A nuclear magnetic resonance study of the ribotrinucleoside diphophate UpUpC

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Received 23 June 1983; Revised and Accepted 28 July 1983

ABSTRACT

500 MHz ^{1}H, 67.89 MHz ^{13}C and 80.97 MHz ^{31}P-NMR studies are reported on the ribotrinucleoside diphosphate UpUpC, the triplet codon corresponding to the amino acid phenylalanine. Complete spectral assignments are given and conformational parameters for the backbone and the furanose rings are determined. All three nucleotide units show a near-balance for the N/S equilibrium with a slight preference for the N-type ribose (~60%). The backbone conformation around the C3'-03' bonds show a preference for the trans domain, while the orientation aroung the C5'-05' bonds is predominantly trans.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy has yielded considerable information about conformational details of nucleic acids in solution (1,2). The majority of studies have employed ^{1}H-NMR and in particular proton-proton and proton-phosphorus couplings to extract structural information (3,5), while only few deal with ^{13}C or ^{31}P-NMR. ^{13}C-NMR spectroscopy is particularly useful for the determination of the sugar phosphate backbone conformation via carbon-phosphorus coupling constants (6,7), which can easily be determined from proton decoupled ^{13}C-NMR spectra. To date most ^{1}H-NMR studies on oligoribonucleotides containing complete assignments of base and sugar protons have dealt with dimers (8,10), and only a few trimers (10,12) and two tetramers (13,14) have been investigated. (For a recent review on ^{1}H-NMR studies see ref. 15). The few ^{13}C-NMR studies all deal with dimers or homopolymers (16, 18).

As part of an investigation into the structural and dynamic aspects of codon-anticodon interactions in solution, we have carried out a ^{1}H, ^{13}C and ^{31}P-NMR study on the oligoribonucleotide, UpUpC, the triplet codon for the amino acid phenylalanine. To the best of our knowledge, this study represents the first example of a ^{13}C-NMR investigation of a ribotrinucleoside diphosphate.

EXPERIMENTAL

UpUpC was synthesized from the suitably protected nucleosides according to the 1-hydroxybenztriazole phosphotriester approach (19). It was deblocked in a series of three steps and purified by anion-exchange chromatography on Sephadex A-25. The purity of the isolated oligoribonucleotide was controlled by HPLC on an anion-exchange column. The purified product could be completely digested by ribonuclease T_2 to give uridine-3'-monophosphate and cytidine in a ratio of 2:1. The sample was feeeze dried extensively from 99.6% D_2O and finally dissolved in 99.96% D_2O buffer containing 10 mM potassium phosphate, 500 mM KCl, pH* 6.5 (meter reading uncorrected for the isotope effect on the glass electrode). Prior to use all glassware was heated to 200°C for 4 h to inactivate possible traces of ribonuclease. The concentration of UpUpC employed for the ^{1}H-NMR spectra was 10 mM, and for the ^{13}C and ^{31}P-NMR spectra 20 mM.

^{1}H-NMR spectra were recorded at 500 MHz on a Bruker AM 500 spectrometer (spectral width 4000 Hz, 8 to 32 K data points). A two dimensional J-resolved spectrum (20) was also recorded at 500 MHz and 5°C for the sugar region of the spectrum, using a spectral width of 2400 Hz in the chemical shift axis (4 K data points) and ± 20 Hz in the J axis (128 data points).

^{13}C-NMR spectra were recorded at 67.89 MHz on a Bruker WM 270 spectrometer (spectral width 12195 Hz with 16 K data points for the complete spectrum, and spectral width of 4000 Hz with 16 K data points for the ribose carbon region) using an interpulse time of 5 s.

^{31}P-NMR spectra were recorded at 80.96 MHz on a Bruker WM 200 spectrometer (spectral width 2000 Hz, 8 K data points) using an interpulse time of 5 s. In order to determine the irradiation frequency for the selective decoupling experiment a ^{1}H-NMR spectrum was recorded using the decoupling coil as observation channel.

