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Modified bases in tRNA: the structures of 5-carbamoylmethyl- and 5-carboxymethyl uridine

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ABSTRACT

The crystal structures of two nucleosides, 5-carbamoylmethyluridine (1) and 5-carboxymethyluridine (2), were determined from three-dimensional x-ray diffraction data, and refined to R=0.036 and R=0.047, respectively. Compound 1 is in the C3'-endo conformation with χ= +5.2° (anti), ψ^{co}= +63.4° and $\psi^{c\alpha}$= +180.0° (tt); 2 is in the C2'-endo conformation with χ= +49.4° (anti), ψ^{co}= -60.5° and $\psi^{c\alpha}$= +60.0° (gg). For each derivative, the plane of the side chain substituent is skewed with respect to the plane of the nucleobase; for 1, the carboxamide group is on the same side of the uracil plane vis à vis the ribose ring; for 2, the carboxyl group is on the opposite side of this plane. No base pairing is observed for either structure. Incorporation of structure 1 into a 3'-stacked tRNA anticodon appears to place O8 within hydrogen bonding distance of the O2' hydroxyl of ribose 33, which may limit the ability of such a molecule of tRNA to "wobble".

INTRODUCTION

An increasing number of pyrimidines found in nature, either in specific nucleic acids or in antibiotics, are characterized by the presence of an alkyl substituent at position 5. Their list includes pyrimidines such as 5-hydroxymethyl cytosine[1] in the DNA of *E. coli* T even phages, 5-hydroxymethyl uracil[2] in the DNA of *B. subtilis* phage SP 8, the antibiotic Bacimethrin[3] from *B. megatherium*, 5-(4',5'-dihydroxypentyl)uracil[4] from *B. subtilis* phage SP 15, and the universal nucleoside pseudouridine[5] in loop IV of tRNAs involved in protein synthesis.

In addition several modified uridines possessing a carboxymethyl side chain at position 5 are widely distributed in tRNAs in both procaryots and eucaryots. The β-D-ribonucleosides of 5-carboxymethyl-[6], 5-carbomethoxymethyl-[7], 5-carbamoylmethyluracil[8], the respective 02'-methyl-[9] as well as some 2-thio-derivatives[10] have been isolated and characterized[11]. Interestingly these nucleosides, when present, occupy the "wobble" position of various anticodon sequences[12].

The effects of the 5-alkyl groups in these pyrimidine nucleotides remain to a large extent unexplained. The presence of such groups must certainly influence the immediate and perhaps the overall structures of polynucleotides in which they occur. A knowledge of the detailed structural features and conformation of such nucleosides could illuminate that point. In this context we report here our results of an x-ray crystallographic study of 5-carbamoylmethyl uridine (ncm^5U) and 5-carboxymethyl uridine (cm^5U)[13].

EXPERIMENTAL

Well formed crystals of 5-carbamoylmethyl and 5-carboxymethyl uridine were selected for data collection. The experimental details are summarized in Table 1. The program MULTAN[14] was used for structure determinations. Scattering factors were from the International Tables for X-ray Crystallography[15] and for the hydrogen atoms those of Stewart[16] et al. The heavier atoms were refined anisotropically and the hydrogen atoms isotropically. The final R values are .036 for 5-carbamoylmethyl uridine and .047 for 5-carboxymethyl uridine. Table 2 contains the final coordinates and the structure factors are deposited.

The bond distances and angles are similar to one another and agree well with those found in other uracil derivatives[17]. The ribose ring of the carboxymethyl derivative is in the C2'endo conformation and has an χ angle of 49.4° (anti). The ribose ring of the carbamoylmethyl uridine is C3'endo and has the low value for χ (5°) normally associated with C3'endo sugar rings. The conformation of the C4'-C5' bond in the carboxymethyl derivative

