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Carbon-13 NMR in conformational analysis of nucleic acid fragments. 3. The magnitude of torsional angle ε in d(TpA) from CCOP and HCOP NMR coupling constants

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ABSTRACT

Carbon-13 NMR spectra of the deoxyribonucleotide d(TpA), 3',5'-cyclic AMP and 3',5'-cyclic dAMP were measured. It is shown that the different substitution of C2' in deoxyribonucleotides versus ribonucleotides does not affect the vicinal C2'-C3'-O3'-P coupling to a measurable extent. Therefore, the same set of Karplus parameters may be used for the C2'-C3'-O3'-P couplings in ribonucleotides and in deoxyribonucleotides.

Vicinal carbon-phosphorus and proton-phosphorus coupling constants are used to calculate the magnitude of the torsion angle ε(C4'-C3'-O3'-P), which amounts to 195° in the trans conformer and to 261° in the gauche(-) conformer.

INTRODUCTION [1,2]

Two new parametrizations of the Karplus equations for vicinal NMR coupling constants in CCOP (eq. 1) and HCOP (eq. 2) fragments were introduced in a previous paper[3]:

$$^3J_{CCOP} = 6.9\cos^2\phi - 3.4\cos\phi + 0.7 \quad (1)$$

$$^3J_{HCOP} = 15.3\cos^2\phi - 6.1\cos\phi + 1.6 \quad (2)$$

The parameters for equations (1) and (2) were derived simultaneously, using a large dataset of coupling constants $^3J_{CCOP}$ and $^3J_{HCOP}$ of oligoribonucleotides. The $^3J_{CCOP}$ coupling constants were measured along three different molecular fragments, i.e. C2'-C3'-O3'-P, C4'-C3'-O3'-P and C4'-C5'-O5'-P. In ribose residues the first two fragments are identical with respect to the character and number of electronegative substituents, whereas in the latter fragment the central C5' bears two protons in contrast to the central C3' which bears one carbon atom and one proton. The possible effect of this substitution on $^3J_{CCOP}$ was necessarily ignored in the parametrization procedure. However, judging by analogy from the much better studied $^3J_{HCCH}$ system[4] such an effect, if present, would be relatively small.

The question arises whether or not the aforementioned Karplus parameters are applicable to DNA fragments. In the case of deoxyribonucleotides, the C2'-C3'-O3'-P fragment is substituted differently compared to ribonucleotides, i.e. in the former the hydroxyl group on C2' is replaced by hydrogen. It should be noted that the electronegative substituent in question (OH) is located on one of the coupling nuclei (C2'), and little is known of the effect of such a substituent upon the coupling constant. Davies and Sadikot[5] have estimated from C1'-C2'-O2'-P couplings in 2',3'-cyclic mononucleotides that substitution of a proton located on the coupled ^{13}C by a nitrogen causes an increase of 1.1 Hz in the vicinal $^{3}J_{CCOP}$. However, their estimate is based solely upon couplings observed in 2',3'-cyclic nucleotides. It is well known that the magnitude of bond angles (which is not the same in 3',5'-cyclic nucleotides and open chain oligonucleotides) affect the magnitude of vicinal coupling constants[6]. Therefore, results obtained from these compounds should be viewed with some caution.

The present paper deals with the possible effect of a change of substitution pattern of C2' from a comparison of 3',5'-cyclic AMP with 3',5'-cyclic dAMP and from an analysis of couplings obtained from d(TpA) at a number of temperatures. The experiments suggest the absence of a measurable effect of a 2' oxygen substituent on $J_{C2'-P}$. Moreover the results obtained for d(TpA) allow for the determination of the magnitude of backbone angle ε (C4'-C3'-O3'-P) in this compound.

MATERIALS AND METHODS

NMR samples

The compounds studied were synthesized via a modified phosphotriester approach[7]. D_2O solutions were prepared with nucleotide concentrations ranging from 20-100 mM; a 5 mm tube, containing 0.4 ml of solution, was used for d(TpA) and a 10 mm tube, containing 1.2 ml of solution was used for c-dAMP and c-AMP. A trace of EDTA was added in order to neutralize paramagnetic contaminations, and the pH was adjusted to 7.5 (± 0.5) (meter reading). Tetramethylammonium chloride (TMA) served as an internal reference. Chemical shifts are measured relative to the central peak of TMA and can be converted to the dioxane scale by means of the relationship: $\delta_{dioxane} = \delta_{TMA} - 11.50$.