^{1}H chemical shifts are reported with respect to 4,4-dimethyl silapentane-1-sulfonate(DSS); ^{13}C chemical shifts with respect to the methyl group of DSS (which is -1.1 ppm from trimethylsilane); and ^{31}P chemical shifts with respect to 85% orthophosphoric acid (as external standard).

RESULTS AND DISCUSSION

The one dimensional ^{1}H-NMR spectrum of UpUpC at 278 K (Fig. 1A) was completely assigned by comparison with published data on the constituents and extensive spin-spin decoupling experiments. The U(1) group of resonances can easily be identified since its H5'/H5" signals do not contain any ^{1}H-^{31}P coup-

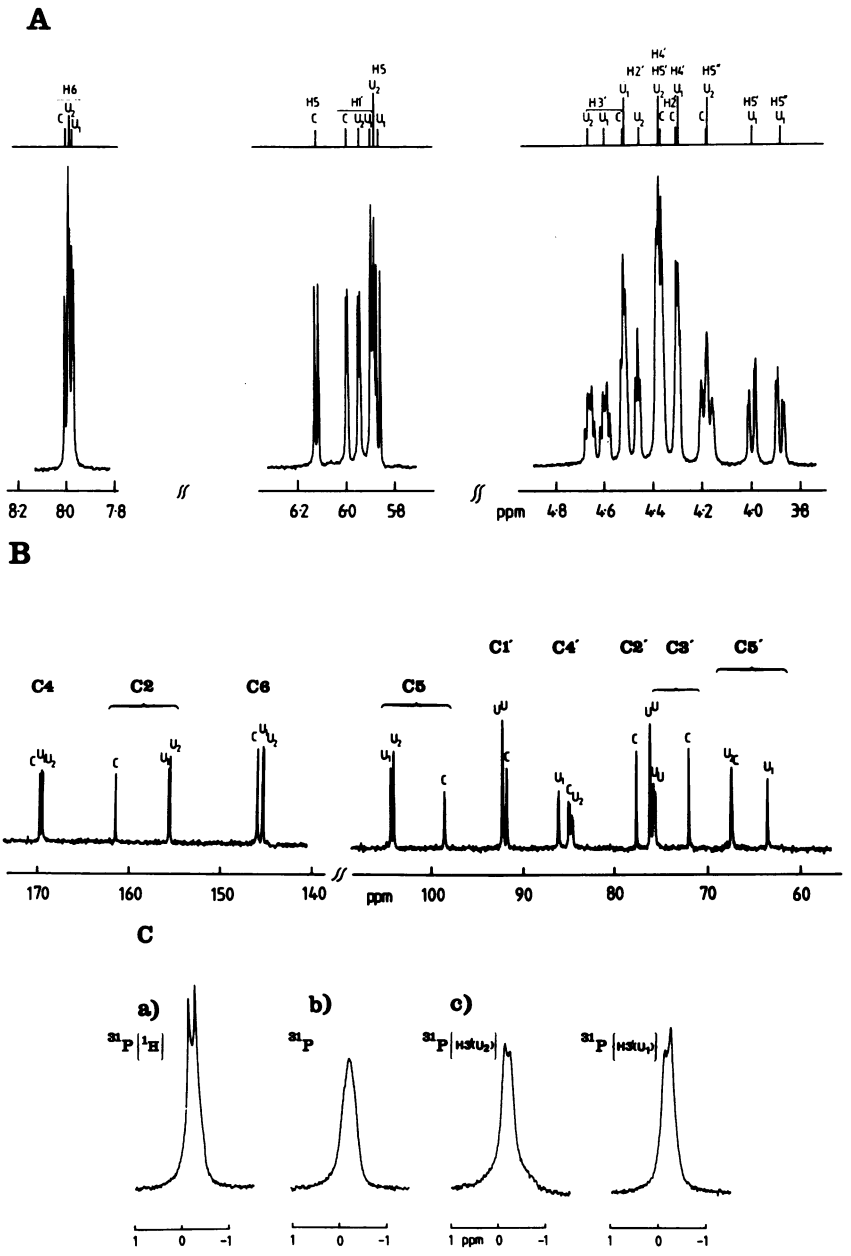

Fig. 1

NMR spectra of the ribotrinucleoside diphosphate UpUpC. A: 500 MHz ^{1}H-NMR spectrum at 278 K. B: 67.89 MHz ^{31}C-NMR spectrum (^{1}H decoupled) at 273 K. C: 80.96 MHz ^{31}P-NMR spectrum at 278 K: a) ^{1}H decoupled, b) coupled, c) selectively decoupled

Table 1.

^{1}H-^{1}H and ^{1}H-^{31}P coupling constants (in Hz) of UpUpC at 278 K

	U(1)	U(2)	C(3)
1',2'	3.9	4.2	3.7
2',3'	5.7	5.1	5.2
3',4'	5.5	5.5	5.5
3',P3'	7.8	9.4	-
5',P5'	-	4.1[a]	4.1[a]
5",P5'	-	3.8[a]	3.8[a]
Σ 1'2' + 3'4'	9.4	9.7	9.2

[a] Exact determination not possible because of overlapping signals

lings and are characteristically shifted to the high field end of the ribose proton region. Similarly the H3' resonance of C(3) can be identified. All assignments based on decoupling experiments were confirmed by means of a two dimensional correlated spectrum. ^{1}H-^{1}H coupling constants as well as ^{1}H-^{31}P coupling constants were determined directly from either the one-dimensional spectrum (aromatic protons and H1' protons) or a two-dimensional J-resolved spectrum of the ribose spectral region (Table 1).

