Vol. 151, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1988 Pages 608-614

UNUSUAL STRUCTURAL FEATURES OF 2',3'-DIDEOXYCYTIDINE, AN INHIBITOR OF THE HIV (AIDS) VIRUS*

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Received January 12, 1988

The structure and conformation of 2',3'-dideoxycytidine, a potent inhibitor of the human immunodeficiency virus, was determined by X-ray crystallography. The nucleoside crystallizes in the tetragonal space group $P4_12_12$ with cell dimensions $a = b = 8.698(4)$ and $c = 26.155(9)$ Å. Atomic parameters were refined by full-matrix least squares to a final value of $R = 0.037$ for 1926 observed reflections. The conformation of the furanose ring corresponds to the unusual C3'exo/C4'endo ($_3T^4$) pucker, similar to that found in one of the molecules of 3'-azidothymidine (AZT). The glycosidic torsion angle is also smaller than expected. The relevance of these unusual structural features to anti-AIDS activity is assessed.

Acquired immune deficiency syndrome (AIDS)* is a highly lethal disease caused by a retrovirus known as human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) but now designated as human immunodeficiency virus (HIV-1).

3'-Azido-3'-deoxythymidine (AZT) has been administered to patients with AIDS by Yarchoan et al. (1) and some improvement was observed in many of the patients. As a consequence, AZT was rapidly approved by the FDA for use in the therapy of patients with AIDS. These findings stimulated the evaluation of other nucleoside analogs for potential inhibitory activity of retroviruses (2-15). Of the various 2',3'-dideoxynucleoside analogs evaluated for activity

*Publication No. 28512 of the National Research Council of Canada

*Abbreviations: AIDS, acquired immunodeficiency syndrome; AZT, 3'-azido-3'-deoxythymidine; HTLV-III/LAV, human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; HIV-1, human immunodeficiency virus; DDC, d2C, 2',3'-dideoxycytidine.

0006-291X/88 $1.50

against the AIDS virus, Mitsuya and Broder (3) found 2',3'-dideoxycytidine (DDC, d2C), a compound first synthesized by Horwitz and coworkers (16), to be the most potent. Cooney et al. (17) found DDC to be metabolized in HIV-1 infected and uninfected cells to the mono, di- and triphosphates. Starnes and Cheng (15) found 40% of the acid-soluble pool of DDC metabolites to be 2',3'-dideoxycytidine triphosphate. No evidence was found by Cooney et al. (17) or Starnes and Cheng (15) for enzymic deamination of this compound, or by Cooney et al. (17) of phosphorolytic cleavage to cytosine and dideoxyribose-1-phosphate. Mitsuya and Broder (18) reported that DDC was chosen for clinical development not only because it is resistant to deamination and phosphorolytic cleavage, but also because it is well absorbed when administered orally, has uncomplicated pharmacokinetic clearance by the kidney, has no adverse effect on intracellular pyrimidine nucleotides pools and is relatively non-toxic to animals. Recent clinical trials showed that some patients taking DDC have developed peripheral neuropathy, a condition which has been reversed in some cases. At the present time, Broder and Yarchoan are treating AIDS patients by alternating DDC and AZT on a weekly basis (19). In view of its potent anti-AIDS activity, we consider it important to learn more about the physical and chemical properties of DDC. In this report, we describe its three-dimensional structure determined by an X-ray analysis.

METHODS

Crystals of DDC, $C_9H_{13}N_3O_3$, belong to the tetragonal space group $P4_12_12$, and the cell dimensions are $a = b = 8.698(4)$, $c = 26.155(9)$ Å. Three-dimensional X-ray intensity data were collected on a CAD4 diffractometer with Cu Kα radiation. An octant of the reciprocal sphere was measured (θ_{max} = 76°), thus providing Friedel pairs of all hkl reflections. Of the 2064 measurements, 1926 (93.3%) had intensities $>3\sigma(I)$ and were considered observed. The intensities were corrected for Lorentz and polarization factors; absorption corrections were unnecessary ($\mu = 8.7$ cm^{-1}).

