Nucleic Acids Research

A conformational study of adenylyl-(3',5')-adenosine and adenylyl-(2',5')-adenosine in aqueous solution by carbon-13 magnetic resonance spectroscopy.

Thomas Schleich*, Bradford P. Cross*+ and Ian C. P. Smith**.

*Division of Natural Sciences, University of California, Santa Cruz, CA 95064, USA.

ABSTRACT

     The solution conformation of adenylyl-(3',5')-adenosine and adenylyl-(2',5')-adenosine in both the stacked and un-stacked states was studied by carbon-13 magnetic resonance spectroscopy. Large chemical shift differences between the base carbons in the dimers and those in the corresponding monomers are attributed in part to the influence of base-base interaction. Carbon-phosphorus couplings across three bonds revealed the preferred populations for certain backbone rotamers, demonstrating that significant changes in conforma-tion about the C(3')-O and C(5')-O bonds do not occur in the temperature or salt-induced unstacking of adenylyl-(3',5')-adenosine. However, rotations about the C(2')-O and C(5')-O bonds occur in the temperature-mediated unstacking of adenylyl-(2',5')-adenosine.

INTRODUCTION

     Proton nuclear magnetic resonance spectroscopy has been applied with considerable success to problems of nucleic acid structure and conformation (1). The usefulness of ^{13}C NMR in such problems has long been recognized but until recently very little application has been made of this technique (for recent reviews see ref 2-3). Recent improvements and increased avail-ability of instrumentation, the wide range of ^{13}C chemical shifts, the absence of complicated spin-spin splitting patterns under conditions of complete proton decoupling, and the different locations of carbon and hydrogen nuclei, permit the examination of certain conformational features by ^{13}C NMR spectroscopy which are not amenable by ^{1}H NMR spectroscopic techniques alone. In this study the ^{13}C NMR spectra of adenylyl-(2',5')-adenosine (A-A(2',5')), and adenylyl-(3',5')-adenosine (A-A(3',5')) in the stacked and unstacked states

have been analyzed to determine: (a) the usefulness of ^{13}C chemical shifts as monitors of the extent and topography of base-base stacking; and (b) the preferred conformations of the phosphodiester backbone under stacking and unstacking conditions (see Figure 1) with respect to rotations about C(3')-O (C(2')-O), and C(5')-O, as assessed by the angular dependent three-bond ^{13}C-^{31}P couplings.
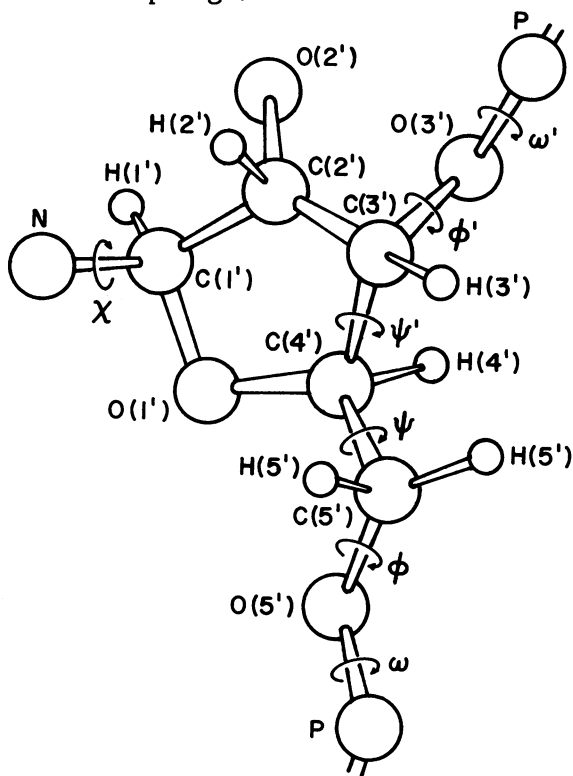
Figure 1. Definition of phosphate backbone torsion angles.

EXPERIMENTAL SECTION

^{13}C NMR spectra were taken with either a JEOL PS-100 or Varian XL-100-15 spectrometer operating at 25.2 MHz in the Fourier transform mode with noise decoupling of ^{1}H at 100 MHz. The free induction decays were stored and subsequently transformed using either a 12K Varian 620/i (PS-100) or a 16K Varian 620/L (XL-100) computer. In most cases the interferograms were multiplied by exponential functions to enhance resolution or signal-to-noise, as appropriate. For determina-

tion of coupling constants, spectral widths of 1000 or 2500 Hz with either 4096 or 8192 data points, respectively, were employed (resolution 0.5 and 0.6 Hz, respectively).

