CONFORMATION OF N-(PURIN-6YLCARBAMOYL)GLYCINE, A HYPERMODIFIED BASE IN tRNA

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Received February 8,1974

Summary. The crystal structure of the potassium salt of N-(purin-6ylcarbamoyl)glycine was determined from three-dimensional X-ray diffraction data. The N^6-substituent is distal (trans) to the imidazole ring, forming an intramolecular hydrogen bond N(glycine)-H---N(1)adenine. This conformation of the N^6-substituent is typical of ureidopurines, and blocks the two sites N^6-H and N^1 of adenine that are normally utilized for complementary base-pairing in the double helical regions of nucleic acids; the internal hydrogen bonding further enhances the shielding of N^1. This blocking of N^6-H and N^1 may be important in enhancing the single stranded conformation of the anticodon loop of tRNA and in preventing the modified adenosine adjacent to the anticodon from taking part directly in codon-anticodon interaction through the complementary base pairing.

Introduction. The ureidopurine derivative N-(purin-6ylcarbamoyl) glycine (PCG)[a] is an analog of N-(purin-6ylcarbamoyl)-L-threonine (PCT) which occupies a position adjacent to the 3'-end of the anticodon in several tRNA's which respond to the codons beginning with adenine (1,2,3). Though PCG has been isolated from enzyme digests of unfractionated yeast tRNA (4), it is not clear whether it occupies a position analogous to that of PCT in tRNA. PCT (and PCG) did not show any cytokinin activity in tobacco and soyabean systems, but several of their analogs were good cytokinins (5,6,7).

[a]Abbreviations used: PCT, N-(purin-6ylcarbamoyl)-L-threonine; PCG, N-(purin-6ylcarbamoyl)glycine; PCTK, the potassium salt of PCT; PCGK, the potassium salt of PCG.

This paper describes the results on PCGK, the potassium salt of PCG, and forms a part of our investigation relating the three dimensional structure of modified components of tRNA with their biological activity.

Methods. PCGK was crystallized as a monohydrate ($C_8H_7N_6O_3K \cdot H_2O$) from 1:3 water-propanol solutions. These crystals are monoclinic, Space group $P\ 2_1/c$ with cell constants at (22±3)C°: a = 14.063(3)Å, b = 7.218(1)Å, c = 14.424(1)Å, β = 129.12(1)°, Z = 4, $\rho_{obsd.}$ = 1.63g.cm^{-3}, $\rho_{calc.}$ (for monohydrate) = 1.66g.cm^{-3}. Three-dimensional intensity data (2242 reflections to the limit 2θ = 160° for CuKα radiation) were collected using a GE-XRD-490 automatic diffractometer and Ross filters by the stationary-crystal stationary-counter method (8). The structure was solved by the multisolution technique (9,10) and refined to an R of 0.058. The least squares method with the block-diagonal approximation was used for the refinement. Individual anisotropic thermal parameters were applied to non-hydrogen atoms and isotropic ones to the hydrogen atoms. The locations of the hydrogen atoms were obtained from electron-density difference maps; their positional parameters were included in the refinement.

Results and Discussion. Figure 1 shows the conformation of the molecule and the internal hydrogen bond between N(11)-H(11) of glycine to N(1) of adenine (N(11)-H(11), 0.85Å; N(11)--N(1), 2.738Å; N(11)-H(11)--N(1), 141°).

The purine, the ureidogroup, the nitrogen N(11), the C^{α}(C(12)) and C^{β}(C(13)) atoms of glycine are nearly coplanar. There is little self-association of the nucleic acid or amino acid components through hydrogen bonding. But the nucleic acid and amino acid components take part in hydrogen bonding to each other; N(6) and N(9)

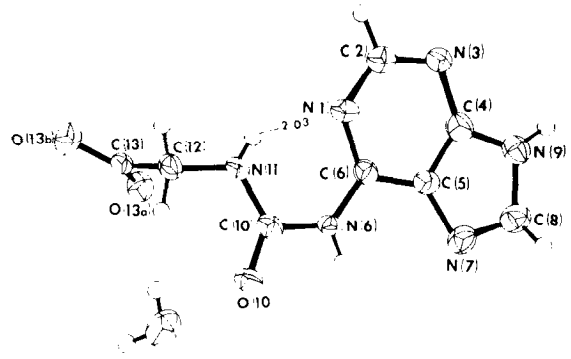

Figure 1.
Conformation of the N^6-substituent in PCGK and the intramolecular hydrogen bond.

are hydrogen bonded to O(13b) and O(13a). Such interactions may serve as models of protein-nucleic acid interactions.

All hydrogens that are covalently bonded to oxygen or nitrogen atoms participate in hydrogen bonding. This structure exhibits stacking and extensive overlapping of the ureidopurine moiety, in a head-to-tail fashion, in planes 3.2Å apart. The potassium ion is coordinated to two water oxygens, the keto oxygens of two ureido groups, two carbonyl oxygens, O(13b), and N(3); the coordination distances range from 2.68Å to 2.91Å. The seven-fold coordination of potassium ion cannot be described in terms of simple geometry. Such seven-fold coordination of potassium ion, though not usual, has been observed in several other structures, (see, for example, Ref.(11)).

The conformation of the substituent on N^6 with respect to the imidazole ring is important, since several N^6-mono-substituted adenines occur in nature both as free nucleosides and as part of macromolecules, possessing interesting biological properties. Due to steric hindrance from N^7, the distal (trans) conformation of the substituent on N^6 with respect to the imidazole ring is the most favorable in these mono-substituted adenines. This

trans conformation of the N^6-substituent, also observed in PCTK (12), will prevent the hydrogen on N^6 from taking part in the "Watson-Crick" base pairing postulated for the double helical regions of nucleic acids (13) but will favor Hoogsteen (14,15) or Haschmeyer-Sobell base (16) pairing. Further, the bulkiness, as well as the particular conformation of the N^6-substituent, partially shields N^1 of adenine from taking part in complementary base pairing, important in codon-anticodon interactions or double helix formation. This shielding of N(1) is assured by the intramolecular hydrogen bond and is virtually complete in PCGK. The close stacking of the bases observed in this structure, together with the blocking of N^6-H and N^1 of adenine suggest that hypermodified bases are important in enhancing the single-stranded conformation for the anticodon loops (17,18,19,20). This blocking of N^6-H and N^1 may be important in preventing any misreading of the codons by bases adjacent to the anticodons. The intramolecular hydrogen bond to N^1 of adenine is interesting in connection with cytokinin activity and will be discussed elsewhere.

Acknowledgements. R.P. is thankful to Dr. D. Harker for valuable discussions, and G.B.C. acknowledges the keen interest and encouragement of Drs. A. Mittelman and G.P. Murphy. Excellent technical help was provided by Mrs. N. Winiewicz and Mrs. G. Hazel. This work in part was supported by a grant from U.S.P.H.S. (CA-14185).

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