Table 1. Crystal data on 5-carboxymethyl uridine and 5-carbamoylmethyl uridine

Formula	$C_{11}H_{14}N_2O_8$	$C_{11}H_{15}N_3O_7$
Crystal system	Orthorhombic	Orthorhombic
Space Group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a	13.360 (3) Å	10.905 (2) Å
b	17.160 (3)	22.380 (3)
c	5.479 (1)	5.114 (1)
V	1256 $A^{\circ 3}$	1248 $A^{\circ 3}$
Cell contents	4 molecules	4 molecules
No. of independent intensity data collected	1364	1274
No. of reflections below threshold value	291	171
Radiation used	CuKα (1.5418 Å)	CuKα (1.5418 Å)
Structure solution	Direct Methods	Direct Methods
Final R value	0.047	0.036
Estimated standard deviations in C-O or C-C bond lengths	.006 Å	.004 Å
Estimated standard deviations for angles	0.4°	0.3°

Table 2. Atomic parameters and their estimated standard deviations for 5-carboxymethyl uridine and 5-carbamoylmethyl uridine

Positional parameters are expressed as fractions of unit cell edges. Anisotropic temperature factors are expressed as: $\exp[-1/4(h^2a^{*2}B_{11}+k^2b^{*2}B_{22}+l^2c^{*2}B_{33}+2hka^*b^*B_{12}+2hla^*c^*B_{13}+2klb^*c^*B_{23})]$. The isotropic temperature factors are of the form $\exp(-B\sin^2\theta/\lambda^2)$ with B values given in $Å^2$. The standard deviation for each parameter, determined from the inverted full matrix, is given in parentheses and applies to the last specified digits. Fractional coordinates are x 10^3 for hydrogen atoms, x 10^4 for other atoms.

A. 5-carboxymethyl uridine

	X	Y	Z	B11	B22	B33	B12	B13	B23
O1'	194(2)	3980(2)	1502(6)	2.64(11)	2.08(10)	2.93(13)	-0.19(10)	-0.27(11)	0.48(12)
O2'	2337(2)	3357(2)	4948(6)	1.86(10)	2.65(11)	3.61(1)	0.35(9)	-0.17(12)	-0.36(12)
O3'	1956(2)	2826(2)	554(7)	3.16(12)	2.85(11)	4.34(1)	-0.39(11)	1.67(14)	-1.36(14)
O5'	-995(2)	2941(2)	4324(7)	2.51(11)	2.48(11)	3.97(1)	-0.00(10)	0.48(13)	0.80(12)
O2	2254(2)	5478(2)	3316(6)	2.00(10)	2.67(11)	3.11(13)	-0.52(9)	0.84(11)	-0.39(12)
O4	348(3)	6516(2)	9295(8)	3.27(14)	4.19(15)	6.78(2)	-0.59(12)	1.40(16)	-3.26(19)
O1CX	-1815(3)	6379(2)	6762(8)	4.54(16)	4.68(15)	5.33(19)	1.94(14)	2.01(16)	2.65(19)
O2CX	-2524(2)	6089(2)	10278(7)	3.24(12)	4.46(15)	3.87(17)	1.79(13)	1.10(14)	0.73(15)
N1	840(2)	4810(2)	4484(7)	2.00(12)	1.47(11)	2.96(16)	-0.11(10)	0.31(14)	-0.24(13)
N3	1267(2)	5989(2)	6259(8)	1.94(12)	1.75(11)	3.51(18)	-0.06(11)	-0.20(14)	-0.58(14)
C1'	1070(3)	4159(2)	2044(8)	2.07(14)	2.13(16)	2.05(17)	0.26(14)	0.20(16)	-0.18(15)
C2'	1346(3)	3481(2)	4157(9)	2.23(16)	2.03(16)	2.20(17)	0.17(13)	0.76(15)	0.06(16)
C3'	1117(3)	2812(2)	2188(9)	2.38(15)	1.73(15)	2.66(19)	-0.38(14)	0.51(17)	-0.35(16)
C4'	175(3)	3155(2)	986(9)	3.23(19)	2.01(15)	2.07(17)	-0.20(15)	0.14(18)	-0.09(16)
C5'	-818(4)	2835(3)	1755(11)	2.68(18)	2.59(18)	3.98(25)	-0.57(16)	-0.31(19)	-0.49(21)
C2	1518(3)	5429(2)	4595(9)	2.13(14)	1.71(15)	2.58(19)	0.04(13)	-0.53(17)	0.20(17)
C4	460(3)	5994(3)	7820(10)	2.08(15)	2.38(17)	3.73(24)	0.27(15)	0.11(18)	-0.51(20)
C5	-224(3)	5338(2)	7555(9)	1.79(14)	2.05(16)	2.48(19)	0.44(14)	-0.24(17)	0.35(15)
C6	-1(3)	4789(2)	5952(9)	1.93(14)	1.61(14)	2.90(19)	0.21(13)	0.51(17)	0.30(16)
C7	-1144(3)	5331(2)	9125(10)	2.49(16)	2.16(16)	3.17(21)	0.49(15)	0.52(18)	0.52(18)
CCX	-1848(3)	5995(3)	8565(10)	1.66(15)	2.77(17)	3.86(22)	0.26(15)	0.04(16)	-0.86(19)