The dinucleoside monophosphates A-A(3',5') and A-A(2',5') were purchased from the Sigma and Miles chemical companies, respectively. D_2O (99.88%) was obtained from Bio-Rad Laboratories and Merck Sharpe and Dohme Canada. Reagent grade $NaClO_4$ (G. F. Smith Co.) was employed. The sample concentrations were usually 0.1 M and were prepared in D_2O (or 4 M $NaClO_4$ in D_2O), adjusted to pD 7.2, lyophilized, and redissolved in D_2O. A capillary of Me_4Si positioned concentrically in a larger tube containing D_2O (or 4 M $NaClO_4$ in D_2O) served as an external reference at each temperature employed. Sample NMR tube diameters were 5, 8, or 12 mm. Chemical shifts and coupling constants are reported with an estimated accuracy of 0.1 ppm and 0.5 Hz, respectively.

RESULTS AND DISCUSSION

In addition to the conformational degrees of freedom available to nucleosides and nucleotides, dinucleoside monophosphates have rotational degrees of freedom about the backbone bonds adjacent to the phosphodiester linkage (see Figure 1). Thus, several aspects of nucleic acid conformation are amenable to investigation at the dinucleotide level that are not present at the monomer level: examples of this include the conformation of the phosphodiester linkage, the mutual orientations of the bases in stacked and unstacked conformations, and the changes in backbone conformation which accompany temperature or denaturant-induced disordering or unstacking of naturally occurring nucleic acids.

^{1}H NMR spectroscopy has provided many details of the solution conformation of adenine dinucleotides (4-7), such as the geometry of the base-base stacking interaction, the prevalent conformation about the glycosyl linkage, and some details of the phosphodiester backbone conformation. These studies and optical activity investigations (8) have suggested that in aqueous solution the naturally occurring dinucleoside monophosphates such as A-A adopt an anti-anti partially-stacked, right handed, incipient helical conformation. Circular di-

chroism studies ($\underline{9}$) suggest that A-A(3',5') is essentially unstacked at 81° C in water or at room temperature in the presence of 4 M NaClO$_4$. ^{1}H NMR studies, however, reveal residual stacking at 95° C for A-A(3',5') ($\underline{4}$).

Assignments. Figure 2 shows a typical ^{13}C NMR spectrum as well as the structure and numbering scheme for the dinucleoside monophosphate A-A(3',5'). The subscripts 1 and 2 refer to nuclei of the 3'- and 5'-adenosinyl moieties, respectively. The observed chemical shift values are presented in Table I.

TABLE I. ^{13}C Chemical shift data (in ppm) for adenosine dinucleoside monophosphates[a]

Compound		C(4)[b]	C(2)[b]	C(6)[b]	C(5)[b]	C(8)[b]	C(1')	C(4')	C(2')	C(3')	C(5')
A-A	3'	149.1	153.5	155.9	119.5	140.9	90.1	84.9	74.8	74.1	61.8
(27°)	5'	148.5	153.0		119.1	140.0	88.6	83.9	75.3	70.6	65.7
A-A	3'	149.4	153.8	156.1	119.7	141.3	90.0	85.3	75.3	74.3	62.3
(37°)	5'	148.9	153.2		119.3	140.3	88.7	84.2	75.4	71.0	66.2
A-A	3'	149.9	154.1	156.6	120.2	141.6	90.1	85.5	c	74.5	62.4
(56°)	5'	149.4	153.7		119.8	140.7	89.0	84.5	75.5	71.2	66.2
A-A	3'	150.5	154.5	157.1	d	d	90.2	85.9	c	74.8	62.9
(81°)	5'	150.0	154.2		d	d	89.3	84.9	75.7	71.6	66.7
A-A[e]	3'	149.5	153.7	156.2	120.2	d	90.1	85.2	c	74.2	62.3
(27°)	5'	148.9	153.2		119.7	d	89.0	83.8	75.3	70.6	66.2
A-A[e]	3'	150.7	153.7	157.4	121.2	d	90.3	86.1	76.2	75.0	62.3
(81°)	5'	150.4	154.7		120.7	d	89.9	84.8	75.8	71.7	67.1
A-A	2'	149.2	153.9	156.4	119.0	141.7	88.8	86.7	78.3	71.6	62.5
(37°)	5'	149.1	153.3	156.2		139.9	88.5	83.5	76.0	70.5	65.3
A-A[f]	2'	150.3	154.7	157.1	d	141.0	89.8	87.9	79.6	72.8	63.7
(80°)	5'		154.2		d		90.0	84.7	77.2	71.7	66.4

[a]Relative to external Me$_4$Si, pD = 7.2 ± 0.2.

[b]Indicates assignment to 3'(2') or 5' moiety of dimer not possible.

[c]Values not obtainable due to overlap of resonances.

[d]Not observable due to long relaxation times.

[e]Solvent is 4 M NaClO$_4$.

