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CONFORMATION OF N^4-ACETYLCYTIDINE, A MODIFIED NUCLEOSIDE OF tRNA, AND STEREOCHEMISTRY OF CODON-ANTICODON INTERACTION

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Summary: The crystal structure of N^4-acetylcytidine (ac^4C) a modified nucleoside of tRNA, has been determined from three-dimensional x-ray diffractometer data. The N^4-substituent is proximal to C(5), quite contrary to expectations from solution studies of N^4-methylcytosine. This orientation of the N^4-substituent will not block Watson-Crick base pairing for reading the third codon by $tRNA_M^{Met}$, and hence the discriminatory function suggested for ac^4C might arise due to non-standard conformation of the polynucleotide backbone of the anticodon around the Wobble base. A common characteristic of the modified nucleosides that occur at the Wobble position is their inability to shield the Watson-Crick base pairing sites; this is quite consistent with the necessity for reading the third base of the codon. This is in sharp contrast to the modifications of the nucleosides adjacent to the 3'-end of anticodons, all of which prevent Watson-Crick base pairing.

Introduction: N^4-acetylcytidine, ac^4C, is a modified nucleoside that occurs at the first position of the anticodon of E. coli $tRNA_M^{Met}$ (1,2), and in the dihydrouridine stem in $tRNA^{Ser}$ (3,4) and $tRNA^{Leu}$ (5). More recently, ac^4C has been identified in 18S rRNA and appears to be conserved in the small ribosomal subunit of eukaryotes (6). Examining the stereochemistry of modified nucleosides adjacent to the 3'-end of anticodons, we have found (7-10) that irrespective of the type of chemical modifications the inability to base pair according to the Watson-Crick base pairing (11) is a common feature shared by all these modified

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nucleosides. On the other hand, none of the modifications on nucleosides that occur at the first position of the anticodon ('Wobble' position (12)), would prevent Watson-Crick base pairing; N^4-acetylcytidine, however, appeared to be an exception (10). Depending on the orientation of the N-acyl group across the C(4)-N(4) bond of cytosine, the base pairing ability of ac^4C is altered. The 'distal' conformation will prevent Watson-Crick base pairing while the 'proximal' one will not. N.m.r. results for m^4C indicate a 20:1 preference for the distal conformation (13,14). If this distal conformation also prevails for ac^4C then this modified nucleoside cannot take part in the codon reading for $tRNA_M^{Met}$ using the standard base pairing, and the genetic code will have to be read as two rather than three letters or a non-standard pairing of ac^4C to G must be used (10). Hence, the stereochemistry of ac^4C is very important and this paper describes the results of our studies on the crystal structure and conformation of ac^4C.

Methods. The nucleoside ac^4C was synthesized using slight modifications of known procedures (15). Crystals of ac^4C ($C_{11}H_{15}N_3O_6$) were obtained from water-propanol solutions. The crystals are orthorhombic, space group $P2_12_12_1$ with cell constants at (22 ± 3)°C: $\underline{a}$ = 18.177(1), $\underline{b}$ = 11.650(1), $\underline{c}$ = 5.692(1)Å; Z = 4, $D_{obsd.}$ = 1.57, $D_{calc.}$ = 1.57 $g \cdot cm^{-3}$. Three dimensional intensity data (1577 reflections to the limit 2θ = 165° for CuKα radiation) were collected using a GE XRD-6 diffractometer and Ross filters and the structure was solved by the multisolution technique (16) and refined to an R of 0.051 using the least-squares method with the block-diagonal approximation. The locations of the hydrogen atoms were obtained from electron-density difference maps; their positional parameters

