300 MHz NMR STUDY ON THE EFFECT OF BASE STACKING ON BACKBONE CONFORMATIONAL FLEXIBILITY IN OXY- AND DEOXY- ADENYL DINUCLEOSIDES

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SUMMARY: The 300 MHz ^{1}H and 40.48 MHz ^{31}P NMR spectra of ApA, dApdA, and several purine nucleotides in aqueous solution have been completely analyzed at various temperatures or concentrations, and the derived coupling constant data interpreted in terms of dynamic conformational parameters by means of a Karplus-type analysis. It is found that base stacking interactions play a key role in reducing conformational flexibility. The C(4')-C(5') and C(5')-O(5') bonds in both oxy- and deoxy- adenyl dinucleosides become increasingly *gauche* conformation with increasing base stacking, while the ribose ring in ApA correspondingly becomes more ^{3}E conformation and the deoxyribose ring in dApdA remains mostly ^{2}E conformation.

INTRODUCTION

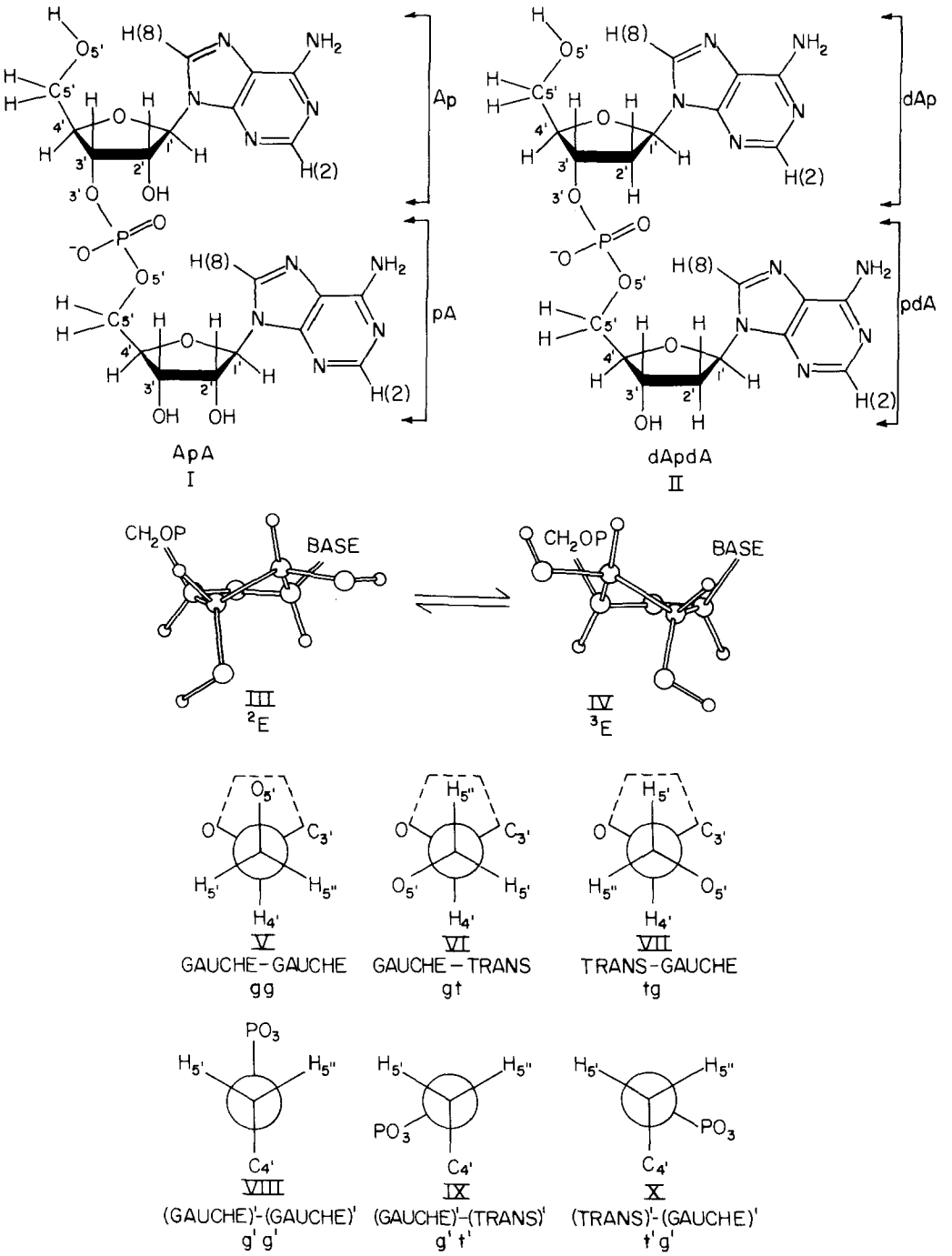
Base stacking interactions have been reported to decrease $J_{1'2'}$ in ApA(I) (1-3) and in 5'AMP (4,5). However no such effect is observed in 5'dAMP and dApdA(II) (3). The availability of 300 MHz ^{1}H and 40.5 MHz ^{31}P NMR systems in the FT mode, in combination with computer simulation, has enabled us to completely analyze the spectra of ApA(I) and dApdA(II) in aqueous solution at several temperatures, and the results have been compared to the effect of concentration on the mononucleotides. A role for base stacking on the conformational dynamics of the backbone is indicated.