[f]Data subject to susceptibility correction of $\underline{ca}$ + .14 ppm.

Assignment of the ribofuranosyl resonances to the 3'-(2'-) or 5'-moieties was made by consideration of the influence of an attached phosphate group ($\underline{10}$-$\underline{12}$): (a) the resonance of the directly-attached carbon increases in chemical shift by $\underline{ca}$.2.5 ppm; (b) the resonances of neighboring carbon atoms are unaffected; (c) the phosphorylated carbon resonance is spin-coupled to the ^{31}P atom of the phosphate by 5.0 ± 1 Hz; (d) the resonances of carbons separated by three bonds are spin-coupled to ^{31}P by 0-10 Hz, depending upon the torsion angles about the C(3')-O (C(2')-O)and C(5')-O bonds. The two C(1')

resonances, which show no spin couplings to ^{31}P (except for
A-A(2',5')), and the two C(4') resonances are assigned by com-
parison with the data for adenosine-3'- and -5'-phosphate (1).
No assignment of the base carbon resonances to the 3'-(2'-) or
5'-moieties can be made without reference to compounds in
which the carbon atoms of only one base have been replaced by
^{13}C. The differences between the base carbon chemical shifts
are indicative of base-base interaction (1-3)
Base-Base Stacking. Two resonances are evident for each of
the base carbons of the dinucleoside monophosphates with the
exception of single resonances for C(6) in A-A(3',5') and
C(5) in A-A(2',5'). Increasing temperature or high concen-
trations of the denaturant NaClO$_4$ have only small effects on
the <u>difference</u> in chemical shift values (Table II) for a par-

TABLE II

Difference between base carbon chemical shifts (in ppm) for
adenosine dinucleoside monophosphates[a]

A-A	(°C)	C(4)	C(2)	C(6)	C(5)	C(8)
3',5'	27°	0.6	0.5	0	0.4	0.9
3',5'	56°	0.5	0.4	0	0.4	0.9
3',5'	81°	0.5	0.3	0	b	b
3',5'	27°c	0.6	0.5	0	0.5	b
3',5'	81°c	0.3	0	0	0.5	b
2',5'	37°	0.1	0.6	0.2	0	1.8
2',5'	80°	0	0.5	0	b	0

[a] $|\delta_{A^-} - \delta_{^-A}|$. Spectral resolution = 0.1 ppm.

[b] Not measureable due to long relaxation time.

[c] Solvent is 4 M NaClO$_4$ in D$_2$O.

ticular base carbon in the 5'- and 3'-moieties. However,
each of the base resonances moves downfield approximately 1.0
to 1.5 ppm for a temperature increase of 54°. A portion of
this (<u>ca</u>. 0.1 ppm) is due to the temperature variation of the
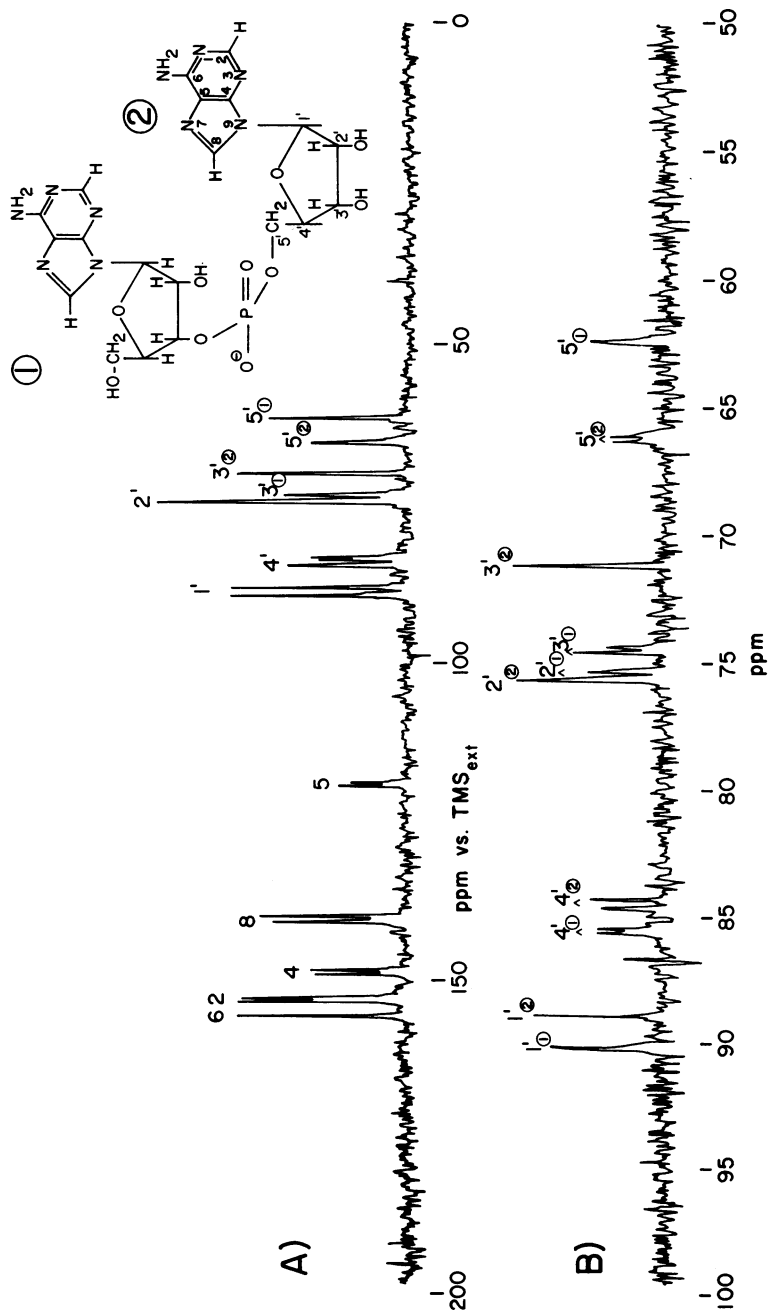susceptibility correction for the external reference (13-14).

