ($\phi' = 0°$ to $-120°$) and the $g^+(\phi')$ domain ($\phi' = 0°$–$120°$).

5′aUMP, 3′aCMP, 5′aCMP). Data were also obtained for two 3′,5′-diphosphates, 3′,5′dCDP and 3′,5′aCDP, in the deoxyribose and arabinose series, respectively. Previous studies of this type have been concerned primarily with ribonucleotides, although we have examined *syn* and *anti* base effects in 2′-deoxyribonucleotides.[13] This represents the first systematic comparison of ribose, 2′-deoxyribose and arabinose derivatives.

To date, seven 3′-ribonucleotide single-crystal structures have been reported.[1,2] No data are available for deoxyribosyl or arabinosyl 3′-nucleotides, or for 3′,5′-diphosphates. Our present study should prove useful for future comparisons of solid-state, solution, and theoretically derived conformations.

EXPERIMENTAL

Materials

The 3′UMP and 3′CMP were purchased from the Sigma Chemical Co. Some phosphates were obtained by protecting and phosphorylating the nucleoside using standard methods.[16] The 5′aUMP was prepared by direct phosphorylation of the unprotected nucleoside following Yoshikawa et al.[17] The 3′,5′aCDP was prepared by direct phosphorylation[18] of cytidine.

Instrumentation

^{13}C spectra were obtained on a Bruker WH-90DS spectrometer (22.63 MHz) equipped with a Nicolet-1180 computer, the NTC-FT1180 software package, and quadrature phase detection. Spectral widths were typically 3800 Hz acquired into 16K data points and zero-filled to 32K. To apodize the free-induction decay (FID), exponential multiplication using a line-broadening (0.5 Hz) was applied. Normally spectra were recorded with complete proton noise decoupling, though some proton-coupled spectra were obtained to facilitate peak assignments. For the purposes of assigning the ^{13}C resonances, ^{1}H-^{13}C chemical-shift correlation experiments were carried out according to Freeman and Morris.[19]

All samples were examined in 10-mm o.d. tubes, at concentrations of 80 mg/mL (or less) in D_2O containing 1% dioxane as internal reference. (Several experiments with 30 mg/mL concentrations revealed no significant concentration dependence of the ^{13}C-^{31}P couplings, in line with earlier observations.[12,13] The probe temperature was regulated at 300 ± 1 K. Paramagnetic ions were removed following the procedure of Cozzone and Jardetzky.[20] The pD was adjusted by the addition of dilute NaOD or DCl and is given as the pH meter reading. The ^{13}C-^{31}P coupling data are provided, in the following sections, in the form of titration curves. The uncertainty in the couplings is in general about 0.2 Hz. The chemical-shift data will be reported later.

Spectral Assignments

The assignment of the ^{13}C resonances of the uracil and cytosine 2′-deoxyribonucleotides followed from the work on 2′-deoxythymidine and its mono- and diphosphates.[13] The assignments of the uracil and cytosine ribonucleotides followed from the work on 3′UMP and 5′UMP by Alderfer and Ts'o,[12] who considered the effects of phosphorylation and phosphate ionization on the chemical shifts. In addition to considering such effects, we obtained an absolute assignment of all furanose carbons from ^{1}H-^{13}C chemical-shift correlation spectra following Freeman and Morris.[19] (This required obtaining the ^{1}H spectra that will be reported later.) Our assignments agree with Alderfer and Ts'o.[12]

The assignment of the uracil and cytosine arabinonucleotides was based on published data for the nucleosides, aU and aC.[21] As in the 3′-ribonucleotides, the chemical shifts of C2′ and C3′, and their ^{13}C-^{31}P coupling constants, were similar, and thus their assignment is not obvious from the ^{13}C spectrum. However, an absolute assignment could be obtained in all cases from ^{1}H-^{13}C chemical-shift correlation spectra. The effects of phosphorylation and phosphate ionization on the ^{13}C chemical shifts supported the assignments.

