THE MOLECULAR STRUCTURE OF THE DISULFIDE OF THE t-RNA CONSTITUENT, 4-THIOURIDINE

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Simultaneously with the discovery of sulfur containing bases in transfer RNA (t-RNA)[1] began a search for the possible role of these minor constituents in the determination and regulation of the secondary structure of t-RNA. It was observed that mild oxidation of sulfur containing soluble RNAs from different sources decreases amino acceptor activity (Carbon et al., 1965) and binding capacity of the t-RNA (Goehler and Doi, 1966). Since these effects could readily be reversed by reduction with thiosulfate, it has been suggested that the mechanism of inactivation and reactivation may involve disulfide formation and reduction, respectively. This was supported by the isolation of 4-thiouridylate disulfide from oxidized tyrosine t-RNA of E. coli (Lipsett, 1967) and further substantiated by studies of the effect of oxidation on the physicochemical properties of this t-RNA (Lipsett and Doctor, 1967). These investigations provided evidence that an oxidation is accompanied by an alteration of the secondary structure of the t-RNA.

In the present study the molecular structure of the disulfide of 4-thiouridine was determined by x-ray diffraction techniques in order to gain further insight into the structural effects of the disulfide formation in t-RNA.

The investigation tends to indicate that a significant change in the

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1) Abbreviations used are as follows: t-RNA, transfer RNA; 4-URS_2, 4,4'-di(thiouridine); 5-$UdRS_2$, di-t-(1-(2'-deoxy-α-D-ribofuranosyl) uracilyl) disulfide; APS_2, di-(2-aminophenyl) disulfide.

secondary structure of the t-RNA would result upon oxidation if the conformation about the disulfide linkage of 4-URS_2 is the same in the polymer as it is in the crystal. The distinct conformation found for this linkage should be taken into account in any attempt to build molecular models of the oxidized form of the t-RNA containing 4-thiouridine residues.

EXPERIMENTAL

Very fine pale yellow needles were derived by recrystallizing the compound (supplied by Cal Biochem. Inc.) from an 80% ethanol-water solution. The following crystallographic data were obtained for these monoclinic needles:

$\underline{a} = 14.480 \pm 0.006$ Å	$D_{measured} = 1.585$ g./cm^3 (by flotation)
$\underline{b} = 15.579 \pm 0.005$ Å	$D_{calc} = 1.592$ g./cm^3 (based on monohydrate)
$\underline{c} = 5.001 \pm 0.002$ Å	Z (no. of molecules in unit cell) = 2
$\beta = 95.97 \pm 0.09^{o}$	Space Group P 2_1

The intensity data were collected on a G.E. XRD-6 diffractometer by the stationary crystal-stationary counter technique using balanced Ni and Co filters. The intensities were converted to structure factor amplitudes by applying Lorentz-polarization and α_1-α_2 splitting corrections. No absorption corrections were applied because of the small size of the crystal utilized (0.7 x 0.03 x 0.05 mm).

The positions of the sulfur atoms were deduced from a sharpened Patterson function, and the other atoms (with the exception of three hydrogens) were subsequently located in Fourier electron density maps. The positional and thermal parameters (non-hydrogen atoms given anisotropic temperature factors) were refined by least squares using a block diagonal approximation. The final R value (usual crystallographic discrepancy index) is 0.079 for all the observed data.

RESULTS AND DISCUSSION

The overall molecular conformation of the compound as viewed down the c-axis of the crystal is illustrated in Figure 1. The structural features observed for this molecule are outlined below.