EXPERIMENTAL METHODS

FT or CW spectra in the ^{1}H configurations were obtained at 100 or 300 MHz and ^{31}P spectra at 40.48 MHz. Details of instrumentation and sample preparation are presented elsewhere (6,7). The assignments of the protons were unequivocally determined from the coupling pattern and computer simulation. The extracted coupling constants are interpreted by means of a Karplus-type analysis in terms of energy minimum conformations about the relevant bonds (III-X) according to standard procedures as have been developed elsewhere (7-15).

RESULTS AND DISCUSSION

Effect of concentration on the dynamic backbone conformation of several purine ribonucleotides. We have previously reported (13) that in 5'AMP, increases in concentration, commencing at 0.05M, cause $J_{1'2'}$ to decrease and $J_{3'4'}$ to correspondingly increase, while $J_{2'3'}$ and the sum $J_{1'2'} + J_{3'4'}$ remain essentially


Ap
pA
ApA
I
dAp
pdA
dApdA
II
CH2OP
BASE
III
2E
IV
3E
V
GAUCHE-GAUCHE
gg
VI
GAUCHE-TRANS
gt
VII
TRANS-GAUCHE
tg
VIII
(GAUCHE)'-(GAUCHE)'
g' g'
IX
(GAUCHE)'-(TRANS)'
g' t'
X
(TRANS)'-(GAUCHE)'
t' g'

Scheme 1

constant. This indicated a loss in ^{2}E(III) ribose ring populations, and a concomitant gain in the ^{3}E(IV) conformer populations. The nucleotides 5'AMP, 5'GMP and 5'IMP at low concentration have similar coupling constants (14), and we have since observed that the coupling constant dependence on concentration in 5'GMP and 5'IMP closely resembles that reported in 5'AMP (see Figure 2 in reference 13). Unusual line broadening of the H(4'), H(5') and H(5'') resonances at the higher concentrations prevents precise determination of the ^{1}H exocyclic couplings, but it is found that the ^{31}P spectra are less broad, and a first order comparison of the ^{31}P spectra at 0.05M and 1.0M, in all three nucleotides, suggests that the coupling constant sum $\Sigma' = J_{5'p} + J_{5''p}$ is less at high concentration, implicating conformational change. Thus, the conformational dynamics of 5'AMP, 5'GMP and 5'IMP are surprisingly similar.

