CRYSTAL AND MOLECULAR STRUCTURE OF THE ACETONIDE OF 5-METHYLAMINOMETHYL-2-THIOURIDINE

A minor constituent of *Escherichia coli* tRNAs

H. KASAI, S. NISHIMURA, H. VORBRÜGGEN+ and Y. IITAKA*

Biology Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo, Japan, +Forschungslaboratorien der Schering AG, Berlin/Bergkamen 1 Berlin 65, Muilerstrasse 170-178, FRG and **Faculty of Pharmaceutical Sciences, Tokyo University, Hongo 7-chome, Bunkyo-ku, Tokyo, Japan*

Received 19 April 1979

1. Introduction

5-Methylaminomethyl-2-thiouridine mnm^5s^2U is a minor constituent of *Escherichia coli* $tRNA_2^{Glu}$ [1,2] and $tRNA_1^{Gln}$ [3]. This nucleoside is located in the first position of the anticodon and recognizes A preferentially to G in the third letter of the codon sequence [1,4]. According to the wobble theory, U in the first position of the anticodon can form a base-pair with either A or G in the codon. Thus it is supposed that the bulky sulfur atom instead of oxygen at position 2 of uridine makes base-pairing with G difficult. Therefore, it is interesting to compare the molecular structure of mnm^5s^2U with those of other uridine derivatives. This communication describes analysis of the crystal structure of a derivative of mnm^5s^2U, 5-methylaminomethyl-2-thiouridine acetonide hydrochloride. The crystal structure of free mnm^5s^2U was reported [5]. There were significant differences between their results and ours. Differences in bond lengths and angles of uracil base seem to be due to differences in the state of protonation at the N(3) atom.

2. Materials and methods

The preparation of mnm^5s^2U used in this study was synthesized as in [6]. The acetonide was prepared by the reaction of mnm^5s^2U with acetone–dimethoxypropane–HCl mixture. The hydrochloride of mnm^5s^2U acetonide thus obtained was purified by recrystallization twice from water, and finally the crystals were grown by slow evaporation of the aqueous solution at room temperature. Single crystals appeared in ~10 days. Data on the crystals are given in table 1.

3. Results and discussion

The crystal structure was deduced by the Patterson method. The packing of molecules and the probable hydrogen bonding are shown in fig.1. An intermolecular hydrogen bond N(3)–H...O(5′) is observed. The sidechain amino group has a great tendency to form a hydrogen bond with electronegative groups. In the present structure, the amino group is hydrogen bonded to the 2 chloride anions with N–H...Cl distances of 3.062 and 3.081 Å as shown in fig.1. It does not form the hydrogen bonded 8-membered ring

Table 1
Data on crystals of 5-methylaminomethyl-2-thiouridine acetonide hydrochloride

Formula	$C_{14}H_{19}N_3O_5S{\cdot}HCl$
Mol. wt	379.7
System	Orthorhombic
a	9.690 (6)
b	35.437 (20)
c	5.172 (3)
Space group	$P2_12_12_1$
Z	4