In order to undertake a detailed pseudorotational analysis of the furanose ring the spin-spin coupling constants have to be determined for a series of temperatures (14); however, some information concerning the ribose ring conformation can be obtained from the H1' doublet splitting. Based on the simple approximation N(%) = 100 x (10 - $J_{1'2'}$/10) (30), we find 61% N for U(1), 58.5% N for U(2) and 63.4% N for C(3); using a graphical method based on $J_{2'3'}$ + $J_{3'4'}$ (21) almost the same result is obtained: 61% N for U(1), 58% N for U(2) and 61% N for C(3). The percentage N conformer found for all three nucleotide units in UpUpC is thus much smaller than that found in trimers like ApApC or ApCpC (14). Since a high proportion of the N conformer is associated with the stacked A-RNA conformation (14) this indicates that the trimer UpUpC does not predominantly adopt such a conformation.

Assignment of the ^{13}C-NMR spectrum (Fig. 1B) was carried out on the basis of published data for mono- and dinucleotides (6,17,22). C2' and C3' resonances of U(1) and U(2) were assigned via the temperature dependence of their

Table 2.

^{13}C Chemical shifts of UpUpC at 273 K and of monoribonucleosides U and C (shifts are quoted in ppm relative to the methyl group of DSS)

	U(1)	U(2)	C(3)	U [a]	C [a]
C2	154.1	153.8	159.9	155.0	159.9
C4	168.4	168.3	168.7	169.8	168.5
C5	104.8	104.5	98.7	104.7	98.5
C6	143.4	143.3	144.0	144.0	144.0
C1'	92.1	92.1	91.6	91.8	91.3
C2'	75.5	75.5	77.0	76.0	76.4
C3'	75.1	75.1	71.2	71.9	71.7
C4'	85.8	84.4	84.6	86.5	86.1
C5'	62.4	66.5	66.5	63.2	63.2

[a] From ref. 23; shifts converted to the DSS scale

chemical shifts. While the C2' resonance shifts only slightly with temperature, the C3' resonance shifts by ∿0.8 ppm from being on the high field side of the C2' resonance at 273 K to low field at 323 K. A comparison between our ^{13}C-NMR shifts for UpUpC and those reported by Kruska and Blonski (23) for nucleosides (Table 2) shows their close similarity, thus again pointing towards a low amount of stacking in this trimer. Introduction of a phosphate at either C3' or C5' leads to downfield shift of ∿3 ppm, which is in agreement with the reported effects of phosphorylation on the chemical shift of dinucleotides (17).