Effect of temperature on the dynamic backbone conformation of ApA. The data on ApA(I, Table I) show a significant temperature dependence of all the coupling constants. It is clear from the magnitude of the couplings (7, 11-14) that sizeable populations of both ^{2}E(III) and ^{3}E(IV) conformer exist.[1] The $J_{1'2'}$ coupling in both the Ap and pA fragments (I), decreases by approximately 1 Hz, while $J_{3'4'}$ increases by 1 Hz, and $J_{2'3'}$ and the sum $J_{1'2'} + J_{3'4'}$ remain essentially constant (Table 1). This not only shows a net increase in ^{3}E population, but the specific behavior of these coupling constants suggests that the dihedral angles in the 'pure' ^{2}E(III) and 'pure' ^{3}E(IV) conformers have not significantly changed, but rather only the position of the equilibrium is different (12-14). The practical consequence of this is that *changes* in ribose ring population may be computed with less error than the actual populations (14, 15). Thus the coupling constant changes indicate $\simeq$ 10% increase in ^{3}E(IV) conformer populations as the temperature is decreased from 72° to 12°. Hruska and Danyluk (1) have previously reported that $J_{1'2'}$ of the mononucleotides 5'AMP and 3'AMP is not temperature dependent in this temperature range. Fang et al. (3) have reported that $J_{1'2'}$ is 5.5 and 5.2 Hz in 5'AMP and ApA when dissolved in the "destacking agent" DMSO. The change in ribose ring conformation obviously depends on base stacking interactions (1-5).

The dynamic conformer distribution about the exocyclic C(4')-C(5') bond (V-VII) may be determined from the coupling constant sum $\Sigma = J_{4'5'} + J_{4'5''}$

[1] In aqueous solution the *ribose* pucker is generally treated qualitatively, and may be considered to include sizeable $^{3}_{2}T$, ^{3}E, $^{3}_{4}T$ as well as $^{2}_{3}T$, ^{2}E and $^{2}_{1}T$ conformations (12).

TABLE I

Coupling Constants for ApA, 3'AMP, 5'AMP, dApdA, d3'AMP, d5'AMP[a]

Comp. →	Ap of ApA				pA of ApA				3'AMP	5'AMP	dAp of dApdA			pdA of dApdA			3'dAMP	5'dAMP
Temp. →	12	27	40	72	12	27	40	72	27	27	27	40	72	27	40	72	27	27
J in Hz																		
H(1')-H(2')	3.8[b]	4.2[b]	4.4[b]	4.8[b]	4.0[b]	4.3[b]	4.4[b]	4.6[b]	6.2	5.7	8.7[c]	8.5[c]	8.7[c]	7.0[c]	6.8[c]	6.9[c]	8.0	7.3
H(1')-H(2'')	--	--	--	--	--	--	--	--	--	--	5.5[c]	5.7[c]	6.0[c]	6.7[c]	6.7[c]	7.1[c]	6.2	6.3
H(2')-H(2'')	--	--	--	--	--	--	--	--	--	--	-14.0	-14.0	-14.6	-13.9	-14.1	-14.6	-14.2	-14.1
H(2')-H(3')	(5.2)	(5.2)	5.2	5.2	4.8	5.1	5.1	5.1	5.2	5.2	5.6	5.6	6.2	6.6	6.8	7.0	5.9	6.3
H(2')-P(3')	--	--	--	--	--	--	--	--	1.1	--	--	--	--	--	--	--	0.8	--
H(2'')-H(3')	--	--	--	--	--	--	--	--	--	--	2.3	2.2	2.5	4.1	4.1	4.1	2.9	3.4
H(3')-H(4')	5.4	5.0	4.8	4.4	5.2	4.9	4.8	4.6	2.9	3.7	2.4	2.2	2.2	4.0	4.0	4.0	2.5	3.0
H(3')-P(3')	(7.6)	(8.0)	(8.4)	(8.8)	--	--	--	--	7.9	--	5.6	5.8	6.2	--	--	--	7.4	--
H(4')-H(5')	2.2	2.3	2.4	2.6	(2.5)	(2.5)	2.8	2.8	2.4	3.1	3.3	3.1	3.4	2.5	2.5	2.5	3.1	3.8
H(4')-H(5'')	3.1	3.8	3.7	4.0	2.5	3.5	3.6	4.2	3.3	3.1	3.3	4.1	4.6	2.8	3.0	3.8	3.7	3.8
Σ[d]	5.3	6.1	6.1	6.6	5.0	6.0	6.4	7.0	5.7	6.2	6.6	7.2	8.0	5.3	5.5	6.3	6.8	7.6
H(4')-P(5')	--	--	--	--	2.2	2.0	1.8	1.8	--	1.7	--	--	--	2.5	2.5	2.5	--	1.7
H(5')-P(5')	--	--	--	--	(3.7)	(4.3)	4.5	4.9	--	5.0	--	--	--	3.8	3.8	4.6	--	5.2
H(5'')-P(5')	--	--	--	--	3.7	4.3	4.5	4.9	--	5.0	--	--	--	3.6	3.6	4.6	--	5.0
Σ'[e]	--	--	--	--	7.4	8.6	9.0	9.8	--	10.0	--	--	--	7.4	7.4	9.2	--	10.2
H(5')-H(5'')	-13.0	-12.8	-12.8	-12.8	-11.4	-11.6	-11.7	-11.6	-12.7	-12.0	-12.0	-12.0	-13.3	-12.0	-12.0	-12.6	-13.1	-13.0

