CONFORMATION AND POSSIBLE ROLE OF HYPERMODIFIED NUCLEOSIDES ADJACENT TO 3'-END OF ANTICODON IN tRNA: N-(PURIN-6-YLCARBAMOYL)-L-THREONINE RIBOSIDE

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Summary. The crystal structure of N-(purin-6-ylcarbamoyl)-L-threonine riboside was determined from three-dimensional x-ray diffraction data. The N^6-substituent is distal (trans) to the imidazole ring, leading to a bifurcated hydrogen interaction involving two intramolecular contacts with the hydrogen on N(threonine): a hydrogen bond to N(1) of adenine and a close contact to the hydroxyl oxygen of threonine. The conformation of the molecule and the internal hydrogen bond completely block the two sites N^6-H and N^1 of adenine from taking part in the Watson-Crick base pairing. This inability to base pair according to the Watson-Crick scheme appears as a common structural feature in all modified bases adjacent to the 3'-end of anticodons. These results, along with Crick's hypothesis for codon recognition, suggest that the hypermodified bases adjacent to the anticodons may be important in (i) preventing the misreading of the codons by bases adjacent to anticodons and (ii) promoting a single stranded conformation for the anticodon loops.

Introduction. The hypermodified base N-(purin-6-ylcarbamoyl)-L-threonine (PCT)[a] occurs in several tRNA's (1,2) which respond to the codons beginning with adenine (3,4,5). This modified base occupies a position adjacent to the 3'-end of the anticodons in tRNA's mentioned above (3,4). Other hypermodified bases such as N^6-(Δ^2-isopentenyl) adenine (IPA) and its 2-methylthio

[a]Abbreviations used: PCT, N-(purin-6-ylcarbamoyl)-L-threonine; PCTR, the riboside of PCT: PCTK, the K^+-salt of PCT; PCG, N-(purin-6-ylcarbamoyl)-glycine; PCGK, the K^+-salt of PCG; IPA, N^6-(Δ^2-isopentenyl)-adenine; 2-MT-IPA, the 2-methylthio analog of IPA.

analog (2-MT-IPA) occur in an analogous position in tRNA's responding to codons beginning with uracil (6,7,8). PCT did not show any cytokinin activity in tobacco and soyabean systems, but several of their analogs were good cytokinins (9,10,11). This paper describes the results of our studies on the riboside of PCT (PCTR), and forms a part of our investigation relating the three-dimensional structure of modified components of tRNA with their biological activity.

Methods. After many attempts, one crystal of PCTR ($C_{15}H_{20}O_8N_6$) was obtained from an aqueous solution. This crystal is monoclinic, space group $P2_1$ with cell constants at (22 ± 3)°C: a = 10.447(1), b = 16.894(2), c = 5.001(1), β = 94.69(5)°, Z = 2, $\rho_{calc.}$ = 1.56g.cm^{-3}. Three-dimensional intensity data (2036 reflections to the limit 2θ = 165° for CuKα radiation) were collected using a GE XRD-6 diffractometer and Ross filters by the stationary-crystal stationary-counter method (12). The structure was solved by a combination of vector search (13), multisolution (14,15) and trial and error methods and refined to an R of 0.04 using the least squares method with the block-diagonal approximation. Individual anisotropic thermal parameters were applied to the non-hydrogen atoms. The locations of the hydrogen atoms were obtained from electron-density difference maps; their positional parameters and individual isotropic thermal parameters were included in the refinement.

