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^{1}H NMR STUDY OF THE SUGAR PUCKER OF 2',3'-DIDEOXYNUCLEOSIDES WITH ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) ACTIVITY[1]

B. Jagannadh, D. V. Reddy and A. C. Kunwar

Indian Institute of Chemical Technology, Hyderabad 500 007, India

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SUMMARY - The sugar ring conformations of 2',3'-dideoxyribosyladenine (ddA), 2',3'-dideoxyribosylcytosine (ddC), 2',3'-dideoxyribosylguanine (ddG), 2',3'-dideoxyribosylhypoxanthine (ddI), 3'-azido-2',3'-dideoxyribosylthymine (AZT), 3'-azido-2',3'-dideoxyribosyluracil (AZU) and 3'-fluoro-2',3'-dideoxyribosylthymine (FddT) have been investigated by ^{1}H NMR spectroscopy. While the sugar ring in FddT exists almost totally in C2'-endo geometry, other nucleosides show equilibrium between sugar puckers of C3'-endo family (N-type) and C2'-endo family (S-type). For unsubstituted dideoxynucleosides C3'-endo conformer is favoured (≃ 75%), whereas for AZT and AZU both the conformers have almost equal populations. Unlike X-ray diffraction studies, the NMR results do not support the suggestion that C3'-exo sugar puckers are desirable for the anti-HIV activity of these nucleosides.

3'-Azido-2',3'-dideoxyribosylthymine (AZT) is still the only FDA approved drug for the treatment of the dreaded disease acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV) (**1,2**). However, cellular toxicity and viral resistance limits the efficacy of AZT. This has lead to considerable research activity directed towards finding out an effective treatment of AIDS. 2',3'-Dideoxynucleosides (ddNs) and their azido and fluoro analogues appear to be the most promising chemotherapeutic drugs at present. Strong recommendations have been made to use 2',3'-dideoxyribosylcytosine (ddC) and 2',3'-dideoxyribosylhypoxanthine (ddI), in addition to AZT, in the most severe cases of AIDS (**3**). X-ray diffraction studies show that a majority of the HIV-active nucleosides have unusual, though very similar, ribosyl geometry, implicating an important role for it in the anti-HIV activity (**4**). With an aim to gain insight into the conformational features of the furanose ring, responsible for anti-HIV activity, ^{1}H NMR studies have been carried out on 2',3'-dideoxyribosyladenine (ddA), ddC, 2',3'-dideoxyribosylguanine (ddG), ddI, 3'-azido-2',3'-dideoxyribosyluracil (AZU) and 3'-fluoro-2',3'-dideoxyribosylthymine (FddT) (Figure 1) in aqueous solutions. The data on AZT (**5**) have been reanalysed in the light of the results on these ddNs.

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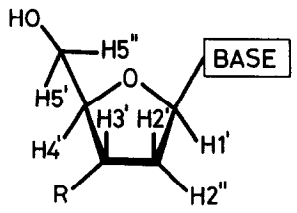

	BASE	R
ddA	ADENINE	H3"
ddC	CYTOSINE	H3"
ddG	GUANINE	H3"
ddI	HYPOXANTHINE	H3"
AZU	URACIL	N_3
FddT	THYMINE	F

Figure 1. The structure of 2',3'-dideoxynucleosides.

EXPERIMENTAL

All the compounds investigated have been obtained from National Cancer Institute, USA and were used without further purification. NMR measurements were performed on Varian UNITY 400 NMR spectrometer equipped with a Sun 360 computer. ^1H NMR spectra were recorded in D_2O on 0.02-0.03 molar solutions at room temperature (nominally 21°C). The protons in the sugar rings are usually strongly coupled and the spectral parameters were obtained by analysing the spectra with the help of LEQUOR program **(6)**. The relevant vicinal indirect couplings ($^3J_{H-H}$) between the protons in the furanose ring are given in the Table 1. The errors of all the couplings are less than 0.1 Hz.