RESULTS

Geminal ^{13}C-^{31}P Coupling Constants

The pH dependence of the geminal couplings, J(C3′-P3′) and J(C5′-P5′) are shown in Fig. 2. Though the C5′ and C3′ are primary and secondary carbons, respectively, the two sets of data are similar [overall range: 4.3–5.1 Hz for J(C3′-P3′); 3.9–5.3 Hz for J(C5′-P5′)]. No significant dependence on the type of sugar or base is apparent. Decreases of about 0.5 Hz occur upon ionization of the phosphate in the pH 5–7 range, in line with the secondary pK_a values (5.7–6.3) reported for 3′- and 5′-ribo- and deoxyribonucleotides.[20] (No determinations of phosphate pK_a values for arabinonucleotides are known to us; the titration curves in Figs. 2–4 suggest that these are, to within 0.5 pH units, identical to those of the ribo- and deoxyribonucleotides.) Similar behavior has been noted for other 3′-, 5′-, and 3′,5′-phosphates.[12,13] An unusually large (7.0-Hz) J(C5′-P5′) coupling has been noted in 3′,5′-cyclic nucleotides.[10,22]

Vicinal ^{13}C-^{31}P Coupling Constants

The pH dependence of the vicinal J(C2′-P3′) and J(C4′-P3′) couplings are shown in Fig. 3, while Fig. 4 shows the pH dependence of their *SUM*. It is important to note that in each figure a discontinuous numbering of the vertical axis was used to separate the titration curves according to the nature of the sugar and to reveal trends that would otherwise be masked by

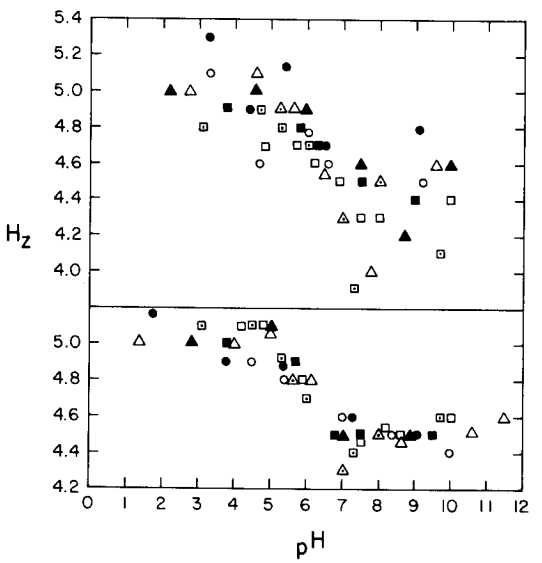

Fig. 2. The pH dependence of the geminal coupling constants J(C5′-P5′) (top) and J(C3′-P3′) (bottom) (uncorrected for deuterium isotope effect). Circles: ribonucleotides; squares: arabinonucleotides; triangles: 2′-deoxyribonucleotides. Solid symbols: uracil base; open symbols: cytosine base. Dotted open symbols: 3′,5′-diphosphates.

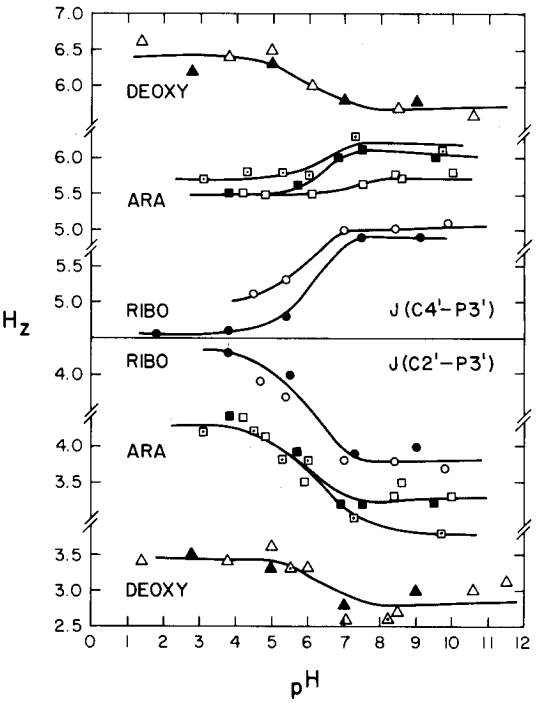

Fig. 3. The pH dependence of the vicinal coupling constants J(C4′-P3′) (top) and J(C2′-P3′) (bottom) for a series of 3′-nucleotides and 3′,5′-diphosphates (uncorrected for deuterium isotope effect). Symbols as in Fig. 2.

the considerable overlap. Since this magnifies differences between the three series, it is important to note that the three sets of data are confined to the following, *nonoverlapping* ranges: J(C2′-P3′): 2.6–4.4 Hz; J(C4′-P3′): 4.6–6.6 Hz; *SUM:* 8.8–10.1 Hz.