Results and Discussion: Figure 1 illustrates the conformation of the molecule and the bifurcated hydrogen interaction involving the hydrogen H(N11). The distal conformation of the molecule with the substituent on N(6) turned away from N(7) enables HN(11) to form an internal hydrogen bond to N(1) of adenine. (N(11)-

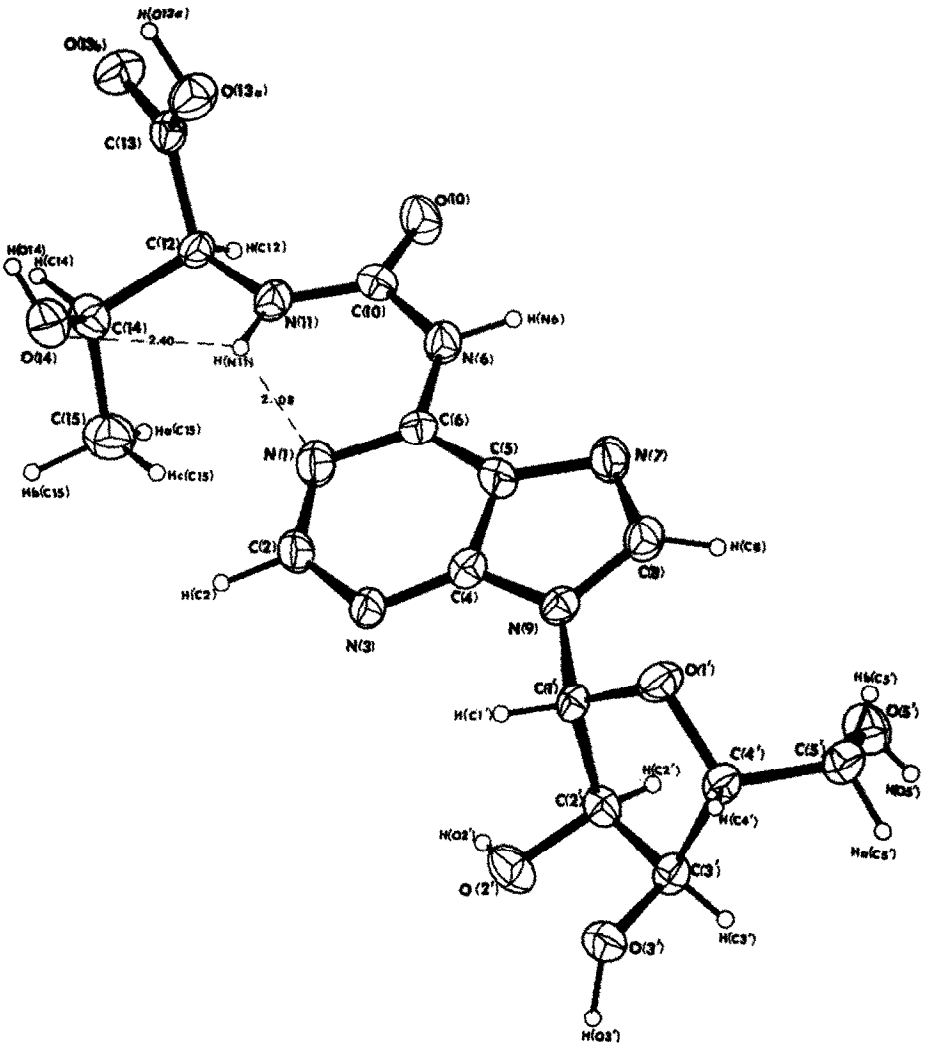

Figure 1
Conformation of the PCTR molecule and the intramolecular hydrogen bond.

H(N11) = 0.87Å, (N11)···N(1), 2.709Å, N(11)-H(N11)···N(1) = 128°); the particular conformation assumed by the threonine moiety affords a short contact between H(N11) and the hydroxyl oxygen O(14) of the threonine group. The atoms N(11), H(N11), N(1) and O(14) involved in this bifurcated hydrogen interaction are in a plane; such bifurcated interactions have been observed in a number of structures and have been discussed (16,17,18,19). The PCTR mole-

cule exhibits the anti conformation; the torsion angle χ_{CN} around the glycosidic bond (20,21) is 33°. The sugar ring has the C(2') endo conformation with the pseudo rotation parameters (22) τ_m = -35.5° and P = 145.1°. The conformation about the C(4')-C(5') bond is gauche-gauche; the torsion angles for O(5') with respect to O(1') and C(3') around the C(4')-C(5') bond are -63.7° and 57.3° respectively.