	X	Y	Z	B
HO2'	244(5)	368(3)	633(13)	5.2(15)
HO3'	191(4)	244(4)	-41(12)	4.6(16)
HO5'	-98(5)	246(5)	479(16)	6.9(19)
HO2X	-289(4)	647(4)	1000(13)	4.3(15)
HN3	172(5)	634(4)	654(15)	5.7(18)
HC1'	164(4)	438(3)	172(11)	3.1(12)
HC2'	90(3)	334(2)	532(8)	1.2(8)
HC3'	103(3)	227(2)	293(8)	1.3(8)
HC4'	38(4)	308(3)	-59(10)	2.5(10)
H15'	-83(4)	231(3)	145(10)	2.8(11)
H25'	-138(4)	310(3)	87(12)	4.0(13)
HC6	-45(4)	431(3)	582(10)	3.1(11)
H1C7	-88(4)	532(3)	1000(11)	3.3(12)
H2C7	-150(3)	489(3)	877(9)	1.6(9)

B. 5-carbamoylmethyl uridine

	X	Y	Z	B11	B22	B33	B12	B13	B23
O1'	8607(2)	1478(1)	-3867(4)	3.00(9)	2.24(8)	1.15(8)	0.23(7)	0.36(7)	0.82(7)
O2'	5996(2)	1710(1)	-1513(5)	2.27(9)	3.25(10)	3.39(11)	0.00(8)	-0.76(9)	0.25(10)
O3'	7254(2)	2794(1)	-359(5)	3.20(9)	2.62(10)	1.99(9)	0.52(8)	0.02(8)	-0.49(8)
O5'	10234(2)	2288(1)	-5774(5)	2.94(9)	3.79(12)	2.17(9)	0.38(9)	1.03(9)	0.54(9)
O2	7892(2)	468(1)	2955(5)	2.52(9)	2.81(10)	1.34(9)	-0.52(7)	-0.11(8)	-0.23(9)
O4	10849(2)	131(1)	6366(6)	3.34(10)	4.03(12)	2.31(11)	0.38(9)	-0.23(10)	1.29(10)
O8	12282(2)	1512(1)	5980(5)	3.25(9)	3.55(12)	2.14(9)	0.29(9)	-0.12(9)	-0.61(9)
N1	8734(3)	941(1)	1817(5)	2.45(10)	2.22(10)	1.56(10)	0.04(9)	-0.17(10)	0.16(9)
N3	9003(3)	292(1)	4464(6)	2.78(11)	2.12(10)	2.04(11)	-0.15(9)	0.08(10)	0.49(9)
N8	13886(3)	953(2)	5206(7)	2.79(12)	4.55(16)	3.46(15)	0.69(12)	-0.48(13)	-0.73(13)
C1'	7888(3)	1283(2)	-1028(6)	2.44(12)	2.62(14)	1.32(12)	0.02(11)	-0.24(11)	0.24(10)
C2'	7063(3)	1696(2)	86(6)	2.19(11)	3.01(14)	1.42(12)	-0.14(11)	0.08(11)	0.07(11)
C3'	7887(3)	2242(1)	-389(6)	2.15(12)	2.60(14)	1.24(12)	0.16(11)	-0.08(10)	-0.16(10)
C4'	8468(3)	2112(1)	-2950(6)	2.38(12)	2.24(12)	1.24(12)	0.17(10)	-0.02(11)	-0.02(10)
C5'	9692(3)	2413(2)	-3285(7)	2.56(13)	2.99(14)	1.91(13)	0.09(11)	0.33(12)	-0.11(12)
C2	8214(3)	570(1)	3824(7)	2.72(13)	2.14(12)	1.42(12)	-0.17(11)	-0.11(11)	-0.04(11)
C4	10253(3)	377(2)	4637(7)	2.72(13)	2.20(14)	1.93(14)	0.51(11)	0.04(13)	0.08(11)
C5	10753(3)	761(1)	2646(7)	2.52(12)	2.12(12)	1.78(14)	0.19(12)	-0.27(11)	-0.27(11)
C6	9989(3)	1020(2)	324(7)	2.33(12)	2.46(14)	1.64(13)	0.21(11)	0.12(11)	0.02(11)
C7	12126(3)	811(2)	3434(7)	2.62(13)	2.93(14)	1.84(14)	0.54(12)	0.00(13)	-0.31(12)
C8	12724(3)	1119(2)	4728(7)	2.58(13)	2.64(14)	1.99(14)	0.39(12)	-0.03(13)	0.09(12)