At pH < 5.0 the J(C2′-P3′) couplings are similar for the ribose and arabinose derivatives (~4.3 Hz), whereas those for the deoxyribose derivatives are smaller (~3.4 Hz). Secondary ionization of phosphate in the pH 5–7 range induces a decrease (~1.5 Hz) for both 3′UMP and 3′CMP. The pH-induced decreases for 3′aUMP and 3′aCMP are smaller (~1.0 Hz). In the arabinose series, the pH-induced decreases in J(C2′-P3′) show a small dependence on the presence of a 5′-phosphate (~1.5 Hz in the 3′,5′aCDP plot). For the 2′-deoxyribose molecules, small decreases in J(C2′-P3′) (~0.5 Hz) are seen upon secondary ionization of the 3′-phosphate. Overall, the nature of the base (U or C) has little effect on J(C2′-P3′).

The J(C4′-P3′) couplings show a greater dependence on structure. At pH < 5.0 they fall in the range 4.6–6.6 Hz, with the ribonucleotide values at the lower end, the deoxyribonucleotides at the upper end, and the arabinonucleotides in midrange. In contrast with J(C2′-P3′), J(C4′-P3′) for the ribose and arabinose molecules experience phosphate ionization-induced *increases* in the pH 5–7 range. The increase for the ribonucleotides

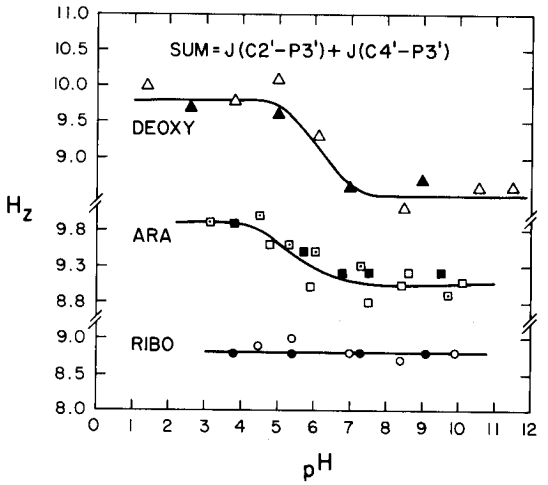

Fig. 4. The pH dependence of the *SUM* = *J*(C4′-P3′) + *J*(C2′-P3′) for a series of 3′-nucleotides and 3′,5′-diphosphates (uncorrected for deuterium isotope effect). Symbols as in Fig. 2.

(1.0–1.3 Hz) matches the decrease in *J*(C2′-P3′), with the result that their *SUM* is essentially independent of pH in the range examined (Fig. 4). The ionzation-induced increases for the arabinose molecules are smaller (0.3–0.5 Hz), and coupled with the larger pH-induced decreases in *J*(C2′-P3′), they lead to a pH-dependent *SUM* whose magnitude decreases (0.5–1.0 Hz) in the pH 5–7 range. On the other hand, for the deoxyribose molecules, *J*(C4′-P3′) like *J*(C2′-P3′) decreases (0.5–1.0 Hz) in the pH 5–7 range. This parallel dependence of the individual couplings is reflected in the strong dependence of their *SUM* on the ionization state of the 3′-phosphate.

Note that secondary phosphate ionization tends to reduce, for the three types of sugar derivatives, differences in the individual couplings and in the *SUM* values. Note also that overall the nature of the pyrimidine has little influence on the couplings (<0.5 Hz). The largest base effects are seen for *J*(C4′-P3′) for 3′UMP and 3′CMP at pH < 5.0, and for 3′aUMP and 3′aCMP at pH > 7.0.

DISCUSSION

Electronegativity Effects

One factor complicating interpretation of the vicinal ^{13}C-^{31}P coupling constants in terms of the C3′-O3′ conformational preferences is the electronegativity effect of the 2′-hydroxyl on *J*(C2′-P3′). In the ribonucleotides and arabinonucleotides, C2′ and C4′ are identically bonded: two carbons, one oxygen, and one hydrogen atom. Thus, the *J*(C2′-P3′) and *J*(C4′-P3′) couplings for the two series of molecules can be compared in conformational terms with minimal concern for electronegativity corrections (assuming

the C2′ configuration is not important). However, replacement of the 2′-hydroxyl by a hydrogen in the deoxynucleotides should lead to an increase in J(C2′-P3′). No theoretical estimates of the magnitude of this effect are known to us.