One-dimensional NMR Spectroscopy

^{13}C NMR spectra were recorded on a Bruker WM-200 WB spectrometer (operating at 50.3 MHz), and on a Bruker WM-300 spectrometer (operating at 75.3 MHz). Both spectrometers are equipped with a doubly-tuned 10 mm probe for ^{31}P decoupling purposes. Spectra were recorded on 8K data points, using a two-level decoupling scheme with 1 s relaxation delay; 0.5-1.0 Watt decoupling

power was employed during acquisition. The number of transients recorded was 2.000-50.000, depending on sample concentration; total acquisition times varied between 1 h and 25 h. FIDs were multiplied by a Gaussian window for resolution enhancement purposes, and then zero filled to 128K before Fourier Transformation, resulting in a digital resolution of approximately 0.2 Hz/point in the frequency domain.

Immediately after completion of each ^{13}C spectrum a ^{1}H spectrum of the same sample was recorded, using the decoupler coil, in order to determine the true sample temperature from the chemical shift difference $\delta_{HDO}-\delta_{TMA}$[8]. This internal temperature calibration is essential, because in earlier work it was found that a small temperature increase (varying between 0-5 °C) may occur, even when a two-level decoupling scheme with minimal decoupling power is employed[9].

Two-dimensional NMR spectroscopy

Spectra of c-AMP, c-dAMP and d(TpA) were assigned by means of heteronuclear chemical shift correlation spectroscopy[10,11]. The pulse sequence and phase cycling proposed by Bax[12] was used. Time domain spectra consisted of 2K data points in the t_2-dimension and of 64 data points in the t_1-dimension. Before Fourier Transformation the two-dimensional time-domain spectra were multiplied by a phase-shifted ($1/6\pi$) sine-square window and zero filled to 4K (t_2) and 512 data points (t_1), respectively.

Conformational analysis

Throughout this work use is made of ^{1}H data which have been acquired previously in our laboratories. Accurate ^{1}H-^{31}P coupling constants were determined by means of computer simulations. Conformational properties of the backbone angle ε (C4'-C3'-O3'-P) are calculated from the available coupling constant data, using the interactive non-linear least-squares program APLS[8], which was written in the programming language APL.

Nomenclature

The IUPAC-IUB nomenclature[13] is used throughout this work (Figure 1). Carbon atoms of the sugar ring are numbered according to Figure 2.

RESULTS AND DISCUSSION

3',5'-cyclic AMP and 3',5'-cyclic dAMP

The ^{13}C spectra of c-AMP[9] and c-dAMP were assigned by means of heteronuclear chemical shift correlation spectroscopy. The assignment of the former compound is in agreement with the reassignment made earlier by Uesugi et al.[14], which was based upon chemical shift considerations. Table 1 shows the chemical shifts and coupling constants obtained for both compounds. It is evi-

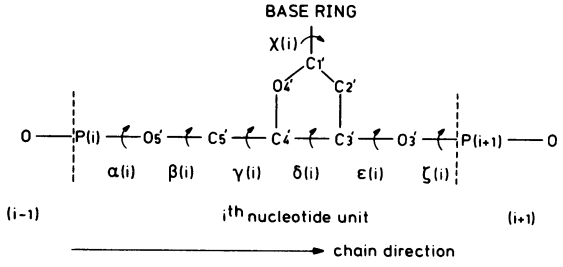

Figure 1: Conformational nomenclature.

dent that all corresponding coupling constants are identical within experimental error (± 0.2 Hz). Because $^{2}J_{C5'-P}$ and $^{2}J_{C3'-P}$ are geminal couplings, they are expected to be sensitive toward changes in bond angles and bond distances. The fact that these two couplings in c-dAMP are identical with those encountered in c-AMP, as well as the fact that $^{3}J_{C4'-P}$ in both compounds is the same, indicates that the six-membered rings in c-AMP and c-dAMP have similar conformations. As far as the sugar ring is concerned, Haasnoot et al.[15] have unambiguously demonstrated from ^{1}H NMR studies the conformational identity of these two compounds.

Having established the conformational correspondence in c-AMP and c-dAMP, the close similarity of $^{3}J_{C2'-P}$ in these two compounds strongly suggests the absence of a measurable effect upon the vicinal $^{13}C-^{31}P$ coupling resulting from the different substitution pattern at C2' in the two compounds. However, for reasons, outlined in the Introduction and in our previous paper[3], results obtained with cyclic nucleoside phosphates should be treated with cau-

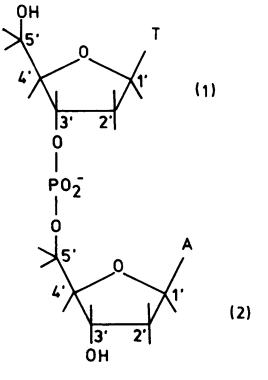

Figure 2: Structure of d(TpA) and carbon numbering of the ribose ring.