[a] Concentrations are 0.05M, pD is 7.4 for ApA and dApdA, and 5.4 for each mononucleotide. The coupling constant error is estimated as ±0.2 Hz, except for the values in parentheses which are less accurate.

[b] The temperature dependence of $J_{H(1')-H(2')}$ shows the same trend as that previously reported (1), except the magnitude of the couplings are different. The original report (1) was a first order analysis of a complex 60 or 100 MHz spectra. Our data has been duplicated with two samples in both CW and FT modes at 300 MHz and is computer fit.

[c] Similar $J_{H(1')-H(2')}$ and $J_{H(1')-H(2'')}$ values obtained elsewhere by first order analysis (3) at 220 MHz.

[d] $\Sigma = J_{H(4')-H(5')} + J_{H(4')-H(5'')}$. [e] $\Sigma' = J_{H(5')-P(5')} + J_{H(5'')-P(5')}$.

(8-10), and an examination of the Σ values (Table 1), interpreted as described elsewhere (8-10), demonstrates that the *gauche-gauche (gg,*V) conformer is preferred in both the Ap and pA components of ApA at all temperatures as in the 3' and 5' mononucleotides. There is a decrease in Σ of 1.3 Hz in the Ap fragment, and 2.0 Hz in the pA fragment (Table 1). Strict usage of the model system (V-VII) translates the coupling constant change as presented in Table 1, to mean a 13-20% increase in the *gg*(V) population at the expense of the *gauche trans (gt, tg,* VI, VII) populations in going from 72° to 12°. This corresponds to the 10% increase in ^{3}E(IV) ribose ring population discussed earlier. It has been reported that the exocyclic linkage in uridine is not temperature dependent (16), and so it is unlikely that we are observing a temperature artifact here. Since the dinucleoside is at a concentration of 0.05M, it is possible that the intramolecular base stacking effect is slightly affected by intermolecular base stacking interactions, but any such effect should be small since no intermolecular effect on Σ in 5'AMP could be detected at concentrations up to 0.1M at 30°(13). The ± 0.2 Hz uncertainty in the magnitude of Σ is the main source of error. A comparison of Σ in 5'AMP and 3'AMP shows a difference of 0.5 Hz (Table 1). Further, the values of 5'AMP and 3'AMP, with the phosphate as a monoanion, show Σ to be intermediate to the Σ range found in the corresponding Ap and pA fragments of ApA in the examined temperature range. Clearly, the formation of the phophodiester linkage connecting the nucleoside pair also has an influence on the conformation about the exocyclic bond.