Our data in Fig. 3 suggest that the electronegativity effects cannot be primarily responsible for the changes in the vicinal ^{13}C-^{31}P couplings of the deoxyribonucleotides relative to the ribo- and arabinonucleotides. For example, at pH < 5.0, the J(C2′-P3′) couplings in the deoxyribose molecules are *smaller* by about 1.0 Hz rather than larger as would be expected if the electronegativity effect dominated. Furthermore, the J(C4′-P3′) couplings in the deoxyribose molecules are larger than in the ribose and arabinose molecules by 1.0–1.5 Hz. These opposing trends in J(C2′-P3′) and J(C4′-P3′) are easily interpreted in terms of conformational changes about the C3′—O3′ bond but are not consistent with a dominant role for any inductive effect of the 2′-hydroxyl.

Additional evidence that inductive effects are of secondary importance is provided by the work on locked 3′,5′-cyclic nucleotides by Lapper et al.[22] who noted that the C2′-P3′ couplings, on the one hand, and the C4′-P3′ couplings, on the other, were identical to within 0.5 Hz for a series of ribose and 2′-deoxyribose derivatives.

Effects of Secondary Phosphate Ionization

The changes in J(C2′-P3′) and J(C4′-P3′) induced by phosphate ionization will reflect (a) electronic (through-bond) effects and (b) conformational effects. The conformational changes will include changes in the relative importance of the $t(\phi')$ and $g^-(\phi')$ domains of the C3′—O3′ bond (Fig. 1) and also in the positions of the minima of the potential energy profile for rotation about C3′—O3′. The electronic effects of phosphate ionization should be similar for the C2′-C3′-O3′-P3′ and C4′-C3′-O3′-P3′ fragments, and thus J(C2′-P3′) and J(C4′-P3′) should show parallel titration behavior if electronic rather than conformational factors were dominant. However, for the ribose and arabinose molecules, phosphate ionization leads to an *increase* in J(C4′-P3′) and a *decrease* in J(C2′-P3′) (Fig. 3). These inverse trends can be understood in conformational (but not electronic) terms. By contrast, both sets of vicinal couplings for the deoxyribose molecules (3′dUMP, 3′dCMP, and 3′5′dCDP) decrease upon phosphate ionization. It would seem, therefore, that electronic factors primarily define the overall trends in the ionization-induced changes for the deoxyribose molecules. We cannot discount, however, a combination of conformational changes (i.e., in $t(\phi')$, $g^-(\phi')$ weighting plus changes in positions of energy minima). These subtle effects of the nature and orientation of the 2′-substituents suggest caution when comparing, in conformational terms, the data for the three series of nucleotides.

Analysis of the C3′—O3′ Bond

Alderfer and Ts'o[12] have discussed the interpretation of the J(C2′-P3′) and J(C4′-P3′) couplings in terms of the $t(\phi')$ and $g^-(\phi')$ populations (Fig. 1). (For steric reasons $g^+(\phi')$ is neglected.[23]) However, x-ray data[1,2] suggest that a continuous range of C3′—O3′ torsion angles, spanning adjacent segments of the $t(\phi')$ and $g^-(\phi')$ domains, are accessible to a 3′-nucleotide. This would result from a low energy barrier at the H3′,P3′ eclipsed orientation ($\phi' = -120°$; Fig. 1). Some theoretical calculations[5,24] reveal a broad energy minimum in the range of ϕ' values defined by the crystal data. If the barrier is small, or absent, in solution, then interpretation of the couplings in terms of "conformer" populations is not valid.[25–27] Until this matter is resolved, we shall interpret our data in a qualitative manner. That is, if J(C4′-P3′) > J(C2′-P3′), we shall say that the C4′,P3′ *trans* situation [$t(\phi')$ domain] is preferred over the C2′,P3′ *trans* situation [$g^-(\phi')$]; if J(C4′-P3′) < J(C2′-P3′), the C2′-P3′ *trans* situation [$g^-(\phi')$] is preferred. To facilitate the comparison, we shall use the ratio J(C4′-P3′)/J(C2′-P3′), denoted by R.