Figure 2. (A) 25 MHz ^{13}C NMR spectrum of A-A(3',5'); (B) Detail of the ribose region of spectrum A.

The ribofuranose resonances sustain a 0.1 to 1.0 ppm downfield change for the same temperature increase. Concentration effects on the ^{13}C chemical shifts of A-A were not observed in the concentration range 0.04 to 0.1 M, demonstrating that the effects of intermolecular association are minimal. In contrast, ^{1}H NMR studies show the occurrence of intermolecular interaction for A-A(3',5') at concentrations less than 0.1 M (4).

It is well documented that in the presence of an external magnetic field a diamagnetic circulation of π-electrons is induced in conjugated systems (15-17). This circulation creates a small magnetic field which can shield or deshield nearby nuclei. Detailed calculations on the sign and magnitude of this effect have been made (16-17). The ring current phenomenon has been observed in the ^{1}H NMR spectra of mononucleotides at high concentration (5) and dinucleoside monophosphates (4). Treating this phenomenon as a simple induced magnetic field effect, one would expect similar variations in the chemical shifts of ^{13}C nuclei. This has in fact been observed in simple systems (18). The stacking of two aromatic moieties introduces an additional complication in the interpretation of ^{13}C NMR spectra because ^{13}C chemical shifts are known to have a much stronger dependence on the nature of the molecular wave function than protons (19). No estimate of the contribution from this effect to ^{13}C chemical shifts of base carbons in a stack has yet been made. A further complication is that solvent molecules may be excluded from the interbase region on formation of stacks. Thus, the changes in ^{13}C chemical shift occurring on base stacking cannot at present be interpreted with any certainty.

Previous studies of the concentration dependence of the ^{13}C NMR spectra of 5'-AMP (1-3) and purine (14) have demonstrated significant changes (0.5 to 1.4 ppm) in chemical shift under conditions where base stacking is indicated by ^{1}H NMR data. One would therefore expect differences in ^{13}C chemical shifts for corresponding nuclei of the 3'-(2'-) and 5'-moieties of base stacked dinucleoside monophosphates, and that these differences should diminish greatly upon destacking.

This is corroborated by the differences found in Table II for
A-A(3',5') and A-A(2',5') and the absence of such differences
in the ^{13}C data for U-U(3',5'), whose uracil moieties do not
sustain a ring current ($\underline{1}$).

The differences in base carbon chemical shifts for corres-
ponding carbon atoms im the 3'-(2'-) and 5'-moieties are inde-
pendent of any corrections for temperature dependence of the
D_2O chemical shift or magnetic susceptibility. Even at 81° C
no significant change in the difference occurs for A-A(3',5'),
suggesting a cancellation of opposing effects. However, for
A-A(2',5') a change of 1.8 ppm in the difference between the
C(8) chemical shifts occurs upon a temperature increase of 43°.
Clearly the stacking patterns of A-A(3',5') and A-A(2',5') are
not identical. This conclusion is in accord with the results
of ^{1}H NMR studies ($\underline{4}$-$\underline{5}$). Interpretation of the chemical shift
data in precise conformational terms must await a better under-
standing of the various influences on the ^{13}C chemical shift.
Backbone Conformation. Various physical studies ($\underline{9}$,$\underline{20}$) and
theoretical predictions ($\underline{21}$-$\underline{23}$) reveal that the properties and
position of the phosphodiester backbone of dinucleoside mono-
phosphates are of crucial importance in defining the overall
conformation of these molecules. To study the conformational
characteristics of the phosphodiester backbone in the presence
and absence of denaturing conditions,the ribose region of the
^{13}C NMR spectra of A-A(3',5') and A-A(2',5') was analyzed to
obtain ^{13}C-^{31}P coupling constants.