	X	Y	Z	B
HC1'	736(2)	87(1)	-179(5)	1.1(6)
HC2'	678(3)	161(1)	187(6)	2.0(6)
HC3'	862(3)	225(1)	105(6)	2.1(6)
HC4'	786(3)	234(1)	-434(6)	2.2(6)
H15'	1023(3)	229(1)	-181(8)	4.1(8)
H25'	959(3)	287(1)	-305(7)	2.8(6)
HO2'	533(3)	211(2)	-188(8)	5.0(9)
HO3'	736(4)	302(2)	81(9)	6.8(10)
HO5'	1075(4)	201(2)	-539(9)	6.5(11)
HN3	859(3)	-1(1)	553(7)	3.6(7)
HC6	1035(3)	129(1)	-47(7)	3.5(8)
H1C7	1243(3)	43(1)	223(7)	3.2(7)
H2C7	1255(3)	104(2)	81(7)	3.6(7)
H1N8	1433(4)	54(2)	424(9)	6.3(11)
H2N8	1438(3)	117(2)	631(8)	4.0(9)

is gauche and that of the carbamoyl derivative, trans.

In the structure of a related minor base, uridine-5-oxyacetic acid methyl ester[18], the side chain is approximately coplanar with the uracil ring. In both of the derivatives we have studied, the planes of the substituent groups are skew with respect to the plane of the nucleobase. The carboxyl group is on the opposite side of the uracil plane vis à vis the ribose ring; the carboxamide is on the same side of this plane. (Figure 1)