At pH < 5.0 (monoanionic phosphate), the near equality of the vicinal ^{13}C-^{31}P couplings for the ribose derivatives (R = 1.1; average over all entries) indicate a balance of the $t(\phi')$ and $g^-(\phi')$ domains; for the arabinose derivatives a slight preference for $t(\phi')$ is indicated (R = 1.3; overall average). Secondary phosphate ionization leads to a stabilization of $t(\phi')$ in the ribose and arabinose series (R = 1.7–2.2 at pH > 7.0). (The most pronounced stabilization appears for 3′,5′-aCDP, presumably a consequence of phosphate–phosphate interaction.) For the deoxyribose molecules a preference for $t(\phi')$ is apparent at pH < 5.0 ($R \sim 1.9$); secondary phosphate ionization has little effect ($R \sim 2.1$ at pH > 7.0). Thus, at pH > 7.0, the R values are similar for the three series of molecules (1.7–2.2), suggesting similar conformational behavior. Also the ϕ' conformation is little affected by the nature of the pyrimidine bases examined. A *syn* pyrimidine base was found[13] to stabilize the $t(\phi')$ domain in a 3′,5′-diphosphate ($R > 10$ in the *syn* 6-methyluracil derivative). A purine base (adenine in 3′AMP) leads to a small destabilization of $t(\phi')$.[12]

Influence of the Sugar Pucker

In general, furanose rings execute an interconversion in solution between the N(3′*endo*) and S(2′*endo*) puckers, the populations of which are estimated from proton coupling constants.[28] It is thought that sugar-pucker-dependent close-contact interactions, involving the oxygen atoms of the 3′-phosphate and the atoms on the sugar ring, should influence the potential energies of the $t(\phi')$ and $g^-(\phi')$ domains[7,29] and hence their relative weightings in solution. In this section we consider some of these interactions qualitatively to see whether the conformational trends suggested by Fig. 3 can be understood in terms of a ϕ', sugar-pucker interdependence. Useful for this discussion are the estimates (Table I) of the 3′-*endo* popu-

TABLE I
Calculated Populations (%) of the *N*(3′-*endo*) Pucker for the 3′-Nucleotides[a,b]

Compound	3′-*endo*(%) pH < 5	pH > 7
3′UMP	56	56
3′CMP	54	57
3′dUMP	37	38
3′dCMP	36	39
3′aUMP	41	51
3′aCMP	38	48

[a] From Ref. 30. Spectra at 90 and 270 MHz.
[b] Assuming $\%N \approx 10J_{3'4'}$; $\%S = 100\% - \%N$, as in Ref. 27.

lations for the 3′-nucleotides obtained from our recent pH study[30] of their proton spectra. In general agreement with earlier (but less complete) data,[23,31,32] the 2′-deoxyribose-3′-nucleotides show a preference (60–70%) for 2′-*endo*, whereas the 3′-ribonucleotides show a near balance (or slight preference for 3′-*endo*). Their pucker is not strongly dependent on the ionization state of the 3′-phosphate. The 3′-arabinonucleotides at pH < 5 resemble the deoxyribose molecules (ca. 60% 2′-*endo*) but show a near balance in 2′-*endo* and 3′-*endo* at pH > 7.

Close Contacts with C5′ Substituents

Sasisekharan et al.[7,29] have shown that in the 3′-*endo* pucker of a ribose or deoxyribose 3′-nucleotide, contacts are made between the O atoms of the 3′-phosphate and the C5′ substituents, which should destabilize large angles in the $g^-(\phi')$ range. Molecular models indicate that similar contacts in the 3′-*endo* pucker should destabilize $g^-(\phi')$ in a 3′-arabinonucleotide. These contacts with C5′ substituents may, however, be discounted as the *dominant* factor determining the trends in the ϕ' orientational preferences suggested by the J(C,P) data in Fig. 3, since, if they were dominant, we would expect the smallest $g^-(\phi')$ weighting for those molecules with the largest 3′-*endo* weighting, namely, the 3′-ribonucleotides. However, at pH < 5 the converse is noted, since the smallest $g^-(\phi')$ weighting is shown by the deoxyribonucleotides that have the smallest 3′-*endo* population. Furthermore, at pH < 5 the arabinonucleotides have a 2′-*endo*,3′-*endo* distribution like that of the deoxyribonucleotides but a $t(\phi')$,$g^-(\phi')$ distribution like that of the ribonucleotides.