The crystal structure was determined by direct methods (20). All hydrogen atoms were located on a difference Fourier map. Atomic parameters were refined by full-matrix least squares with anisotropic temperature parameters for non-hydrogen atoms. The refinement converged at $R = 0.037$ and $R_w = 0.048$ for 1926 observed reflections ($w = 8.0/|F_o|$ for $|F_o| > 8.0$). The coordinates and temperature parameters are listed in Table 1. A list of structure factors is available from the first author.

RESULTS AND DISCUSSION

A stereoscopic view of the DDC molecule is presented in Fig. 1. In view of the unusual conformation which we found in one of the molecules of AZT (21), it is most interesting to examine whether the DDC molecule also has any unusual conformational features. We find that the furanose ring adopts a C3'exo/C4'endo ($_3T^4$) pucker, with a pseudorotation phase angle $P = 207.5°$ and

TABLE 1. Final atomic parameters and their standard deviations

Atom	x	y	z	B_{eq}/B
N1	0.3122(2)	0.7525(2)	0.41029(5)	3.08(6)
C2	0.3178(2)	0.9127(2)	0.40742(7)	3.29(7)
O2	0.2857(2)	0.9757(2)	0.36649(5)	4.42(7)
N3	0.3562(2)	0.9931(2)	0.45007(6)	3.57(7)
C4	0.4005(2)	0.9194(2)	0.49225(7)	3.30(7)
N4	0.4368(3)	1.0024(3)	0.53331(7)	4.60(9)
C5	0.4065(3)	0.7561(2)	0.49459(7)	3.67(8)
C6	0.3575(3)	0.6782(2)	0.45346(7)	3.48(8)
C1'	0.2551(2)	0.6689(3)	0.36444(7)	3.56(7)
C2'	0.3770(3)	0.6429(3)	0.32369(8)	4.57(10)
C3'	0.3506(4)	0.4771(3)	0.30672(8)	4.94(11)
C4'	0.2881(3)	0.4024(3)	0.35460(8)	4.40(9)
O4'	0.1997(2)	0.5244(2)	0.37933(5)	4.21(6)
C5'	0.4105(4)	0.3424(3)	0.39003(10)	5.65(13)
O5'	0.3460(4)	0.3101(2)	0.43877(7)	8.03(14)
HN41	0.471(3)	0.949(3)	0.5607(10)	5.2(6)
HN42	0.415(4)	1.098(4)	0.5336(12)	6.4(8)
H5	0.443(3)	0.708(3)	0.5257(9)	4.0(5)
H6	0.357(3)	0.573(3)	0.4540(9)	4.4(5)
H1'	0.173(3)	0.733(3)	0.3516(9)	4.6(5)
H2'	0.477(4)	0.660(4)	0.3376(10)	6.0(7)
H2"	0.358(3)	0.720(3)	0.2949(11)	6.1(7)
H3'	0.450(4)	0.429(3)	0.2968(11)	5.8(7)
H3"	0.275(3)	0.479(3)	0.2770(10)	5.6(6)
H4'	0.215(3)	0.314(3)	0.3494(9)	5.2(6)
H5'	0.494(4)	0.427(5)	0.3954(13)	8.9(10)
H5"	0.455(3)	0.246(4)	0.3731(11)	6.4(7)
HO5'	0.343(5)	0.220(4)	0.4454(13)	8.7(10)

a maximum amplitude of puckering τ_m = 33.9°. This conformation is quite similar to that of molecule B of AZT (P = 214.5°, τ_m = 36.6°). In the 1987 Cambridge Crystallographic Database (22), which contains well over 600 nucleosides and nucleotides, we find only three similar structures (P = 208 ± 9°) in which the pucker of the furanose ring is not imposed by fusion to another ring. It does not appear likely that this unusual conformation is ascribable to the 2',3'-dideoxy structure of the furanose ring itself. In the