The ^{13}C NMR spectra of mono- and dinucleotides under con-
ditions of complete proton decoupling show several resonances
which are split due to ^{13}C-^{31}P spin-spin coupling (see Figure
2B). From measurements on conformationally rigid 3',5'-cyclic
nucleotides, and by analogy to the relationship between tor-
sion angle and the proton-proton coupling, Lapper et al. ($\underline{24}$)
have proposed the relationship

$$^3J_{C-P} = A\cos^2\gamma \qquad (1)$$

to relate the carbon-phosphorus coupling constant to γ, the
^{13}CCO^{31}P torsion angle. The constant A equals 8 Hz for the

3',5'-cyclic nucleotides corresponding to the maximum value
observed for a <u>trans</u> arrangement (γ = 180°). It is quite
likely that the value of A will vary slightly with the nature
of the compound, as has been found in the analogous relation-
ship for ^1H-^1H couplings (25). The ^{13}C-^{31}P coupling data in
Table III indicate a maximum observed value of 10 Hz for three
bond couplings in the dinucleoside monophosphates. While this
is not necessarily the maximum possible coupling, it represents
the best estimate of A for this type of compound. The
^{31}P-^{13}C couplings across two bonds lie within the expected
range of 5.0 $\pm$ 1.0 Hz.

The three bond ^{13}C-^{31}P couplings may be interpreted in
terms of three models for the conformations about the C(3')-O
(or C(2')-O) and C(5')-O bonds. In a dynamic model one could
consider that a rapid interconversion between classical
staggered conformers (Figure 3) occurs, and that the time
averaged couplings represent the relative populations of the
three allowed conformers (12). Alternatively an equilibrium
between two of the three possible classical staggered con-
formers may be assumed, and finally one could consider the
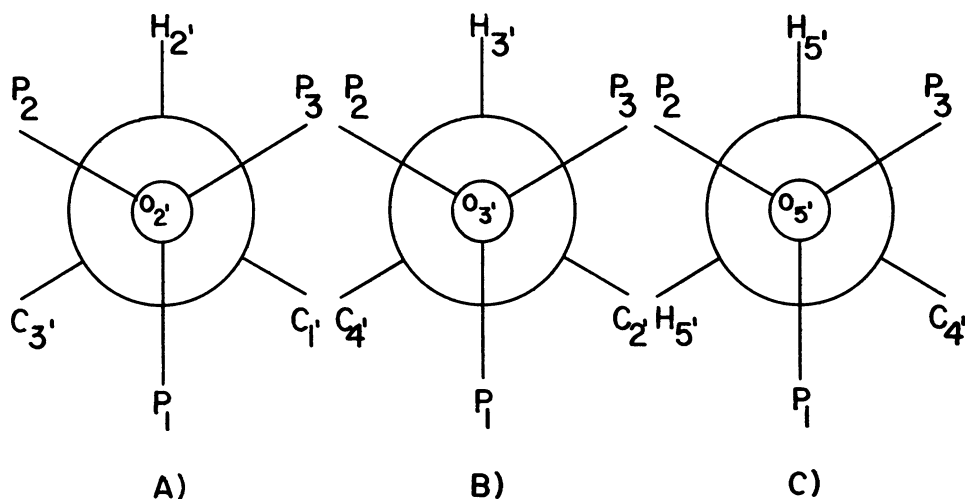possibility of a small range of torsion angles centered around

Figure 3. Classical 60°-staggered rotamer conformations
of the phosphodiester backbone of dinucleoside monophosphates.
(A) 2'-phosphate; (B) 3'-phosphage; (C) 5'-phosphate.

some non-classical torsion angle (other than 60, 180, or 270°)
An additional complication is the possible rapid equilibrium
between stacked and unstacked forms whose relative populations
can vary with conditions of solvent and temperature.

In the dynamic models one formulates expressions for the
^{13}C-^{31}P coupling constants in terms of the populations of the
accessible rotamers and the coupling constants expected for the
conventional gauche (J_g) and trans (J_t) orientations. A first
possibility is to assume rapid interconversion between three
possible rotamers P_1, P_2, and P_3 (see Figure 3B):

$$^3J_{31_{P}-13_{C(2')}} = P_1J_g + P_2J_t + P_3J_g \qquad (2)$$

$$^3J_{31_{P}-13_{C(4')}} = P_1J_g + P_2J_g + P_3J_t \qquad (3)$$

$$1 = P_1 + P_2 + P_3 \qquad (4)$$

Analogous expressions may be derived if one assumes that only
two rotamers, P_2 and P_3, are allowed (P_1 could possibly be
neglected on the basis of X-ray and theoretical studies (7)).
Only one coupling related to rotamers about C(5')-0 is ob-
servable, and hence it is only possible to estimate the sum of
the population of gauche conformers (see Figure 3C):

$$^3J_{31_{P}-13_{C(4')}} = (P_1 + P_3)J_g + P_2J_t \qquad (5)$$

A set of equations analogous to (2)-(4) can be written for
couplings to C(1') and C(3') in A-A(2',5').