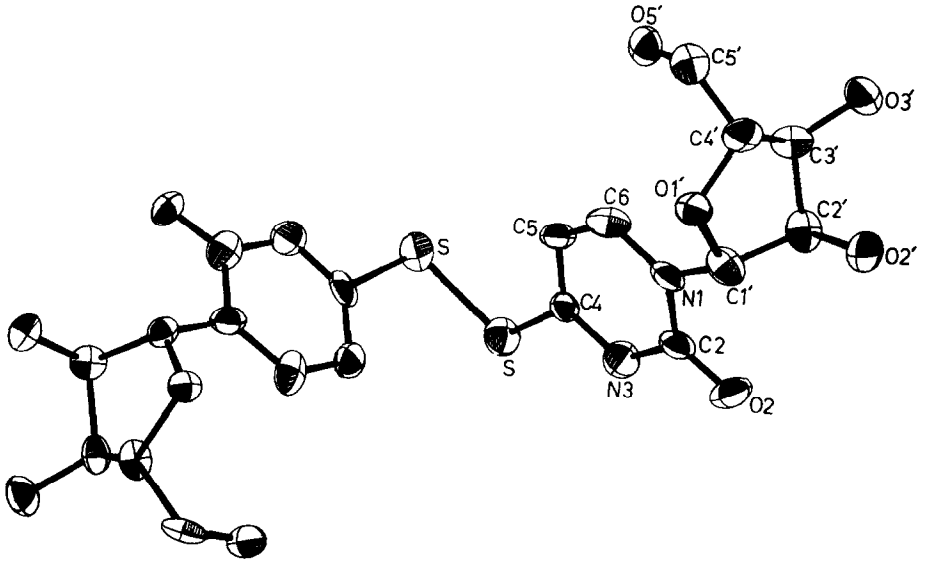

Figure 1. A view of the molecule showing the thermal motion of the various atoms. Drawn by a program written by Johnson (1965).

(1) Within the limits of experimental error, the bond distances and angles of the two nucleosidic residues connected through the S-S bond are similar. They are also in reasonable agreement, where appropriate, with those found in the crystallographic studies of other nucleic acid fragments.

(2) The atoms comprising each of the pyrimidine nuclei are coplanar; but the exocyclic C1' atoms are significantly displaced from the base planes by 0.07 Å and 0.10 Å. The O2 and S atoms attached to one base are essentially coplanar with the heterocyclic ring while in the other pyrimidine system they are removed from the plane by a significant amount,

0.08 Å and 0.06 Å, respectively. The distortions of the exocyclic bonds from the base planes are not unusual, in that similar effects have been observed in other nucleoside and nucleotide crystal structures. These are most likely attributable to intermolecular forces within the crystal, such as hydrogen bonds.

(3) The torsion angles about the glycosidic bonds (C1'-N1), defined as the twist angle between the trace of the plane of the base and the C1'-O1' bond (Donohue and Trueblood, 1960) are -17° and -18°. The anti configuration is typical of all the pyrimidine nucleosides and -tides that have been studied to date, and is in basic agreement with models of the nucleic acids. Likewise the glycosidic bond lengths, 1.48 Å and 1.49 Å are similar in magnitude to those found in other structures.

(4) The furanose rings are both significantly puckered with C3' being displaced endo, i.e., on the same side as C5', from the plane of the other four atoms. The displacements of C3' from the plane of the other four furanose ring atoms are 0.54 Å and 0.58 Å. This type of distortion is commonly found in nucleoside structures (Sundaralingam, 1965; 1968).

(5) The nature of the disulfide linkage between the two nucleosidic residues is of major biological interest. Various parameters obtained for the S-S bond are given in Table I with those of two other structures that are somewhat related. The S-S bond length of 4-URS_2 is similar to that found in many aliphatic disulfides (Hordvik, 1966), but somewhat shorter than the length in the structure of 5-$UdRS_2$ (Shefter et al., 1967).

The torsion angle about the disulfide, ϕ_{ss}, defined in the manner prescribed by Klyne and Prelog (1960) is -87°. Figure 2 is a projected view of a portion of the molecule down the S-S bond showing this twist angle. The left-handed chiral (i.e., minus twist sense) was also found in the crystal structure of 5-$UdRS_2$. The disymmetric nature of the disulfide linkage in 4-URS_2 and 5-$UdRS_2$ concurs with the ORD studies of Irie and co-workers (1968) and Kotick (1968) on these compounds, respectively.