Table 1: Chemical shifts (ppm) and coupling constants (Hz) of c-AMP and c-dAMP.

		C1'	C2'	C3'	C4'	C5'
ribo:	δ	36.232	16.998	21.999	16.500	12.045
	J(CP)	---	8.1	4.5	4.3	7.0
deoxy:	δ	27.904	-20.069	21.043	20.019	12.080
	J(CP)	---	8.2	4.3	4.6	7.0

tion. Therefore an effort was made to estimate the effect of the presence or absence of O2' on the vicinal C2'-C3'-O3'-P coupling constant by means of an independent procedure, as described in the following sections.

Assignment of d(TpA)

A typical ^{13}C NMR spectrum of d(TpA) at 14 °C is shown in Figure 3. The signals of the nuclei that are coupled to phosphorus are shown in inserts. It is seen that even in the low temperature range an excellent resolution is achieved.

The assignment of the ribose resonances in the ^{13}C spectrum of d(TpA) is relatively straightforward. Unlike in ribose derivatives, no ambiguities occur with respect to the identity of the C3' and C2' resonances because the C2' of a deoxyribose resonates at high field. In the ^{13}C NMR spectrum of a dinucleoside monophosphate molecule only one of the two C2' signals is split due to a coupling to ^{31}P. Note that the splitting is resolved at elevated temperatures only. The C3' and C5' signals are split by ^{31}P with two-bond coupling constants of equal

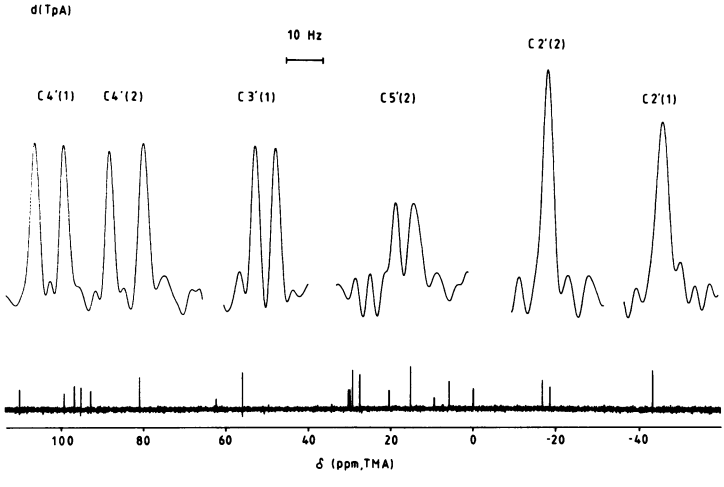

Figure 3: ^{13}C NMR spectrum of d(TpA) at 14°C. The spectrum was recorded in a 5 mm NMR tube at 75.3 MHz.

Table 2: ^{13}C- ^{13}P and H3'-^{31}P coupling constants (Hz) of d(TpA).

T(°C)	J(C4'-P)(1)	J(C4'-P)(2)	J(C2'-P)(1)	J(H3'-P)(1)
1.5	8.8	9.5	a	5.8
5.0	8.5	9.5	a	5.8
7.0	8.5	9.6	a	5.8
14.5	8.3	9.5	a	5.9
19.5	8.0	9.5	a	6.0
30.0	7.9	9.3	a	6.1
37.5	7.7	9.3	2.6	6.3
46.0	7.5	9.3	2.9	6.4
51.0	7.1	9.0	3.1	6.4
56.0	7.2	8.9	3.1	6.5
60.0	6.9	8.9	3.4	6.5
73.0	b	b	3.4	6.7
83.5	b	b	3.5	6.7
92.0	6.6	8.9	3.6	6.8

a) unresolved doublet due to small coupling constant.
b) unresolved due to overlap.

magnitude (5.3 - 5.6 Hz) which are characteristically invariant with temperature. The remaining two doublets are due to the C4' nuclei of the T(1) and A(2) residues. One of these couplings is large (≈ 9 Hz) and relatively constant over the entire temperature range. This behaviour clearly corresponds to that expected for the trans (180°) coupling (C4'-C5'-O5'-P) and therefore the doublet is assigned to C4'(2). The other coupling is much smaller at high temperature but increases with decreasing temperature. This increase in magnitude is approximately equal to the decrease of $J_{C2'-P}$ in the same temperature range. This signal is therefore assigned to C4'(1) (see Table 2 and 3). The present assignment was checked by means of 2D NMR (Figure 4) and is also consistent with the assignment of several longer oligonucleotides by means of 2D NMR[16]. Thus, it appears as a general rule that, in the absence of terminal phosphate groups, the doublet with the largest splitting at high temperature (≈ 9 Hz)

Table 3: Chemical shifts (ppm) of the ribose carbons of d(TpA) at a number of temperatures.