It should be emphasized that the dimers do not necessarily
exist only in the stacked form, and that a rapid interconver-
sion between stacked and unstacked forms takes place. Thus,
the derived conformers are the time averages of the stacked
and unstacked forms.

The phosphorus-carbon coupling constants for the com-

pounds studied are presented in Table III. These values were derived from spectra such as shown in Figure 2B. The predicted values for the <u>gauche</u> and <u>trans</u> rotamers are obtained from equation (1): J_g = 2.5 Hz and J_t = 10 Hz. These values plus equations (2)-(5) (and analogous expressions) permit rotamer populations to be <u>estimated</u>; the results are presented in Table IV.

TABLE III. Phosphorus-carbon coupling constants (in Hz) for adenosine mono- and di-nucleosides[a]

Compound	3'(2')Residue			5'Residue		
	C(1')[b]	C(2')[c]	C(3')[b]	C(4')[c]	C(4')[d]	C(5')
3'-AMP	--	2.8	4.3	4.5	--	--
5'-AMP	--	--	--	--	8.6	4.0
A-A(3',5')	--	4.9	4.9	4.9	8.6	4.9
A-A(3',5') (56°C)	--	e	4.9	4.9	8.5	4.9
A-A(3',5') (81°C)	--	e	4.9	4.9	8.5	4.9
A-A(3',5')[f]	--	e	4.9	3.7	8.6	4.9
A-A(3',5')[f] (81°C)	--	4.9	4.9	4.9	8.5	4.9
A-A(2',5') (37°C	1.0	5.3	3.9	--	10.0	5.0
A-A(2',5') (80°C)	6.5	5.3	2.4	--	8.7	4.9

[a] Spectral resolution = 0.5 Hz and temperature = 28°C unless otherwise indicated.

[b] Conformation-sensitive coupling for 2',5'-dimer.

[c] Conformation-sensitive coupling for 3',5'-dimer.

[d] Conformation-sensitive coupling for both 2',5'- and 3',5' dimers.

[e] Values not available due to overlap of resonances.

[f] Solvent is 4 M NaClO$_4$ in D$_2$O.

TABLE IV. Phosphate rotamer populations in adenosine mono- and di-nucleosides[a]

Compound		3'(2') Residue			5' Residue	
		P_1	P_2	P_3	P_t	P_g
3'-AMP	(28°C)	0.7	0	0.3	--	--
5'-AMP	(28°C)	--	--	--	0.8	0.2
A-A(3',5')	(28°C)	0.4	0.3	0.3	0.8	0
A-A(3',5')	(81°C)[b]	0.4	0.3	0.3	0.8	0.2
A-A(2',5')	(37°C)	1.0	-0.2	0.2	1.0	0
A-A(2',5')	(80°C)	0.5	0.5	0	0.8	0.2

[a] Solution concentration 0.1 M, pD = 7.2. Refer to Figure 3 for the designations of the rotamer populations.

[b] Solvent is 4 M $NaClO_4$ in D_2O.

It is clear from Table III that the preference for rotamers about C(3')-O are very different for 3'-AMP and the 3'-residue of A-A(3',5') regardless of the model assumed. Application of the three state dynamic model to 3'-AMP leads to a strong preference for rotamer 1 (Table IV). For A-A(3',5') each of the allowed rotameric states is populated equally, whereas rotamer 2 in 3'-AMP is virtually unpopulated. It is interesting to note that rotamer 1, the most prevalent rotamer in 3'-AMP, is the one which is predicted (on the basis of conformational energy calculations (26) and X-ray studies (27)) not to be readily accessible. No detectable temperature effects were noted for A-A(3',5'), implying no significant change in the rotamer populations about the OC bonds. Considering the model in which only rotameric states 2 and 3 about C(3')-O are allowed, a two-fold preference for rotamer 3 over rotamer 2 is derived for 3'-AMP; for A-A(3',5') both rotamers 2 and 3 are equally probable. The fixed angle model which is based on a small range of torsion angles centered about a non-classical torsion angle cannot be excluded for 3'-AMP, but is clearly not

appropriate for A-A(3',5') because of the observed equal couplings to C(2') and C(4'). For 3'-AMP, assuming the fixed angle model, two rotamers are possible: (a) $\phi' = 175^O$ - this rotamer is very similar to rotamer 1; (b) $\phi' = 355^O$ - this rotamer can most likely be excluded because of its eclipsed position. (Rotamer angles calculated from $^3J_{31_{P-C(2')}}$ and $^3J_{31_{P-C(4')}}$ differ by 14°.)