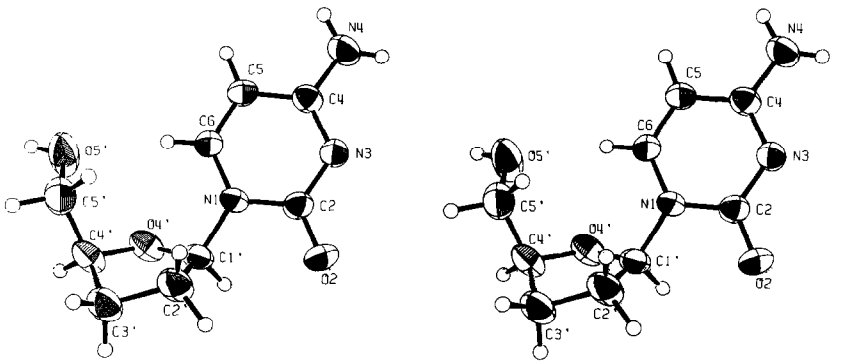

Figure 1. Stereoscopic view of the DDC molecule.

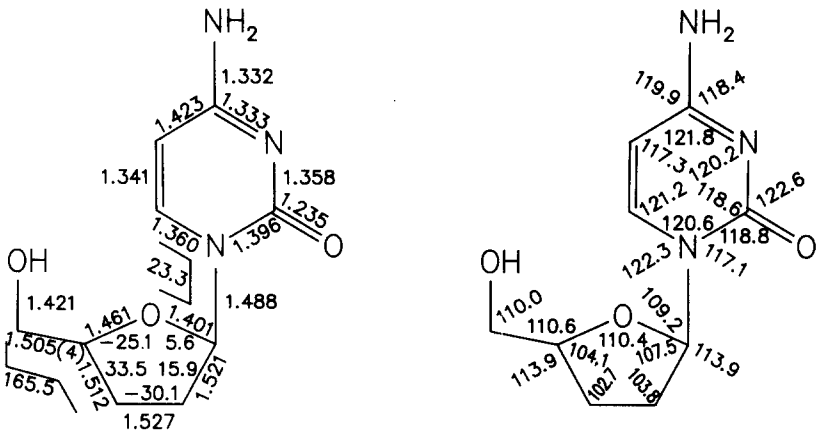

Figure 2. (Left) Bond lengths (in Å) and torsion angles (in deg); unless otherwise indicated, their estimated standard deviations (esd's) are 0.002–0.003 Å and 0.2–0.3°, respectively. (Right) Bond angles; their esd's are 0.15–0.27°.

recently described X-ray analysis of 2',3'-dideoxyribavirin (23) the ring conformations of two independent molecules were reported to be $\underline{P}$ = 4.5°, τ_m = 34.7° and $\underline{P}$ = 3.1°, τ_m = 36.8°. Another unusual conformational feature is the relatively small glycosidic torsion angle, χ_{CN} [C6-N1-C1'-O4'] = 23.3° (Fig. 2). In most nucleosides with a type $\underline{S}$ ring pucker, this angle is in the range 40–65° (24). It may be recalled that in molecule B of AZT the glycosidic torsion angle was also smaller than usual (χ_{CN} = 2.3°). The decrease of this torsion angle is caused by the $_3\underline{T}^4$ pucker of the furanose ring. In this conformation, H5' of the side chain would be very close to H6 if the glycosidic torsion angle was normal. Rotation of the pyrimidine ring about the glycosidic bond relieves this steric interaction and decreases the H6···H5' distance to a tolerable 2.23 Å (the C-H bond lengths were normalized to their nominal value of 1.09 Å). The pyrimidine ring is not exactly planar; the atoms with the largest deviations from the mean plane are C2 (Δ = −0.040(3) Å) and C5 (Δ = −0.031(3) Å). Finally, the $-CH_2OH$ side chain adopts the *anti* conformation which, although not as common as the *gauche*$^+$ rotamer, has been frequently observed in other nucleosides (25).

Fig. 2 shows the geometrical details of the DDC molecule. The bond lengths and bond angles of the pyrimidine moiety are in very good agreement with previously observed values (26). As commonly observed in pyrimidine nucleosides in a low-*anti* glycosidic conformation, the C6-N1-C1' angle is larger than C2-N1-C1'. In the furanose moiety the bond lengths and bond angles are unremarkable and require no comment.

The hydrogen bonds found in this crystal structure are listed in Table 2. The corrected H···A distances were obtained after normalizing O-H, N-H and C-H bond lengths to their nominal values of 0.97, 1.04, and 1.09 Å, respectively.