Elsevier/North-Holland Biomedical Press

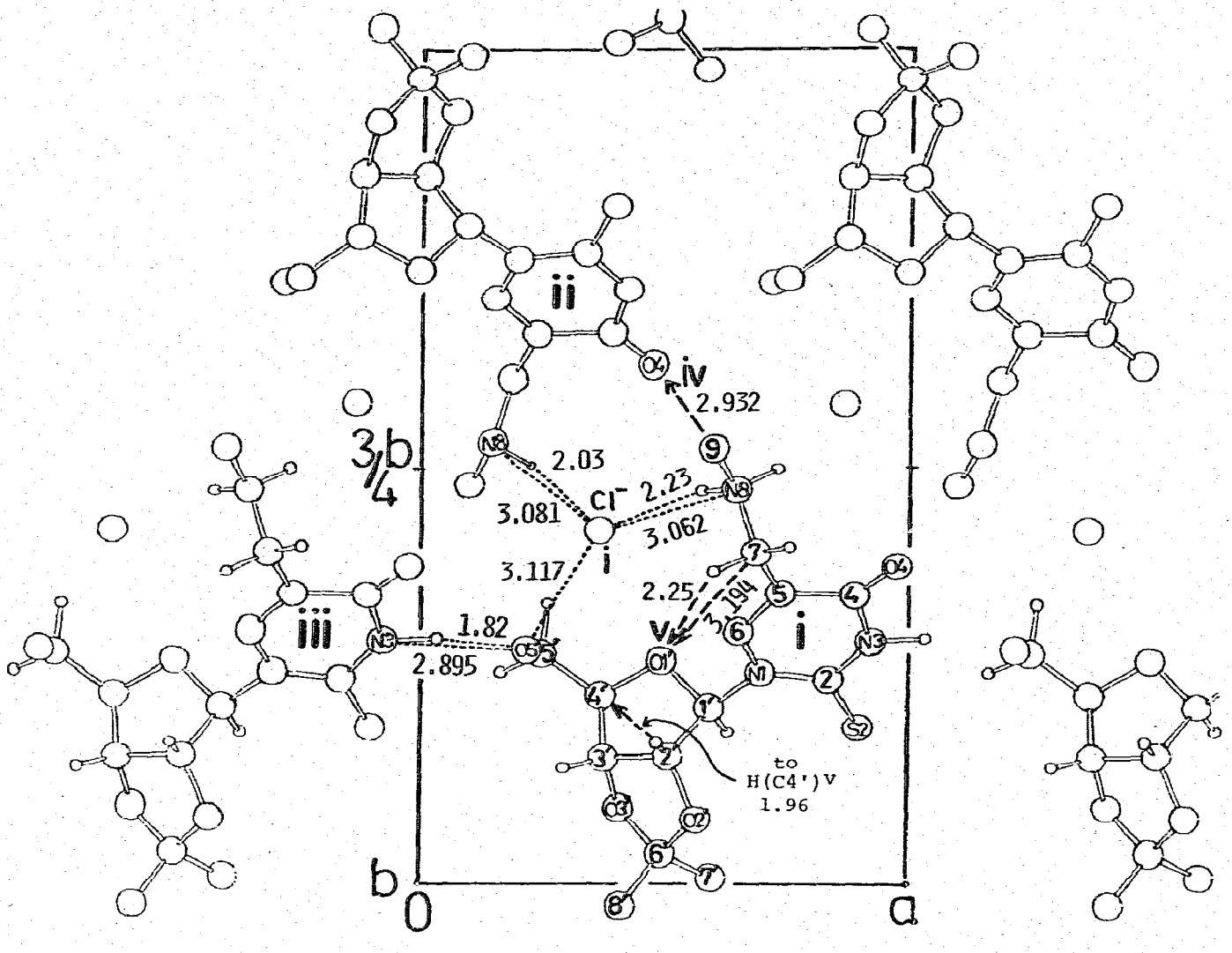

Fig.1. Projection of the crystal structure along the *c* axis. Hydrogen bonds are shown by dotted lines and short intermolecular interatomic distances are shown by broken lines. Symmetry operations are: (i) x, y, z; (ii) $-1/2 + x, 3/2 - y, 1 - z$; (iii) $-1 + x, y, z$; (iv) $-1/2 + x, 3/2 - y, -z$; (v) $x, y, -1 + z$.

system involving O(4) and a water molecule observed in the crystal of the free nucleoside [5].

The plane of the pyrimidine ring and the plane which includes C(5)–C(7)–N(8)–C(9) are vertical to each other. The glycosidic torsion is typically anti; $\chi = 48.5°$. The conformation about the C(4′)–C(5′) bond is *gauche–gauche*; $\phi o1'-o5' = -72.65°$, $\phi c3'-o5' = 45.91°$. The sugar ring has a C(2′)-endo conformation: $\tau_0 = -39.40°$, $\tau_1 = 38.13°$, $\tau_2 = -23.00°$, $\tau_3 = 0.62°$, $\tau_4 = 23.91°$. It is possible that on acetonide formation the conformation of the sugar ring changes from the C(3′)-endo [5] to C(2′)-endo form.