Thus, irrespective of the model applied, it can be concluded that rotamer 2 is highly unfavored for 3'-AMP, but allowed for A-A(3',5').

Considering the rotamers about the O-C(5') bond, regardless of the model used, a strong preference for the C(4')-<u>trans</u> rotamer (rotamer 2 in Figure 3C) is maintained for 5'-AMP, A-A(3',5'), and A-A(2',5'). Increased temperature or 4 M NaClO$_4$ does little to alter this preference except for A-A(2',5') where a decrease in the population of this rotamer is observed at 80^OC.

For A-A(2',5') at 37° we observe a value of 1.0 Hz for $^3J_{31_{P-C(1')}}$. No averaging of J_g and J_t can give this value, consequently we propose that the dynamic model is not appropriate for A-A(2',5') at 37^OC. This is demonstrated by the calculations of negative rotamer populations if the dynamic model is applied to the observed coupling constants. From the fixed angle model we calculate two possible rotameric angles: (a) 169° from H(2') - (a counterclockwise rotation when viewed from O(2') to C(2')); (b) 349° from H(2') - (counterclockwise rotation). The latter rotamer is excluded because of eclipsing with H(2'). (Rotamer angles calculated from $^3J_{31_{P-C(3')}}$ and $^3J_{31_{P-C(1')}}$ differ by only 3°.) Rotamer (a) which is similar to P$_1$ predominates at 37^OC. However, when the temperature is increased to 80^OC a large change in conformation about the O-C(2') bond occurs. The fixed angle assumption is no longer appropriate as no fixed angle can yield the observed couplings to C(1') and C(3'). Applying the dynamic model reveals that rotamers P$_1$ and P$_2$ are about equally preferred, whereas P$_3$ is highly unfavored. It should

be noted that the conformationally insensitive coupling con-
stants $^2J_{P-C(3')}$ and $^2J_{P-C(5')}$ do not change when A-A(3',5')
is perturbed by either temperature or denaturing additive (and
analogously for A-A(2',5')).

The ^{13}C data must be considered in light of the $^{31}P-^1H$
data recently published (6-7) for A-A(3',5'). Similarities
and differences in interpretation are apparent. The most
significant difference between our work and that previously
published occurs for 3'-AMP. Regardless of whether we assume
a dynamic model incorporating two or three allowed rotameric
states we do not observe an equivalence of P_2 and P_3 rotamers;
in fact, if the three state dynamic model is assumed we ob-
tain no P_2 rotamer ($P_2 + P_3$ = 0.3 - this work, <u>versus</u>
$P_2 + P_3$ = 0.7 (7)). In this case the ^{13}C data are more
reliable since they involve two three-bond couplings rather
than the one $^{31}P-^1H(3')$ coupling present in the ^1H spectra.
We do not observe a temperature dependence within experimental
error for the backbone C-P coupling constants; such a depen-
dence has been observed (relatively small) for P-H coupling
constants. This difference is not too surprising in view of
the approximately 2.5 times greater sensitivity of P-H coup-
ling constants to variations in torsion angle. For A-A(3',5')
Sarma and coworkers (6-7) concluded that rotamer 3 predomi-
nates, while we observe equal rotamer populations, yet the
total <u>gauche</u> rotamer populations (0.6 <u>versus</u> 0.7) are in
good agreement. Both ^{13}C and ^1H (6-7) studies are in ex-
cellent accord on the distribution of rotamers about C(5')-0
for the compounds 5'-AMP and A-A(3',5').

The results of this study suggest that minimal rotation
occurs about the backbone torsion angles ϕ and ϕ', of
A-A(3',5') upon additive or temperature induced unstacking.
Analogous ^1H NMR studies (6-7) reveal only small changes in
ϕ, ϕ', and ψ with temperature. Dreiding models of
A-A(3',5') demonstrate that unstacking can occur with ϕ, ϕ',
and ψ held constant by rotations about the 0(3')-P (ω) and
P-0(5') (ω') bonds. By contrast, temperature-mediated un-
stacking of A-A(2',5') must involve rotations about the tor-
sion angle ϕ and the C(2')-0(2') bond. Information about the

torsion angles ω and ω' cannot be obtained by ^{1}H, ^{13}C, or ^{31}P NMR coupling constant investigations; other techniques would have to be employed.