The dynamic conformer distribution about the exocyclic C(5')-O(5') bond (VIII-X) may be monitored from the coupling constant sum $\Sigma' = J_{5'p} + J_{5''p}$ (8-10). The interpretation of the data for Σ' (Table 1) shows that the *gauche'-gauche' (g'g',* VIII) population is strongly preferred in all cases. The observed decrease in Σ' of 2.4 Hz in ApA (Table 1), as treated strictly by the model (VIII-X), indicates a 13% increase in *g'g'*(VIII) population with the decreased temperature (Table 1).

One method of probing the flexibility of the C(3')-O(3') bond is by means of monitoring the magnitude of $J_{3'p}$. Examination of this data in Table 1 demonstrates that on a time-average basis, conformers with a relatively small dihedral angle between H(3') and the phosphate are preferred (17-19). Changes in $J_{3'p}$ and $^{4}J_{2'p}$ (Table 1), as well as ^{13}C analysis (20), show flexibility about this bond.

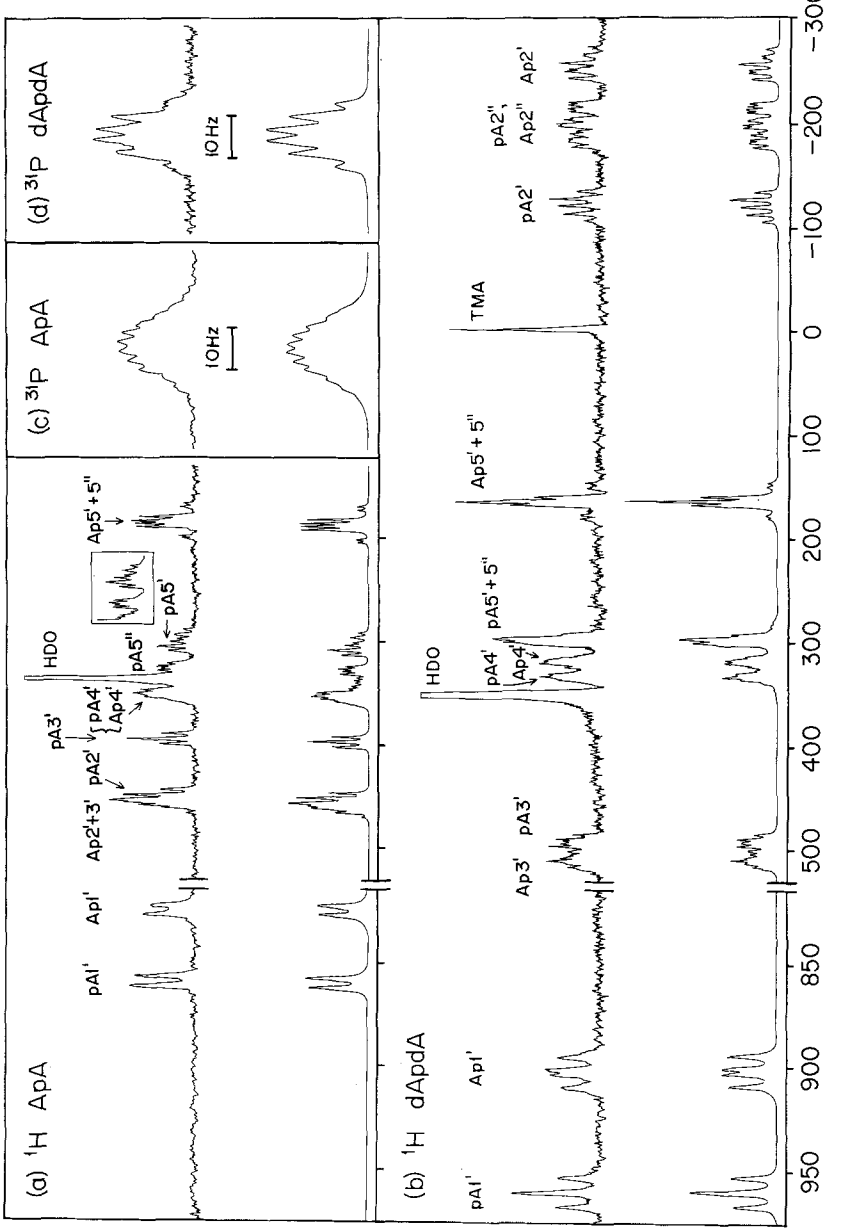


Figure 1. (a-b) 300 MHz continuous wave ^{1}H NMR spectrum of the ribose region (a) ApA and (b) dApdA at 72° respectively with chemical shifts expressed in Hz downfield from internal tetrametylammonium chloride in D_2O. The simulations are presented beneath each NMR spectrum. (a) also contains a F.T. insert of the pA 5' region at 68°. (c-d) The 40.48 MHz ^{31}P FT NMR spectrum of (c) ApA at 40° and (d) dApdA at 27° along with simulations. Concentrations are 0.05M, pD 7.4.

Effect of temperature on the dynamic backbone conformation of dApdA. The coupling constant data for dApdA (Table 1) reveals that the conformation of the deoxyribose ring moiety is only slightly temperature dependent, whereas the exocyclic C(4')-C(5') and C(5')-O(5') conformational bonds show trends similar to those already discussed in ApA.

One should be able to treat the deoxyribose ring qualitatively as a $^2E \rightleftarrows {}^3E$ equilibrium in aqueous solution. The high $J_{1'2'}$ and low values of $J_{2'3''}$ and $J_{3'4'}$ (Table 1) indicate a high ^2E population at all temperatures, but the combined magnitudes of coupling constant data suggests differences, other than population from the ribose series. Repulsion between adjacent C(2') and C(3') hydroxyl groups (21) is not present in the deoxy ring, and further, we have observed that a noticeable fraction of deoxyribonucleosides in the solid state (22-23) are in neither the ^2E or ^3E conformational ranges, but rather are slightly outside these ranges. It appears that the ring conformer potential energy wells are flatter in deoxyribose than in ribose nucleosides.