Properties of the phosphodiester backbone of oligonucleotides are of crucial importance in defining the overall structure of such molecules. In order to extract conformational parameters the ^{13}C-^{31}P coupling constants were determined for UpUpC and analysed. A complete analysis is only possible at high temperature (323 K) at which all ^{13}C-^{31}P coupling constants are resolved. However, extrapolation to a lower temperature was justified due to the general trend in the size of the coupling constants with temperature and the use of several resolved ^{13}C-^{31}P coupling constants determined at temperatures from 273 K to 323 K.

Table 3.

^{13}C-^{31}P coupling constants (in Hz) of UpUpC determined at several temperatures

		273K	283K	293K	303K	313K	323K
U(1)	C2'-P3' [a]	n.r.	n.r.	3.4	3.6	4.0	3.9
	C3'-P3'	5.9	6.8	a	a	4.9	5.4
	C4'-P3'	5.4	4.9	4.9	4.9	4.9	4.9
U(2)	C2'-P3' [a]	n.r.	n.r.	3.4	3.6	4.0	3.9
	C3',P3'	4.4	5.9	a	a	5.4	5.9
	C4',P3'	∿ 15	∿ 13	4.9	4.9	4.9	4.9
	C4',P5'			8.8	8.8	8.8	8.8
	C5',P5'	n.r.	3.9	5.0	6.0	5.9	5.9
C(3)	C4',P5'	10.3	9.8	9.3	9.3	8.8	8.8
	C5',P5'	n.r.	n.r.	4.9	5.4	5.4	4.9

[a] exact determination was not possible due to overlapping signals
n.r. = not resolved

The three bond ^{13}C-^{31}P coupling constants may be interpreted in several ways for the conformations about the C3'-03' and C5'-05' bonds. Based on ^{3}J ^{13}C-^{31}P coupling constants in cyclic nucleotides, a Karplus relationship between the torsion angles β(C5'-05') and ε(C3'-03') and ^{3}J values was demonstrated (6,24). Analysis based upon a dynamic model assuming rapid interconversion between the classical staggered conformers (Fig. 2) considers the time averaged coupling constants as representative of the relative populations of the allowed conformers (16). Alternatively, an equilibrium between fixed conformers can be assumed or non-classical conformations with altered torsion angles can be used for the calculations (17).

Using conventional rotamers the equation $^{3}J_{C,P} = 9.5 \cos^2\theta - 0.6 \cos\theta$ yields values of $^{3}J_{trans} = 10.1$ Hz and $^{3}J_{gauche} = 2.1$ Hz (25). Using the three bond coupling constants, as defined by populations of the conventional rotamers and their theoretical coupling constant values, a set of three expressions for the set of rotamers around the C3'-03' bond allows the deter-

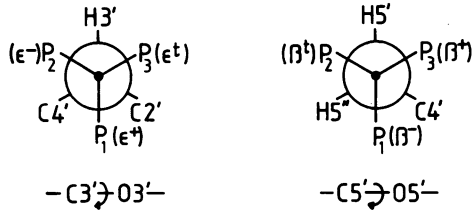

Fig. 2

Newman projections along the backbone angles ε(C3'-03'bond) and β(C5'-05' bond) showing the classical staggered rotamers.