The conformational preferences deduced for the backbone of adenine dinucleotides are compatible with the features of dinucleotide conformation elucidated in previous studies (4-9, 12). Furthermore, the dynamic interpretation would imply that even under favorable conditions for base stacking in A-A(3',5'), the molecule is at least 33% unstacked in the temperature range 25 to 80°C since Dreiding models suggest that base stacking cannot occur for this dinucleotide when the backbone P_2 rotamer about the C(3')-0 bond is present. This is in accord with the observation that extremely low temperatures are necessary to maximize base stacking in dinucleotides (28). Since right-hand to left-hand helical transformations in dinucleoside monophosphates involve primarily rotations about ω and ω' these studies, like others, have not been able to examine this conformational aspect.

ACKNOWLEDGMENTS

**

Division of Biological Sciences, National Research Council, Ottawa, Ontario, Canada K1A OR6

+Present address: International Atomic Energy Agency, P.O. Box 590, A-1011, Vienna, Austria.

REFERENCES
1 Smith, I.C.P., Mantsch, H. H., Lapper, R. D., Deslauriers, R. and Schleich, T. (1973) in "Conformation of Biological Molecules and Polymers", (Proceedings of the Fifth Jerusalem Symposium), Academic Press, New York, pp. 381-401
2 Schleich, T., Cross, B. P., Blackburn, B. J. and Smith, I.C.P. (1975) in "Proceedings of the Fourth Harry Steenbock Symposium", University Press, College Park, Md. (in press)

3 Smith, I.C.P., Jennings, H. J. and Deslauriers, R. (1975)
   Accounts of Chem. Res. 8, 306-313
4 Chan, S. I. and Nelson, J. H. (1969) J. Amer. Chem. Soc.
   91, 168-183
5 Ts'o, P.O.P., Kondo, N. S., Schweizer, M. P., and Hollis,
   D. P. (1969) Biochemistry 8, 997-1029
6 Evans, F. E., Lee, C. and Sarma, R. H. (1975) Biochem.
   Biophys. Res. Commun. 63, 106-114

7 Lee, C., Evans, F. E. and Sarma, R. H. (1975) FEBS Letters
   51, 73-79
8 Bush, C. A. and Tinoco, I., Jr. (1967) J. Mol. Biol. 23,
   601-614
9 Johnson, N. P. and Schleich, T. (1974) Biochemistry 13,
   981-987
10 .' nes, A. J., Winkley, M. W., Grant, D. M. and Robins,
   k. K. (1970) Proc. Natl. Acad. Sci. U.S.A. 65, 27-30
11 Dorman, D. E. and Roberts, J. D. (1970) Proc. Natl. Acad.
   Sci. U.S.A. 65, 19-26
12 Mantsch, H. H. and Smith, I.C.P. (1972) Biochem. Biophys.
   Res. Comm. 46, 808-815
13 Blackburn, B. J., Hruska, F. E. and Smith, I.C.P. (1969)
   Can. J. Chem. 47, 4491-4493
14 Cross, B. P. (1974) Ph.D. Thesis, University of California,
   Santa Cruz
15 Johnson, C. E. and Bovey, F. A. (1958) J. Chem. Phys. 29,
   1012-1014
16 Giessner-Prettre, C. and Pullman, B. (1970) J. Theor.
   Biol. 27, 87-95
17 Giessner-Prettre, C. and Pullman, B. (1970) J. Theor.
   Biol. 27, 341-342
18 Du Vernet, R. and Boekelheide, V. (1974) Proc. Natl. Acad.
   Sci. U.S.A. 71, 2961-2964
19 Ditchfield, R. and Ellis, P. D. (1974) in "Topics in [13]C
   NMR Spectroscopy", Vol. 1, ed. G. C. Levy, pp. 1-46
20 Kondo, N. S., Holmes, H. M., Stempel, L. M. and Ts'o,
   P.O.P. (1970) Biochemistry 9, 3479-3498
21 Yathindra, N. and Sundaralingam, M. (1973) Biopolymers 12,
   297-314
22 Tewari, R., Nanda, R. K. and Govil, G. (1974) J. Theor.
   Biol. 46, 229-239
23 Perahia, D., Pullman, B. and Saran, A. (1974) Biochim.
   Biophys. Acta 340, 299-313
24 Lapper, R. D., Mantsch, H. H. and Smith, I.C.P. (1973)
   J. Amer. Chem. Soc. 95, 2878-2880
25 Sternhill, S. (1969) Quart. Rev. (London) 23, 236-270
26 Pullman, B., Perahia, D. and Saran, A. (1972) Biochim.
   Biophys. Acta 269, 1-14
27 Sundaralingam, M. (1968) Biopolymers 6, 189-213
28 Davis, R. C. and Tinoco, I., Jr. (1968) Biopolymers 6,
   223-242