The magnitude of this angle in the present structure may be accounted for by the minimization of the electron repulsion of the lone pair 3-p electrons of the adjacent sulfur atoms (Pauling, 1949).

One of the distinguishing characteristics of 4-URS_2 with the other two compounds tabulated are the configurations about the C-S bonds. A comparison of the torsion angles, $\phi_{x\text{-}C\text{-}S\text{-}S}$, i.e., the projected angle between the X-C bond and the disulfide bond, shows the marked difference in the configuration of the disulfide linkage of the three structures. In 4-URS_2 the S-S bond almost lies in the plane of the pyrimidine ring and approximately eclipses the C4-C5 bond. In the other two structures the S-S bond is reasonably perpendicular to the ring system.

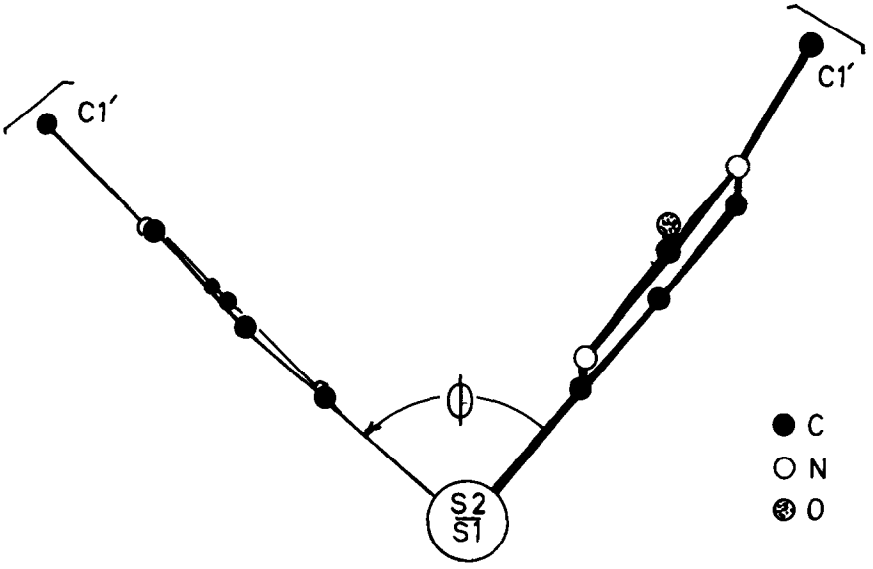

Figure 2.

The denaturation studies of Lipsett and Doctor (1967) indicated that the 4-thiouridine residues of tyrosine t-RNA participate in hydrogen bonding and therefore are most likely part of a helical region of the nucleic acid.

TABLE I

Structural Parameters of Some Disulfides[2)]

Compound	ϕ_{ss} (°)	S-S (Å)	$\phi_{x\text{-}C\text{-}S\text{-}S}$ (°)	C-S (Å)	C-S-S (°)	Reference
4-URS_2	-87.3	2.022 (0.004)	x = N3 179; 175 x = C4 -1; -6	1.79 (0.02)	104 (0.4)	
5-$UdRS_2$	-49.9	2.108 (0.003)	x = C4 -76; -90 x = C6 100; 88	1.76 (0.01)	102 (0.2)	Shefter et al. (1967)
APS_2	±83	2.1	x = C ave. 94	1.8	106	De Mesquita (1967)

2) Standard deviations are given in parenthesis under their respective value where obtained. Accuracy of APS_2 structure much lower than other two.

Considering the present structural findings it can be concluded that considerable strain would not only be imposed on these regions by the changes in the electronic structure of the bases on oxidation but by virtue of the conformation of the disulfide linkage itself. The minimization of the energies would most likely require conformational changes in the macromolecule effecting several residues involved in the maintenance of the secondary structure of t-RNA.

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