Contacts with the C2′ Hydroxyl Group

Pattabiraman et al.[7] have pointed out that in a 3′-ribonucleotide with a 2′-*endo* conformation, close contacts between the 3′-phosphate and the 2′-hydroxyl group destabilize small angles in the $t(\phi')$ domain. This destabilization, occurring during the time the ring spends in 2′-*endo*, would

seem to account for the reduced $t(\phi')$ weighting in the 3′-ribonucleotides, relative to the 3′-deoxyribonucleotides, that is observed at pH < 5. However, this reasoning cannot be extended to the data at pH > 7, since at this higher pH, the ϕ' distributions are similar in the two series even though the riboses retain their significant contribution (ca. 45%) from 2′-*endo*.

In contrast with the situation in ribonucleotides, close contacts between the 3′-phosphate and the 2′-hydroxyl group are not possible in a 2′-*endo* arabinonucleotide. In fact, in the 2′-*endo* pucker the close contacts affecting the ϕ' orientation are identical for an arabinose and a deoxyribose molecule in both the $t(\phi')$ and $g^-(\phi')$ domains, since this pucker provides maximum separation between the 3′-phosphate and the *endo*-2′-hydroxyl. Thus, in solution the $t(\phi')$, $g^-(\phi')$ distribution of an arabinonucleotide and a deoxyribonucleotide should not differ during the times spent in 2′-*endo*. However, molecular models reveal close contacts in a 3′-*endo* arabinonucleotide that should destabilize $t(\phi')$. This destabilization, occurring during the time spent in 3′-*endo*, would seem to account for the decrease in the $t(\phi')$ weighting in the arabinonucleotides relative to the deoxyribonucleotides at pH < 5. But again, the reasoning cannot be extended to the pH > 7 data, at which point their ϕ' distributions are similar, even though the arabinonucleotides retain a significant (and, in fact, enhanced) 3′-*endo* contribution at the higher pH. Thus, we cannot provide a simple rationale of the trends in Fig. 3, which is based on a dominant role for the 2′-hydroxyl group and which applies consistantly over the entire pH range.

Theoretical calculations have revealed a dependence of ϕ' on sugar pucker.[29] Jack et al.[22] have noted, for the nucleotide components of tRNA, that the $t(\phi')$ domain is preferred when the ribose ring has the 3′-*endo* pucker but that $t(\phi')$ and $g^-(\phi')$ are equally acceptable when the sugar is 2′-*endo*. Nmr studies of 3′-*ribo*nucleotides[34,35] point to a similar correlation in solution, namely, that a shift towards a 2′-*endo* pucker should effect a decrease in the $t(\phi')$ weighting. The present study has provided the most complete description of the ϕ' conformational behavior for 3′-nucleotides of the ribose, 2′-deoxyribose, and arabinose series, as well as support for the contention that sugar-pucker-dependent interactions with the 2′-hydroxyl group influence the ϕ' orientation. Furthermore, we could provide, in terms of pucker-dependent 3′-phosphate-2′-hydroxyl contacts, a qualitative description of the gross trends in the J(C,P) data at pH < 5, but we are unable to extend this simple picture to the data at pH > 7 (Fig. 3). In this matter we can only state the obvious—the ionization state of the phosphate, and therefore probably its interaction with the solvent, has some *direct* influence on the energy profile of the ϕ' bond, as well as the *indirect* effect mediated by the sugar pucker. This point is made clear by the absence of any relationship between the titration curves for the J(C,P) data, on the one hand, and that for the sugar puckers (Table I), on the other.

Conformation about the C5′—O5′ Bond

Information about the C5′—O5′ bond of a 5′-nucleotide can be obtained from the vicinal J(C4′-P5′) coupling constant data.[9–13] For our series of 5′-nucleotides in the ribose (5′UMP, 5′CMP), deoxyribose (5′dUMP, 5′-dCMP), and arabinose (5′aUMP, 5′aCMP) series, this coupling falls in the narrow range of 8.2–8.7 Hz over the entire pH range considered. This points to a preference (70–80%) for the *trans* orientation of the P5′-O5′-C5′-C4′ fragment, with little dependence on the nature of the sugar.[13] These results are in line with published ^{1}H-^{31}P coupling constant data.[32]

SUMMARY

This study dealt mainly with the pH dependence of the vicinal J(C2′-P3′) and J(C4′-P3′) coupling constants for pyrimidine 3′-nucleotides in the ribose (3′UMP, 3′CMP), deoxyribose (3′dUMP, 3′dCMP), and arabinose (3′aUMP, 3′aCMP) series. The data provide information about the influence of the sugar on the orientation about the C3′—O3′ bond.

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