mination of their relative populations

$$^{3}J\ (C2'\text{-}P3') = P_1 J_g + P_2 J_t + P_3 J_g$$

$$^{3}J\ (C4'\text{-}P3') = P_1 J_g + P_2 J_g + P_3 J_t$$

$$1 = P_1 + P_2 + P_3$$

Since there is only one $^{13}C\text{-}^{31}P$ coupling constant observable for the C5'-05' bond, only the sum of the population of gauche conformers versus trans conformers can be determined from

$$^{3}J\ (C4'\text{-}P5') = (P_1 + P_3)J_g + P_2 J_t$$

At 323 K the conformation about the C5'-05' bond of both U(2) and C(3) is approximately 84% β^t. Lowering the temperature leads to an increase in the percentage of the trans conformer as judged by the increase in ^{3}J(C4'-P5') for C(3). At the lowest temperature employed the conformation has to be entirely trans since the coupling constant is already slightly larger than its theoretically determined limiting value (10.25 Hz as compared to 10.1 Hz).

Analysis of the conformation about the (C3'-03') bond is more complex. Assuming the simple dynamic model with three conventional rotamers their distribution at 323 K is calculated as $P_1 = 0.18$, $P_2 = 0.47$, $P_3 = 0.35$; thus $\varepsilon^t = 35\%$, $\varepsilon^- = 47\%$ and $\varepsilon^+ = 18\%$ for both U(1) and U(2). In contrast to previous calculations on dimers (17), we do not find a substantial population of the ε^+ conformer. However, since theoretical studies (26,27) and X-ray determinations (28,29) show that the ε^+ conformer is virtually non-existent, the analysis may also be performed according to a two rotamer model in which the ε^+ rotamer is depopulated and non classical gauche rotamers assumed (17). Using this approach we find for U(1) and U(2) $\varepsilon^t = 56\%$ and $\varepsilon^- = 44\%$. Since not all coupling constants are resolved at low temperatures it is not possible to calculate rotamer populations at 273 K. However, ^{3}J(C4'-P3') for U(1) is

larger at 273 K than at high temperatures, and thus it can be concluded that the proportion of the ε^t rotamer increases with lowering the temperature. Since it is the ε^t rotamer that allows a maximum of base stacking, this increase in coupling constants can be interpreted as being due to an increased stacked conformation of UpUpC at low temperatures.

The proton decoupled ^{31}P-NMR spectrum shows two resonances of similar chemical shift (-0.25 and -0.11 ppm) for the two phosphodiester linkages, whereas the proton coupled spectrum does not resolve the two resonances due to the ^{1}H-^{31}P coupling and consists of a broad peak of ∿25 Hz linewidth. In order to assign the two peaks to either the UpU or the UpC phosphate we made use of the assigned ^{1}H-NMR spectrum for a double resonance experiment. The H3' proton resonances of U(1) and U(2) are well separated at the low field of the sugar proton region and thus ideally suited for selective ^{1}H irradiation in order to collapse the coupling, which is by far the largest proton-phosphorous coupling observed in nucleotides. Using this approach, irradiation of the lowest field U(2)H3' proton resonances leads to a sharpening of the lower field ^{31}P resonance (at 0.11 ppm) which is therefore assigned to the UpC phosphate. Similarly, irradiation at the position of the U(1)H3' resonance sharpens the high field ^{31}P resonance, (at -0.25 ppm) which is therefore assigned to the UpU phosphate. This experiment is illustrated in Fig. 1C.

SUMMARY

In contrast to the well stacked A-RNA helix fragments found for purine containing trimers, UpUpC, as characterised by NMR parameters, shows a near balance for the N/S equilibrium of the furanose ring with a slight preference for the 3'-endo conformation at low temperatures, a strong preference for the trans orientation ε^t around the C5'-O5' bond and slight preference for the ε^t domain around the C3'-O3' bond. This indicates a substantial amount of conformational flexibility, probably due to a rapid equilibrium of the stacked and unstacked states.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council (GMC, AMG and BJK), the Lister Institute of Preventive Medicine (GMC) and the Max-Planck Gesellschaft (LWM). GMC is a Lister Institute Research Fellow. The ^{1}H and ^{31}P NMR spectra were recorded on the AM 500 and WM 200 spectrometers of the Medical Research Council Biomedical NMR Centre at the National Institute for Medical Research.

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