Following the same interpretative approach as that discussed for ApA, the exocyclic linkage may be monitored. The data (Table 1) show that the *gg*(V) and *g'g'*(VIII) conformations are preferred in all cases, and also that these conformer populations increase with increasing base stacking, in spite of the substantial difference between the ribose and deoxyribose ring conformational behavior. The $J_{3'p}$ coupling decreases with decreasing temperature (Table 1), which indicates smaller time-averaged dihedral angles between the coupled nuclei.

Extrapolation to polynucleotide conformations in aqueous solution. From the conformational trends exhibited at the mononucleotide and dinucleoside levels, it should be possible to make a few predictions on secondary structure in aqueous solution at the nucleic acid level. Chan and Nelson's study on ApA (2) reveals that this dinucleoside is not completely unstacked even at 95°, and is not completely stacked at 5°. It is expected that in a completely stacked state, the coupling constant trends are more magnified than those observed in the temperature range of 12°-72°. One might further expect a state such as in the pAp part of ApApA to exhibit even a larger effect and actually *single-stranded* Poly A[2] does have an unusually high, but not necessarily pure, ^3E conformation (5)

The completely polymerized and base stacked situation is the structure in a Watson-Crick-type double helix. The available data suggest a very high

[2] *Single* stranded poly purines such as Poly A seem to possess more base stacking than pyrimidines such as Poly U or Poly C (24).

^3E ribose ring population for such a structure. If one attempts to extrapolate $J_{1'2'}$ of ApA to a 'pure' ^3E state, i.e., $J_{1'2'} \simeq 1$ Hz, assuming the trends observed in the 72°-12° range will continue, then the corresponding coupling constant sums Σ and Σ' would be very low, and such low values could only be interpreted as showing overwhelming *gg*(V) and *g'g'*(VIII) population. On the other hand, any regions which have little base stacking should have a flexible $^2E \rightleftarrows {}^3E$ equilibrium with a flexible backbone in solution. Since the temperature dependence of Σ and Σ' is so similar between ApA and dApdA, helical DNA may also be mainly *gg-g'g'*. The present findings are compatible with the report that helical RNA is ^3E *gg-g'g'* and DNA is ^2E *gg-g'g'* in the solid state (22-23)!

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