A SYN CONFORMATION FOR INOSINE, THE WOBBLE NUCLEOSIDE IN SOME tRNA's

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SUMMARY: The crystal structure of an orthorhombic form of inosine was determined from three-dimensional X-ray diffraction data. There are two crystallographically independent inosine molecules, both of which assume a syn conformation that is different from the conformations found in other crystalline forms of inosine. Apparently, inosine has considerable conformational freedom, a property that may be required at the "wobble" position of anticodon triplets.

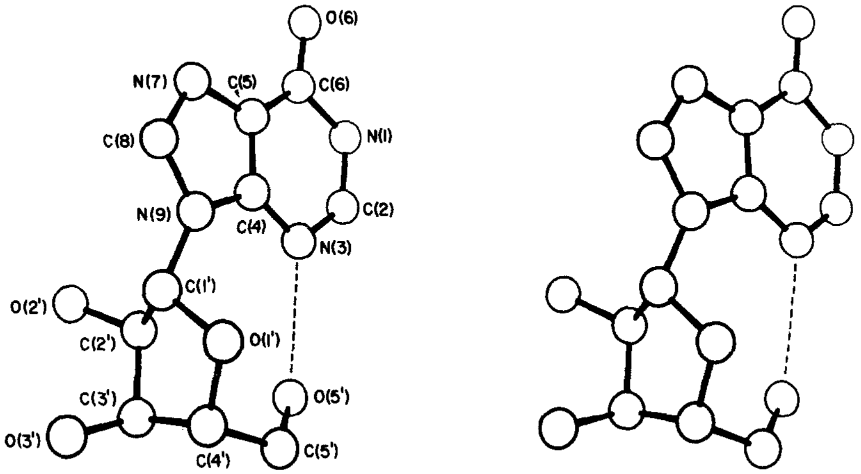
Inosine occurs at the 5'-end (the "wobble" position (1)) in the anticodon triplets of several tRNA's (2). A detailed knowledge of the conformational properties of this nucleoside is necessary for understanding the factors involved in codon-anticodon interactions, and for model-building studies of tRNA. X-ray studies of two crystal forms of inosine have been reported (3,4). In this paper we describe the structure of a third crystal form, in which inosine assumes a conformation that is considerably different from those found in the other crystal structures.

METHODS

Orthorhombic inosine was crystallized from aqueous solution, at 20°C, as transparent needles elongated along (001). The nucleoside crystallizes in space group $P2_12_12_1$, with $\underline{a}$ = 13.261(2), $\underline{b}$ = 21.285(2) and $\underline{c}$ = 8.097(1) Å. There are eight molecules in the unit cell and thus two crystallographically independent molecules in the asymmetric unit. Intensities for 1884 unique reflections were measured on an automated diffractometer, by using copper Kα radiation. Trial coordinates for all 38 nonhydrogen atoms were obtained by direct methods, with the use of the computer program MULTAN (5). Coordinates of the nonhydrogen atoms, along with anisotropic temperature factors, were refined by least-squares. All hydrogen atoms were located in difference Fourier maps calculated during the latter stages of refinement, and were included in structure factor calculations, but not in the least-squares refinement. The final R index ($=\Sigma||Fo|-|Fc||/\Sigma|Fo|$) for all reflections is 0.065.

RESULTS AND DISCUSSION

The crystal structure of orthorhombic inosine is nearly identical to (isomorphous with) that of 6-thiopurine riboside (6-TPR), which has been described in detail by Shefter (6). The hydrogen-bonding pattern is the same as that of 6-TPR, except for differences in the hydrogen-bond lengths. As in the 6-TPR structure, all hydrogen atoms that are covalently bonded to oxygen or nitrogen atoms participate in hydrogen-bonding and there are several close C-H---O contacts. The purine moieties of the two independent inosine molecules are stacked in the $\underline{c}$ direction with an interplanar spacing of about 3.3 Å. The stacking pattern is essentially the same as that of 6-TPR, which stacks with a slightly larger interplanar spacing of about 3.5 Å.



INOSINE - 1

INOSINE - 2

Figure 1.

Conformations of the two crystallographically independent inosine molecules (hydrogen atoms are not shown).