Temp.(°C) Carbon	1.5	19.5	37.5	56.0	73.0	92.0
C4'(1)	30.563	30.456	30.358	30.273	30.186	30.122
C4'(2)	30.252	30.178	30.127	30.115	30.075	30.055
C1'(1)	29.524	29.524	29.546	29.594	29.603	29.629
C1'(2)	27.732	27.747	27.803	27.906	27.959	28.028
C3'(1)	20.756	20.465	20.183	19.909	19.722	19.533
C3'(2)	15.383	15.277	15.219	15.203	15.225	15.254
C5'(2)	9.582	9.495	9.465	9.447	9.470	9.473
C5'(1)	5.915	5.860	5.814	5.782	5.764	5.750
C2'(2)	-16.750	-16.736	-16.704	-16.669	-16.648	-16.632
C2'(1)	-18.607	-18.598	-18.546	-18.509	-18.443	-18.394

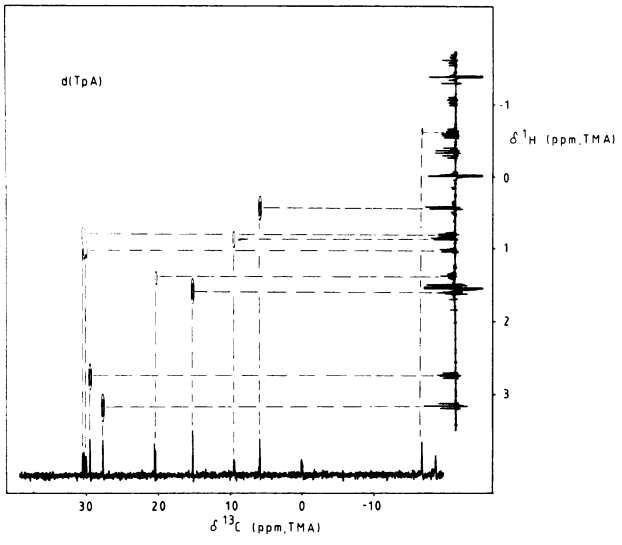

Figure 4: Two-dimensional heteronuclear chemical shift correlation spectrum of d(TpA) at 27°C. The spectrum was recorded at 50.3 MHz. A ^{1}H spectrum (300.3 MHz) is shown along the vertical axis (f1), a ^{13}C spectrum (50.3 MHz) is shown along the horizontal axis (f2). Only the ribose region is shown.

should be assigned to the C4' of the 3' terminus, whereas the doublet with the smaller splitting should be assigned to the C4' of the 5' terminus of dinucleosides monophosphates.

The Karplus parameters for C2'-C3'-O3'-P couplings in d(TpA)

The temperature dependence of the coupling constants $^{3}J_{C4'-P}(1)$, $^{3}J_{C2'-P}(1)$ and $^{3}J_{H3'-P}(1)$ is of special interest, as this property can give insight into the conformational characteristics of the backbone torsion angle ε. X-ray investigations[17] and a lanthanide shift study[18] strongly suggest that in the solid state as well as in solution only the trans rotamer (ε^{t}) and the gauche^{-} rotamer (ε^{-}) are present (Figure 5). In other words, ε^{+} appears to be forbidden. Therefore, in the following the existence of a two-state equilibrium for ε ($\varepsilon^{t}/\varepsilon^{-}$) is assumed.