TABLE 2. Distances and angles for hydrogen bonds

		Distances (Å)			Angles (deg)
D A	at	D···A	H···A	H···A_{corr}	D-H···A
N4-HN41···O2	½-x, ½+x, ¼+z	2.978(3)	2.09(3)	1.96	167(2)
N4-HN42···O4'	y, x, z̄	2.958(2)	2.62(3)	2.58	105(2)
N4-HN42···O5'	y, x, z̄	3.268(4)	2.45(3)	2.28	161(2)
C6-H6···O5'	x, y, z	3.226(3)	2.32(2)	2.15	169(2)
O5'-HO5'···N3	x, -1+y, z	2.774(3)	1.98(4)	1.82	170(4)

The N4-HN42···O5' hydrogen bond is relatively weak because the proton is also donated to O4' (asymmetric bifurcation). Intramolecular C6-H···O5' bonds have been frequently observed in nucleosides and are known to stabilize the gauche+ rotamer of the side chain (24). The present case is unusual in view of the trans conformation of the $-CH_2OH$ group. This hydrogen bond is possible only because of the rare ${}_3T^4$ pucker of the furanose ring and the relatively small glycosidic torsion angle.

BIOLOGICAL CONSIDERATIONS

The detailed mechanism of the anti-AIDS activity of DDC is not yet clear. Lacking a 3'-OH group, DDC is obviously a chain terminator and was shown to be incorporated into DNA (15). Yet, as in the case of AZT, it is not certain whether chain termination or inhibition of the retroviral reverse transcriptase by the triphosphate is more important. In contrast to AZT, DDC does not have a 3'-substituent which would facilitate binding to the reverse transcriptase (21). The absence of a polar 3'-substituent would also decrease the molecule's capacity to bind to kinases which would explain why DDC is a much poorer substrate for deoxycytidine kinase than deoxycytidine (4). Furthermore, the conformation of DDC may also play a role in the anti-AIDS activity of the nucleoside. It, too, may influence the rate of phosphorylation by kinases. The rare conformation which we found in this crystal structure analysis reflects a higher than usual potential energy state of the molecule and may therefore represent the transition state in which DDC or its triphosphate interact with enzymes (21). The fact that both AZT and DDC have similar unusual conformations is remarkable, but whether these structural features can be correlated with anti-AIDS activity cannot be assessed at the present time.

The toxicities produced in humans by these two antiviral agents are markedly different. AZT toxicity is primarily one of bone marrow suppression

(anemia, leukopenia, neutropenia) as well as nausea, myalgia and very severe headaches, whereas DDC produces very painful peripheral neuropathy as well as a body rash. This clearly implies that, in addition to their involvement with reverse transcriptase and viral DNA synthesis, these drugs interact with other sites in vivo. The molecular basis for their toxicities is presently unknown. We hope, however, that our results and those of future similar studies will lead to structural modifications which will afford anti-AIDS drugs with retained antiviral activity but decreased clinical toxicity.

ACKNOWLEDGEMENTS

All calculations were performed with the NRCVAX system of programs (27). Figure 1 was drawn with the ORTEP program of Johnson (28). T.S.L. and W.H.P. acknowledge support by USPHS grant CA05262 from the National Institutes of Health.