Figure 1 shows the nearly identical conformations of the two crystallographically independent inosine molecules, designated as inosine-1 and inosine-2. The molecules are in the *syn* conformation; the torsion angle ϕ_{CN}, which defines the conformation about the C(1')-N(9) bond (7), is 121.9° for inosine-1 and 125.5° for inosine-2. These ϕ_{CN} values are close to a minimum predicted for the *syn* region in the nonbonded, potential energy curve of inosine, as calculated by Wilson and Rahman (8). The ribose moieties are in the C(2')-*endo* conformation, with atom C(2') displaced about 0.6 Å from the plane of the other four atoms of the ribose rings. The conformation about the C(4')-C(5') bond of the ribose moieties is *gauche*-*gauche* (9) [i.e. the C(5')-O(5') bond is *gauche* with respect to the C(4')-O(1') bond and also *gauche* with respect to the C(4')-C(3') bond]; for inosine-1 ϕ_{OO} = 62.7° and ϕ_{OC} = 56.7°, and for inosine-2 ϕ_{OO} = 57.6° and

ϕ_{OC} = 64.2°. Both molecules display O(5')---N(3) intramolecular hydrogen bonds, with an O(5')---N(3) distance of 2.80 Å for inosine-1 and 2.89 Å for inosine-2.

In addition to the orthorhombic form of inosine, the crystal structures of inosine dihydrate (3) and a monoclinic form of anhydrous inosine (4) have also been determined. Like the orthorhombic form of inosine, inosine dihydrate crystallizes with two independent molecules in the asymmetric unit. Thus conformational data are now available for inosine in five different crystalline environments. These data are summarized in Table 1. In all cases, the ribose moieties are puckered with either C(2')-endo or C(3')-endo, and the conformation about the C(4')-C(5') bond is either gauche-gauche or gauche-trans. The greatest amount of variability occurs in ϕ_{CN}, the torsion angle about the C(1')-N(9) glycosidic linkage. For two of the inosine molecules, the glycosidic torsion angle falls within the conventional anti range (7) which is centered at about ϕ_{CN} = -30°; for two others this angle falls into the conventional syn range (7) which is centered at about ϕ_{CN} = 150°; and, for still another inosine molecule, ϕ_{CN} assumes the unusual value of -121°. Considering only these crystallographic data, it is apparent that inosine can assume at least four distinctly different conformations:

(1) ϕ_{CN} anti, C(2')-endo, ϕ_{OO} gauche and ϕ_{OC} gauche;
(2) ϕ_{CN} anti, C(3')-endo, ϕ_{OO} gauche and ϕ_{OC} trans;
(3) ϕ_{CN} syn, C(2')-endo, ϕ_{OO} gauche and ϕ_{OC} gauche; and
(4) $\phi_{CN} \cong -121°$, C(2')-endo, ϕ_{OO} gauche and ϕ_{OC} gauche.

In the "wobble" position of anticodon triplets, inosine can pair with adenine, uracil or cytosine at the third position

Table 1.

Conformational data for the three crystalline forms of inosine (g = *gauche* and t = *trans*).

STRUCTURE	ϕ_{CN}	PUCKER OF RIBOSE	CONFORMATION ABOUT C(4')-C(5')	
			ϕ_{OO}	ϕ_{OC}
Inosine Dihydrate				
Molecule-1	-121°	C(2')-*endo*	55° (g)	64° (g)
Molecule-2	-48 (*anti*)	C(2')-*endo*	73 (g)	47 (g)
Monoclinic Inosine	-11 (*anti*)	C(3')-*endo*	75 (g)	169 (t)
Orthorhombic Inosine				
Molecule-1	122 (*syn*)	C(2')-*endo*	63 (g)	57 (g)
Molecule-2	125 (*syn*)	C(2')-*endo*	58 (g)	64 (g)

of codon triplets (1), and it is possible that the nucleoside must undergo conformational changes in order to form these different base pairs. Although inosine may have less conformational freedom when incorporated into polynucleotides (10), it is now clear that the unsubstituted nucleoside is flexible enough to assume various conformations in different crystalline environments.

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