The bond lengths and bond angles are listed in tables 2 and 3, respectively. The structures of various uridine derivatives have already been determined by X-ray analyses, and the bond lengths and angles of these derivatives are also listed in tables 2, 3 for comparison [5,7–10]. As seen in these tables, the differences in the bond lengths and angles of the base from those observed in free mnm^{5}s^{2}U [5] are quite significant. The shortening of the C(4)–O(4) and

Table 2
Comparison of the bond lengths (Å) in bases

Bond distances (Å)	mnm^5s^2U acetonide, HCl	mnm^5s^2U free	mcm^5s^2U	s^2U	m^5U	Br^5U
N(1)–C(2)	1.396(11)[a]	1.42	1.37	1.368	1.377	1.40
C(2)–N(3)	1.380(12)	1.30	1.31	1.360	1.376	1.37
N(3)–C(4)	1.402(13)	1.37	1.47	1.388	1.384	1.39
N(1)–C(6)	1.385(12)	1.35	1.40	1.381	1.361	1.35
C(4)–C(5)	1.505(13)	1.47	1.39	1.435	1.437	1.43
C(5)–C(6)	1.348(12)	1.33	1.39	1.337	1.345	1.36
C(2)–X[b](2)	1.652(10)	1.72	1.74	1.677	1.196	1.23
C(4)–O(4)	1.169(13)	1.27	1.18	1.228	1.223	1.23
N(1)–C(1′)	1.475(11)	1.45	1.50	1.500	1.481	1.49
C(5)–C(7)	1.484(13)					
C(7)–N(8)	1.473(11)					
N(8)–C(9)	1.503(13)					
Reference		[5]	[7]	[8]	[9]	[10]

[a] Estimated SD
[b] X = S or O

C(2)–S bonds by 0.10 and 0.07 Å, respectively, and the lengthening of the C(2)–N(3) bond by 0.08 Å and of many other bonds by ~0.03 Å, as compared with those in free mnm^5s^2U, reflect the greater contributions of the di-keto form to the present structure. This may be the consequence of protonation of the N(3) atom of mnm^5s^2U in the hydrochloride, which is also reflected in the widening of the C(4)–N(3)–C(2) angle by ~6°. The greater bond angles (120°–126°) subtended at the terminals of the presumed double bonds, O–C(4)–N(3), O–C(4)–C(5), S–C(2)–N(1), S–C(2)–N(3), C(6)–C(5)–C(4), C(6)–C(5)–C(7) and C(5)–C(6)–N(1) may also reflect the contribution of the di-keto form to the present structure.

Table 3
Comparison of the bond angles (°)

Bond angles (°)	mnm^5s^2U acetonide, HCl	mnm^5s^2U free	mcm^5s^2U	s^2U	m^5U	Br^5U
C(6)–N(1)–C(1′)	118.0(7)	122.4	118	121.3	121.5	120.0
C(2)–N(1)–C(1′)	119.8(7)	120.1	124	117.8	116.8	117.5
C(2)–N(1)–C(6)	122.2(7)	117.4	118	120.8	121.6	122.0
N(1)–C(2)–N(3)	113.8(8)	120.3	121	116.0	114.1	114.0
N(1)–C(2)–X(2)	124.2(7)	117.2	120	123.4	123.0	123.8
N(3)–C(2)–X(2)	122.0(7)	122.4	119	120.7	122.9	121.7
C(4)–N(3)–C(2)	130.1(8)	123.8	128	126.6	127.2	127.8
C(5)–C(4)–N(3)	111.1(8)	115.9	107	114.4	115.4	112.8
C(5)–C(4)–O(4)	126.4(9)	122.6	135	126.4	125.4	125.8
N(3)–C(4)–O(4)	122.5(9)	121.3	118	119.2	119.2	121.4
C(6)–C(5)–C(4)	120.1(8)	118.3	129	119.5	117.6	121.4
C(5)–C(6)–N(1)	122.6(8)	123.8	118	122.6	124.2	121.3
C(6)–C(5)–C(7)	121.7(8)					
C(4)–C(5)–C(7)	118.1(8)					
N(8)–C(7)–C(5)	114.2(8)					
C(9)–N(8)–C(7)	112.6(7)					
Reference		[5]	[7]	[8]	[9]	[10]