There is no stacking of the bases in the crystal structure. All polar hydrogens, bonded to oxygen or nitrogen atoms participate in hydrogen bonding; in addition, there is a C-H···O contact from C(1') to O(13b) of another molecule.

The trans orientation of the N^6-substituent with respect to the imidazole ring is found to occur in the neutral PCTR, and in anionic PCTK (23) and PCGK (24) forms of the ureidopurine molecule. This molecular conformation has important consequences on its biological properties, both as a free nucleotide and as a part of the anticodon loop in tRNA (23). This distal conformation of the modified base, the internal hydrogen bonding to N(1) and the particular conformation assumed by the threonine moeity block the two sites N^6-H and N^1 of adenine from taking part in the Watson-Crick base pairing (25) postulated for the double helix conformation and for codon recognition (26). This inability to base pair according to the Watson-Crick pairing is shared to varying degrees by the modified and hypermodified bases adjacent to and on the 3'-end of the anticodons. The anticodon-adjacent modified bases are all purines; they are 6-methyladenine, 2-methyladenine, IPA, 2-MT-IPA, PCT, 1-methylguanine, base Y, and 1-methylinosine (from the compilation of the known sequences of tRNA in Ref. 27). The crystallographic studies on IPA (28), 2-MT-IPA (29), and 6-methyladenine (30) have shown that in these molecules, the

N^6-substituent is trans to the imidazole ring, presumably due to the steric repulsion from N(7) (23). Consequently, N(6) and N(1) are blocked from taking part in the Watson-Crick base pairing. The methyl group in the 2-position of 2-methyladenine will impair the A-U base pairing, due to steric repulsion between the methyl group on adenine and the keto oxygen on the 2-position of uracil (Figure 2). In 1-methylguanine and 1-methylinosine, N(1) is not available for hydrogen bonding and the keto oxygen on C(6) in these purines is prevented from approaching appropriate base-pairing hydrogen bond donor by the bulky methyl group on N(1). Base Y, and the

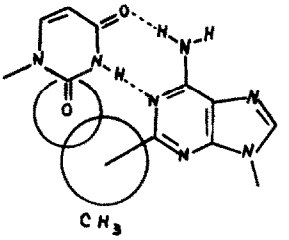

Figure 2
The A-U base pairing is impaired by the bulky methyl group on the 2-position of adenine.

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newly isolated N^6-methyl derivative of PCT (31) cannot base pair according to the Watson-Crick Scheme due to their chemical nature. Different chemical modifications of the bases may give rise to different functional roles for these bases, but a feature which appears to be common to all these is their inability to base pair according to the Watson-Crick Scheme. It is known that modified nucleosides play an important role in the ribosomal binding and in codon-anticodon interaction (32,33,34,35,36). From the genetic code, it has been inferred (26) that, in the first two positions of the codons, the four bases are clearly distinguished, and their

associations are restricted to the Watson-Crick pairs. It is important that no mistakes should occur in reading the first two bases of the codons. Any misreading at the first two position of codon reading may be prevented by the conformation of the anticodon loop (36). Tetranucleotide binding studies (37,38) along with suppression of frameshift mutations with an altered anticodon loop (39, 40,41) emphasize the importance of the chemical structure and the conformation of the anticodon loops. Our investigations show that a common characteristic of all the anticodon adjacent modified bases is their inability to take part in the Watson-Crick base pairing, and suggest that their function is to prevent misreading of the codons. Several tRNA's do not possess modified bases adjacent to the anticodons on the 3'-side; it is possible that in these tRNA's, proper codon reading is achieved by a selective three-dimensional conformation of the anticodon loop alone. The conformation of the ApApA molecule (42) which contains a helical section on the 5'-end and a sharp loop involving the 3'-end adenosine is interesting in this connection. In tRNA's, where such a preferential conformation may not be possible, modification of the bases adjacent to the anticodons can prevent misreading.

The inability of the modified bases to take part in the Watson-Crick Base pairing should promote a single stranded and flexible conformation for the anticodon loops. In this connection, it is interesting to note the C(2')-endo pucker of the sugar in the structure of PCTR.

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