In the first step of the conformational analysis of the C3'-O3' backbone angle any assumptions should be avoided concerning the validity of the Karplus parameters of equation (1) for the C2'-C3'-O3'-P fragment in deoxyribonucleotides. Therefore, only $J_{C4'-P}$ and $J_{H3'-P}$ are used as input data for equations (1) and (2). Using twelve sets of these two couplings recorded at twelve different temperatures (24 experimental data) as input for the least squares program APLS one is able to calculate two torsion angles (ε^{t} and ε^{-}), as well as the posi-

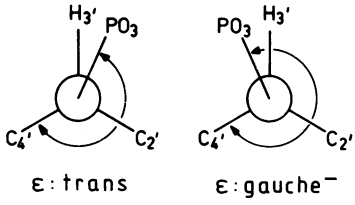

Figure 5: The two possible rotamers for backbone angle ε (C4'-C3'-O3'-P).

tion of the ($\varepsilon^t/\varepsilon^-$) equilibrium at each of the twelve temperatures (i.e. 14 parameters). The results are shown in Table 4. An ε^t angle of 195° and an ε^- angle of 261° are calculated. Relative populations of ε^t conformer range from 82% at 275 K to 58% at 365 K. Obviously, the ε^t conformer remains predominant at all temperatures and its population increases when the temperature is lowered.

At this point an estimation of the effect upon $J_{C2'-P}$ of the different substitution of C2' in deoxyribonucleotides versus ribonucleotides can be obtained in the following way. Assuming the existence of trigonal projection symmetry, the C2-P torsion angle is calculated to be 75° and 141° in the ε^t and ε^- conformer, respectively. Using these values in combination with equation (1) the coupling constant $J_{C2'-P}$ of the pure ε^- and ε^t conformers can be predicted. The values obtained in this way are J = 0.3 Hz for the trans conformer and J = 7.5 Hz for the gauche$^-$ conformer. Utilizing calculated rotamer populations at a number of temperatures (vide supra) one can now estimate $J_{C2'-P}$(est) at each temperature and compare the results with the experimental couplings $J_{C2'-P}$(exp). This comparison is shown in Table 5. Although accurate couplings

Table 4: Calculated ε(t) and ε(-) torsion angles and molfractions of ε(t) conformer of d(TpA).

T(°C)	%t (±2)
1.5	82
5.0	79
7.0	79
14.5	76
19.5	74
30.0	72
37.5	69
46.0	67
51.0	64
56.0	64
60.0	62
92.0	58
ε(t):	195°
ε(-):	261°

Table 5: Experimental coupling constants J(C2'-P,exp) and estimated coupling constants J(C2'-P,est) based on Karplus parameters of eqn. 1.

T(°C)	J(C2'-P,exp)	J(C2'-P,est)
37.5	2.6	2.5
46.0	2.9	2.7
51.0	3.1	2.9
56.0	3.1	2.9
60.0	3.4	3.0
92.0	3.6	3.3

could only be measured at elevated temperatures, it is evident from inspection of Table 5 that $J_{C2'-P}$(est) agrees surprisingly well with $J_{C2'-P}$(exp).

The above finding strongly suggests that a 2' oxygen does not affect the C2'-P coupling to a measurable degree. Consequently, at the present level of sophistication, the same Karplus parameters may be used for C2'-C3'-O3'-P fragments in ribonucleotides and deoxyribonucleotides. This conclusion differs from that reached by Davies and Sadikot[5].

CONCLUSIONS

In summary, in two cases no effect upon the vicinal CCOP coupling constant of the oxygen substituent on C2' can be detected:

1) In c-AMP and c-dAMP, where the torsion angle of interest (C2'-C3'-O3'-P) amounts to approximately 180°.
2) In d(TpA), where the torsion angle of interest amounts to approximately 75° in the most abundant trans conformer and to approximately 140° in the less abundant gauche$^-$ conformer.

Therefore, it appears appropriate to apply the parameters of equation (1) to C2'-P couplings in deoxyribonucleotides.

Although in the present work ε^t and ε^- angles were obtained from $J_{C4'-P}$ and $J_{H3'-P}$ only, there is no reason to exclude $J_{C2'-P}$ in deoxynucleotides from consideration in future investigations. In the case of d(TpA) the following values are found: ε^t = 195° and ε^- = 261°. Further work on a number of other DNA constituents is now in progress. It appears that the new values obtained for ε^t are slightly larger than previously thought[19]. Moreover, a base-sequence dependent variation of ε^t is seen. These results will be discussed in a subsequent paper[16].

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2. Abbreviations used: NMR, Nuclear Magnetic Resonance; TMA, tetramethylammonium chloride; EDTA, ethylenediaminotetraacetic acid; c-AMP, adenosine 3',5'-cyclic monophosphate; c-dAMP, 2'-deoxyadenosine 3',5'-cyclic monophosphate; d(TpA), 2'-deoxythymidilyl-(3',5')-2'-deoxyadenosine.
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