In crystals of free mnm^{5}s^{2}U, protonation of the basic secondary amino group causes deprotonation of N(3) [5], while in the hydrochloride protons are fully supplied by hydrochloride and the nucleoside adopts the protonated form. All the uridine derivatives given in tables 2, 3 with no such extra basic groups in the crystal, are protonated at the N(3) atom. This is similar to the case in tRNA molecules.

Comparison of the bond lengths with those of other uridine derivatives listed in table 2 indicates the elongation of the C(4)–C(5) bond can be attributed not to S(2) substitution but to methylaminomethyl substitution at position 5, because the bond length in s^{2}U is similar to those of other uridine derivatives and the length in free mnm^{5}s^{2}U is significantly more than in these derivatives, though it is still shorter than in the present structure. Furthermore, in mcm^{5}s^{2}U, the C(4)–C(5) bond is shorter than in other uridine derivatives.

Marked differences were also observed between the lengths of C(2)–N(3), N(3)–C(4) and C(2)–S(2) bonds in mnm^{5}s^{2}U (acetonide, HCl) and mcm^{5}s^{2}U, although both compounds are 5-substituted 2-thiouridine derivatives. In particular, the longer C(2)–N(3) bond and shorter C(2)–S(2) bond in mnm^{5}s^{2}U (acetonide, HCl) than in mcm^{5}s^{2}U suggest that the contribution of the di-keto form is greater in mnm^{5}s^{2}U than in mcm^{5}s^{2}U. In this connection it should be mentioned that mcm^{5}s^{2}U and mnm^{5}s^{2}U have slightly different coding properties; i.e., mcm^{5}s^{2}U specifically recognizes A but not G [4], while mnm^{5}s^{2}U recognizes G slightly as well as A [1]. It is tempting to speculate that this characteristic of mnm^{5}s^{2}U is due to the stronger hydrogen bonding capacity of the pyrimidine base effected by the methylaminomethyl side chain. The C(2)–S(2) distance in mnm^{5}s^{2}U, acetonide, HCl (1.652 Å) is similar to that in s^{2}U (1.677 Å) [8], whereas in mcm^{5}s^{2}U it is significantly longer (1.74 Å). On the other hand, the C(2)–O(2) distances of other typical uridine derivatives are ~1.2 Å [9,10]. These results may account for the preferential recognition of A by mnm^{5}s^{2}U and mcm^{5}s^{2}U. The difference in the coding properties of mnm^{5}s^{2}U and mcm^{5}s^{2}U may also be explained by the difference in bond length C(2)–S(2), because S(2) may be used by hydrogen bonding with G in the wobble position.

As described before, the very short C(4)–O(4) and C(2)–S lengths and rather longer C(2)–N(3) and N(3)–C(4) lengths in mnm^{5}s^{2}U (acetonide, HCl) compared with those in all other derivatives except mcm^{5}s^{2}U, indicate a greater shift of the keto–enol tautomerism to the keto form. This form is essential for hydrogen bonding of the base with A in the normal Watson-Crick base pair as well as with G in the wobble position. In free mnm^{5}s^{2}U, on the other hand, there is a shift to the enol form, as evidenced by elongation of the C(4)–O(4) and C(2)–S bonds and shortening of the C(2)–N(3) bond.

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