RESULTS AND DISCUSSION

The geometry of the furanose ring is normally described by the concept of pseudorotation with phase angle (P) and puckering amplitude (ϕ_m) **(7,8)**. Usually NMR data are analysed with fast equilibrium between two broad class of conformers; the N-type (referring to north in the pseudorotation wheel) , having a phase angle of pseudorotation P(N) between -90° to 90° and a puckering amplitude $\phi_m(N)$ and the S-type (referring to south), characterised by 90° < P(S) < 270° and ϕ_m (S). The experimental couplings are thus averaged over two sugar puckers, defined by P(N), P(S), $\phi_m(N)$, $\phi_m(S)$ and the populations of the two conformers X_N and X_S. The "generalised Karplus equations", obtained from several X-ray crystallographic studies, for various nucleosides are available and provide the relation between the puckering parameters and the couplings **(9,10)**. Since such relations are not available for ddNs, the equations for 2'-deoxyribonucleosides were used. The additional relations involving H3" have been derived by assuming that the dihedral angles of H3" differ from those for H3' by 120°. Thus providing eight equations for the vicinal couplings in unsubstituted ddNs.

The furanose rings in ribonucleosides and 2'-deoxyribonucleosides contain three and five $^3J_{H-H}$ respectively. For such systems the determination of all the five puckering parameters is not always possible and requires making physically meaningful approximations **(9,11,12)**. However, in systems with five couplings it

Table 1.

Relevant vicinal couplings (Hz) in 2',3'-dideoxynucleosides in aqueous solution

Coupling constant	Nucleosides						
	ddA	ddC	ddG	ddI	FddT	AZU	AZT[a]
$^3J_{H1'-H2'}$	3.4	3.3	3.4	3.0	9.3	6.0	6.4
$^3J_{H1'-H2''}$	6.9	7.0	7.0	6.9	5.7	6.7	6.7
$^3J_{H2'-H3'}$	8.1	8.2	8.2	8.2	5.3	7.7	7.2
$^3J_{H2'-H3''}$	3.9	3.4	3.8	3.5	-	-	-
$^3J_{H2''-H3'}$	10.0	10.4	10.1	10.6	1.4	5.5	5.2
$^3J_{H2''-H3''}$	8.2	8.2	8.3	8.2	-	-	-
$^3J_{H3'-H4'}$	8.3	9.4	8.4	8.5	1.5	5.4	4.9
$^3J_{H3''-H4'}$	6.6	5.9	6.6	6.5	-	-	-

[a] in DMSO (from Ref 5).

is tempting to solve the five simultaneous equations to obtain all the puckering parameters. Such an exercise requires a careful interpretation of the data and a perfect fit between experimental and calculated couplings should not be the only criterion for deriving these parameters. On the other hand, unsubstituted ddNs have eight independent $^3J_{H-H}$ in the sugar rings and provide a system where it may be possible to determine all the puckering parameters using a least squares procedure. Unfortunately, despite this overdeterminacy, all the parameters could not be determined independently. The values of ϕ_m were quite unreasonable, being more than 50° for one conformer and less than 20° for the other, due to very large correlation coefficients (-0.99) between ϕ_m(N) and ϕ_m(S)(**13**). Structural parameters have therefore been obtained by taking the following line of action.

The values of ϕ_m for nucleosides and nucleotides commonly lie in a narrow range and quite often the reported values of ϕ_m(N) and ϕ_m(S) are very similar (**10,14,15**). Therefore in our calculations ϕ_m(N) and ϕ_m(S) were set to be equal. The results are presented in Table 2. All the unsubstituted ddNs have ϕ_m in the range 34.8° to 37.2°, within 2.5° of the average value of 34.7° reported for thirteen ddN structures by X-ray diffraction (**4**). The major conformer (≃ 75%) has P(N) biased towards C2'-exo conformers for purine nucleosides (ddA,ddG and ddI) while for ddC (pyrimidine nucleoside) it is closer to C3'-endo conformer. The minor conformer (≃ 25%) has P(S) around 175° for ddNs with purine bases while for ddC it has a value of 139°. Thus for ddA, ddG and ddI, C2'-exo/C3'-endo and C2'-endo/

Table 2.