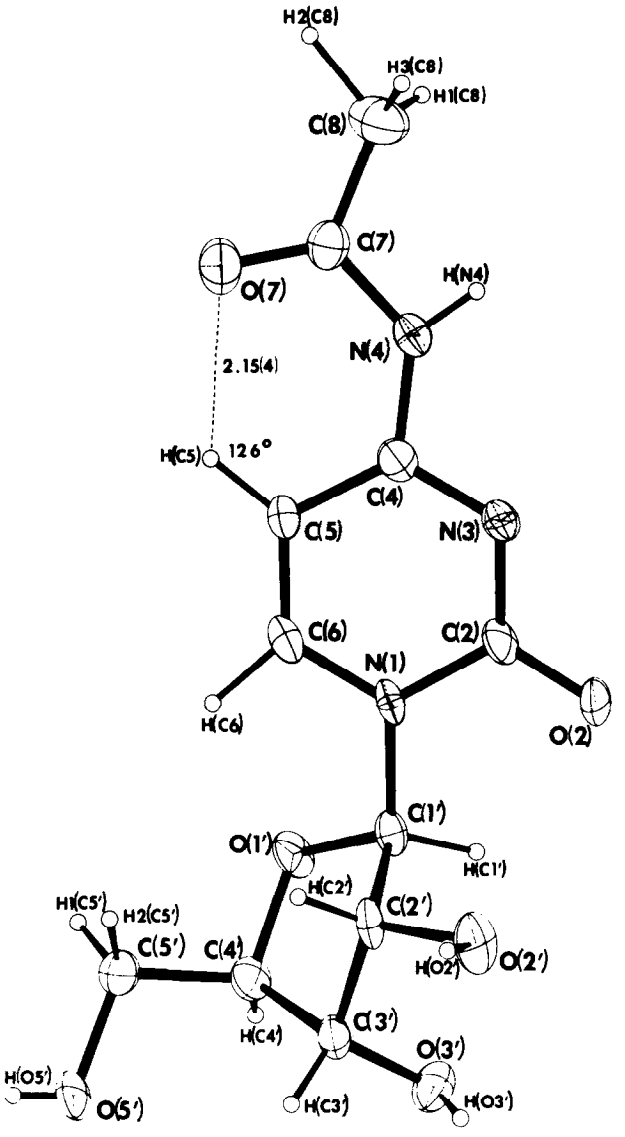

Figure 1. Conformation of N^4-acetylcytidine. Note that the substituent on N(4) is oriented proximal to C(5). The conformation across C(4')-C(5') is gauche$^-$.

and individual isotropic thermal parameters were included in the refinement.

Results and Discussion. Figure 1 illustrates the conformation of the molecule. The 'proximal' conformation of the molecule with

the substituents on N(4) turned towards C(5) will enable H(N4), N(3) and O(2) to take part in Watson-Crick base pairing with G, in spite of the modifications. This molecule exhibits the anti conformation with χ_{CN} = 33.4°. The sugar ring has the C(2')-endo pucker. The conformation across the C(4')-C(5') bond is not the preferred gauche$^+$ conformation, but the gauche$^-$ conformation (O(5') is trans to O(1') and -gauche to C(3')). There is no base pairing of the cytidine bases. All polar hydrogens take part in hydrogen bonding.

The proximal orientation of the N^4-substituent is quite unexpected, in view of the n.m.r. results (13,14) on the conformation of m^4C. Since an acetyl group is bulkier than a methyl group, it was felt that the preferred conformation of ac^4C will be distal. Further, model building studies indicated short contacts from H(C5) to the acetyl in the proximal orientation. Consequently, the observed proximal conformation and the H(C5) to O(7) contact of 2.15(5)Å (C(5)-H(C5)···O(7) angle is 126°) in the crystal leave no alternative but to regard the intramolecular H(C5)···O(7) contact as not repulsive but attractive giving rise to the formation of a planar six-membered ring (C(7)-N(4)-C(4)-C(5)-H(C5)···O(7)). In this connection, it is interesting to note that the more acidic H(C6) usually takes part in hydrogen bonding (17-19); in this structure the electron withdrawing ability of N-acetyl group probably has polarized H(C5) also sufficiently to take part in hydrogen bonding (19). Our n.m.r. studies indicate that the H(C5) proton which usually has a δ value of 6.04 for C moved downfield to 7.25 for ac^4C indicating an increasing acidity for this proton. Also lack of H···O=C repulsion has been used to explain the preferred conformation of acetates of cyclohexanols (20,21). Thus, it is possible that the proximal conformation

observed in the crystal is also the preferred conformation in solution and could prevail in tRNA also. Therefore, it appears that the functional role of the N^4-acetyl group is not necessarily aimed at preventing the Watson-Crick base pairing.

The modified nucleosides that have been found to occur at the Wobble positions are Gm, I, "Q", Cm, ac^4C, cmo^5U, mcm^5U, mo^5U, mnm^5s^2U and mcm^5s^2U (from our compilation of about 70 tRNA sequences). Examination of the stereochemistry of these nucleosides (10) readily shows that a common characteristic of all these modifications is their inability to shield the Watson-Crick base pairing sites. This inability to shield the Watson-Crick base pairing is quite consistent with the necessity for reading the third base of the codons and unlike the effect of the modifications on the nucleosides adjacent to the 3'-end of anticodons whose role is possibly to define the reading frame in the anticodon loop for reading the genetic code (7,10). The presence of the modified nucleoside ac^4C seems to be quite specific for $tRNA_M^{Met}$ in E. coli and it has been suggested (2) that its uniqueness might be related to the discriminative function of $tRNA_M^{Met}$ which decodes only the internal AUG codon.

It is interesting to note that E. coli $tRNA_F^{Met}$ with a CAU anticodon and an unmodified adenosine adjacent to it on the 3'-end wobbles in the first codon position (can read GUG as well as AUG) (22), yeast $tRNA_F^{Met}$ containing t^6A adjacent to the anticodon reads only AUG (23) and E. coli $tRNA_M^{Met}$ with modified nucleoside ac^4C at the Wobble position and t^6A adjacent to the anticodon reads only the internal methionine codon (22,24). A comparison of the association constants of these three tRNA with ApUpG shows (25) that E. coli $tRNA_M^{Met}$ has the lowest value. The reduction in the bonding strength which seem to arise due to

modification of cytosine, can be due to several causes such as the prevention of base pairing of ac^4C to G or non-standard conformations for the polynucleotide chain adjacent to ac^4C. Our results show that ac^4C has the proximal conformation and a non-standard gauche$^-$ orientation across the C(4')-C(5') bond. If these conformations persist at the polynucleotide level also, the alteration in the conformation of the back-bone from the standard helical conformations will be important for the selective responses of these methionine tRNA's. Model building studies are in progress to evaluate the selective responses of these different species of methionine tRNA's (9).

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