REFERENCES

1. Yarchoan, R., Weinhold, K.J., Lyerly, H.K., Gelmann, E., Blum, R.M., Shearer, G.M., Mitsuya, H., Collins, J.M., Myers, C.E., Klecker, R.W., Markham, P.D., Durack, D.T., Nusinoff-Lehrman, S., Barry, D.W., Fischl, M.A., Gallo. R.C., Bolognesi, D.P., and Broder, S. (1986) Lancet 1, 575-580.
2. Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Nusinoff-Lehrman, S., Gallo, R.C., Bolognesi, D., Barry, D.W., and Broder, S. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 7096-7100.
3. Mitsuya, H., and Broder, S. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 1911-1915.
4. Balzarini, J., Pauwels, R., Herdewijn, P., De Clercq, E., Cooney, D.A., Kang, G.-J., Dalal, M., Johns, D.G., and Broder, S. (1986) Biochem. Biophys. Res. Commun. 140, 735-742.
5. Baba, M., Pauwels, R., Herdewijn, P., De Clercq, E., Desmyter, J., and Vandeputte, M. (1987) Biochem. Biophys. Res. Commun. 142, 128-134.
6. Balzarini, J., Pauwels, R., Baba, M., Robins, M.J., Zou, R., Herdewijn, P., and De Clercq, E. (1987) Biochem. Biophys. Res. Commun. 145, 269-
7. Balzarini, J., Robins, M.J., Zou, R., Herdewijn, P., and De Clercq, E. (1987) Biochem. Biophys. Res. Commun. 145, 277-283.
8. Dahlberg, J.E., Mitsuya, H., Blam, S.B., Broder, S., and Aaronson, S.A. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 2469-2473.
9. Hamamoto, Y., Nakashima, H., Matsui, T., Matsuda, A., Ueda, T., and Yamamoto, N. (1987) Antimicrob. Agents Chemother. 31, 907-910.
10. Herdewijn, P., Balzarini, J., De Clercq, E., Pauwels, R., Baba, M., Broder, S., and Venderhaeghe, H. (1987) J. Med. Chem. 30, 1270-1278.
11. Lin, T.S., Chen, M.S., McLaren, C., Gao, Y.S., Ghazzouli, I., and Prusoff, W.H. (1987) J. Med. Chem. 30, 440-444.
12. Lin, T.S., Schinazi, R.F., Chen, M.S., Kinney-Thomas, E., and Prusoff, W.H. (1987) Biochem. Pharmacol. 36, 311-316.
13. Lin, T.S., Schinazi, R.F., and Prusoff, W.H. (1987) Biochem. Pharmacol. 36, 2713-2718.
14. Mitsuya, H., Jarrett, R.F., Matsukura, M., DiMarzo Veronese, F., DeVico, A.L., Sarngadharan, M.G., Johns, D.G., Reitz, M.S., and Broder, S. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 2033-2037.
15. Starnes, M.C., and Cheng, Y.C. (1987) J. Biol. Chem. 262, 988-991.
16. Horwitz, J.P., Chua, J., Noel, M., and Donatti, J.T. (1967) J. Org. Chem. 32, 817-818.

17. Cooney, D.A., Dalal, M., Mitsuya, H., McMahon, J.B., Nadkarni, M., Balzarini, J., Broder, S., and Johns, D.G. (1986) Biochem. Pharmacol. 35, 2065-2068.
18. Mitsuya, H., and Broder, S. (1987) Nature 325, 773-778.
19. Dagani, R. (1987) Chem. Engineer. News 65(47), 41-49.
20. Main, P., Hull, S.E., Lessinger, L., Germain, J.-P., Declercq, J.-P., and Woolfson, M.M. (1978) MULTAN 78, University of York, England, and University of Louvain, Belgium.
21. Birnbaum, G.I., Giziewicz, J., Gabe, E.J., Lin, T.-S., and Prusoff, W.H. (1987) Can. J. Chem. 65, 2135-2139.
22. Allen, F.H., Kennard, O., and Taylor, R. (1983) Acc. Chem. Res. 16, 146-153.
23. Sanghvi, Y.S., Hanna, N.B., Larson, S.B., Robins, R.K., and Revankar, G.R. (1987) Nucleosides Nucleotides 6, 761-774.
24. Birnbaum, G.I., and Shugar, D. (1987) In Topics in Nucleic Acid Structure (S. Neidle, Ed.), Part 3, pp. 1-70. Macmillan, London.
25. deLeeuw, H.P.M., Haasnoot, C.A.G., and Altona, C. (1980) Isr. J. Chem. 20, 108-126.
26. Taylor, R., and Kennard, O. (1982) J. Am. Chem. Soc. 104, 3209-3212.
27. Gabe, E.J., Lee, F.L., and Le Page, Y. (1985) In Crystallographic Computing 3 (G.M. Sheldrick, C. Kruger, and R. Goddard, Eds.), pp. 167-174. Clarendon Press, Oxford.
28. Johnson, C.K. (1976) ORTEPII, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, TN.