Puckering parameters for 2',3'-dideoxynucleosides

Nucleoside	P(N) (o)	P(S) (o)	ϕ_m(N) (o)	X_S	RMS error (Hz)	ED_{50}[a] (μ_m)
ddA	- 4	171	35.2	0.27	0.27	6.4
ddC	7	139	37.2	0.25	0.31	0.3
ddG	- 3	169	34.8	0.26	0.28	7.6
ddI	- 6	181	35.1	0.23	0.32	10.0
FddT	-14[b]	157	35.6	0.92	0.13	0.001
AZU	-17	140	32.3	0.53	0.07	0.43
AZT[c]	- 6	154	33.0	0.57	0.08	0.003

[a] 50% effective dose on human MT-4 cells infected with HIV (from Ref 18).

[b] Very large error in its determination, because of small X_N.

[c] in DMSO.

C3'-exo conformers exist in equilibrium with P very close to the ideal values for two twist conformers ${}_2^3T$ (P(N)=0°) and ${}_3^2T$ (P(S)=180°). As mentioned, for ddC the results are somewhat different. However, calculations peformed with P(N)=0° and P(S)=180° still provide an acceptable rms error of 0.43 Hz and show that even for ddC an equilibrium between C2'-exo/C3'-endo family and C2'-endo/C3'-exo family puckers is consistent with the experimental couplings. This points out the need for proper estimation of errors in these parameters. Further calculations show that variations of P(N) by about ±15° and P(S) by ±40° still result in acceptable rms errors (< 0.5 Hz) of the fits. The larger error in P(S) is the outcome of a small value of X_S.

AZT, AZU and FddT (Table 1) have only five vicinal couplings and the above approach was again followed, as independent determination of ϕ_m(N) and ϕ_m(S) is not possible due to strong correlation between them (**13**). For AZT and AZU the parameters obtained are very similar despite the fact that they have been investigated in two different solvents. These systems show equilibrium between almost equal populations of C2'-exo/C3'-endo and C1'-exo/C2'-endo conformers. It is however noticed that for S-type conformers a C2'-endo/C3'-exo pucker still results in very acceptable fits between experimental and calculated couplings. Thus AZU and AZT sugar geometries also show an equilibrium between C3'-endo family and C2'-endo family conformers. FddT on the other hand exists predominently in the S-type (C2'-endo) conformer with X_S = 0.92, ϕ_m = 35.6°, P(S) = 157° and P(N) = -14° (with small values of X_N, P(N) is not very accurately determined). In fact calculations performed with only one conformer provided P(S) = 160°, ϕ_m (S) = 33.7° with rms error of 0.46 Hz.

X-ray crystallographic studies show that the sugar ring conformations of ddA, ddC, FddT, AZT and AZU are centred around C3'-exo (**4,16**). It was suggested, that the C3'-exo conformation may be an important feature for the anti-HIV activity of these nucleosides since it places the 5'-OH group at the right place for a proper interaction with the kinases leading to the phosphorylation of the ddNs (**4**). Laser Raman study of ddC and AZT on the other hand have indicated that they adopt a predominently N-type (C3'-endo family) conformation. The NMR results for ddC are consistent with these findings while AZT shows equal preference for the N-type and the S-type conformations (**17**).

From our study, some trends can be observed. With the exception of ddC, there is an increase in the anti-HIV activity (ED_{50}) (**18**) of the nucleosides with X_S (Table 2). The 3'-substituted ddNs show a bias for the S-type conformers (53% to 92%) while the unsubstituted ddNs have a predominance of the N-type conformers ($\simeq$ 75%), which is in contrast to the results obtained in the crystalline state. The presence of a polar 3'-substituent plays an important role in the stabilization of the S-type conformations due to *guache* effects (**19**) and X_S increases with the polarity of the substituent. Though these results show some unusual conformational features, definitive conclusions about the nature of the sugar pucker necessary for anti-HIV activity are difficult to make. It will therefore be necessary to study a large number of nucleosides, which are HIV-inhibitors, in order to arrive at more general and meaningful conclusions.

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