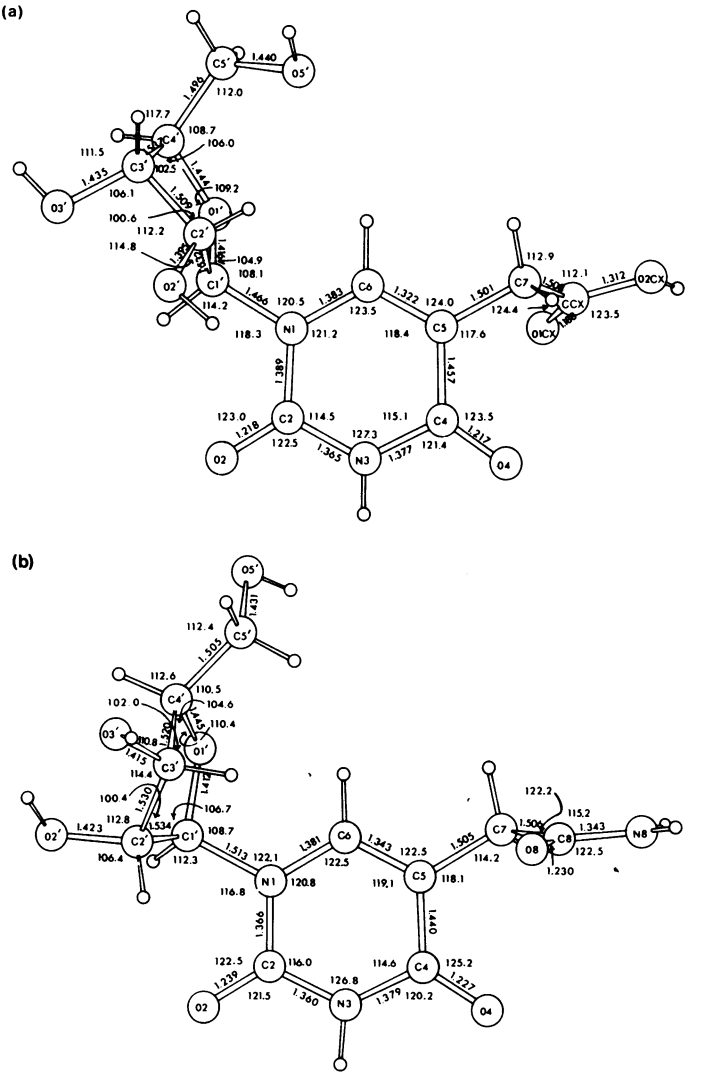

Fig. 1. Bond distances and angles of (a) 5-carboxymethyl uridine and (b) 5-carbamoylmethyl uridine.

RESULTS

The Molecular and Crystal Structures

The structural features of both the carboxymethyl and carbamoylmethyl uridine derivatives are summarized in Figure 1 which shows the distances, angles and conformations of the molecules and in Table 3 which summarizes the conformational features of the molecules.

In both crystal structures (Figures 2a, b, Table 4) the uracil bases are arranged in the "herringbone" fashion found in many uridine structures[18,19]; there is no base pairing. In the carbamoylmethyl derivative there are infinite spirals of hydrogen bonds connecting the N3 of one uracil ring to the O2 of another. Another hydrogen bonded spiral connects the amide (N8) to a symmetry related O4. The O1' of the ribose points at a base in an adjacent plane as has been found in other nucleoside structures[20]. In the carboxymethyl derivative, the N3 and O2 of the uracil rings are hydrogen bonded to symmetry related ribosyl O3' and O2' hydroxyl atoms as was seen in the structure of uridine 5-oxyacetic acid[18].

DISCUSSION

The specificity of certain tRNAs for recognition of multiple codons differing in the third letter only, has been consistent with the predictions of the "wobble" hypothesis[21]. Results from such earlier studies and the

Table 3. Conformation Angles

Angle	Atoms Involved				Values (°)	
					5-carboxymethyl uridine (C2' endo)	5-carbamoylmethyl uridine (C3' endo)
	A	B	C	D		
χ_{CN}	O1'	C1'	N1	C6	+ 49.4 (anti)	+ 5.2 (anti)
ψ_{OO}	O1'	C4'	C5'	O5'	- 60.5 (gauche)	+ 63.4 (trans)
ψ_{OC}	C3'	C4'	C5'	O5'	+ 60.0 (gauche)	+ 180.0 (trans)
τ_0	C4'	O1'	C1'	C2'	- 25.7	+ 10.0
τ_1	O1'	C1'	C2'	C3'	+ 39.6	- 30.7
τ_2	C1'	C2'	C3'	C4'	- 37.4	+ 38.7
τ_3	C2'	C3'	C4'	O1'	+ 23.7	- 34.2
τ_4	C3'	C4'	O1'	C1'	+ 1.4	+ 15.4
	C4	C5	C7	CCX(C8)	+ 64.5	- 67.7
	C5	C7	CCX(C8)	O1CX(O8)	+ 16.1	- 30.8

The nomenclature used is that used by Sundaralingam[46]. Each conformation angle is defined in terms of 4 consecutive atoms ABCD. The positive sense of the rotation is clockwise from A to D while looking down the BC bond. The atoms in parentheses refer to 5-carbamoylmethyl uridine.

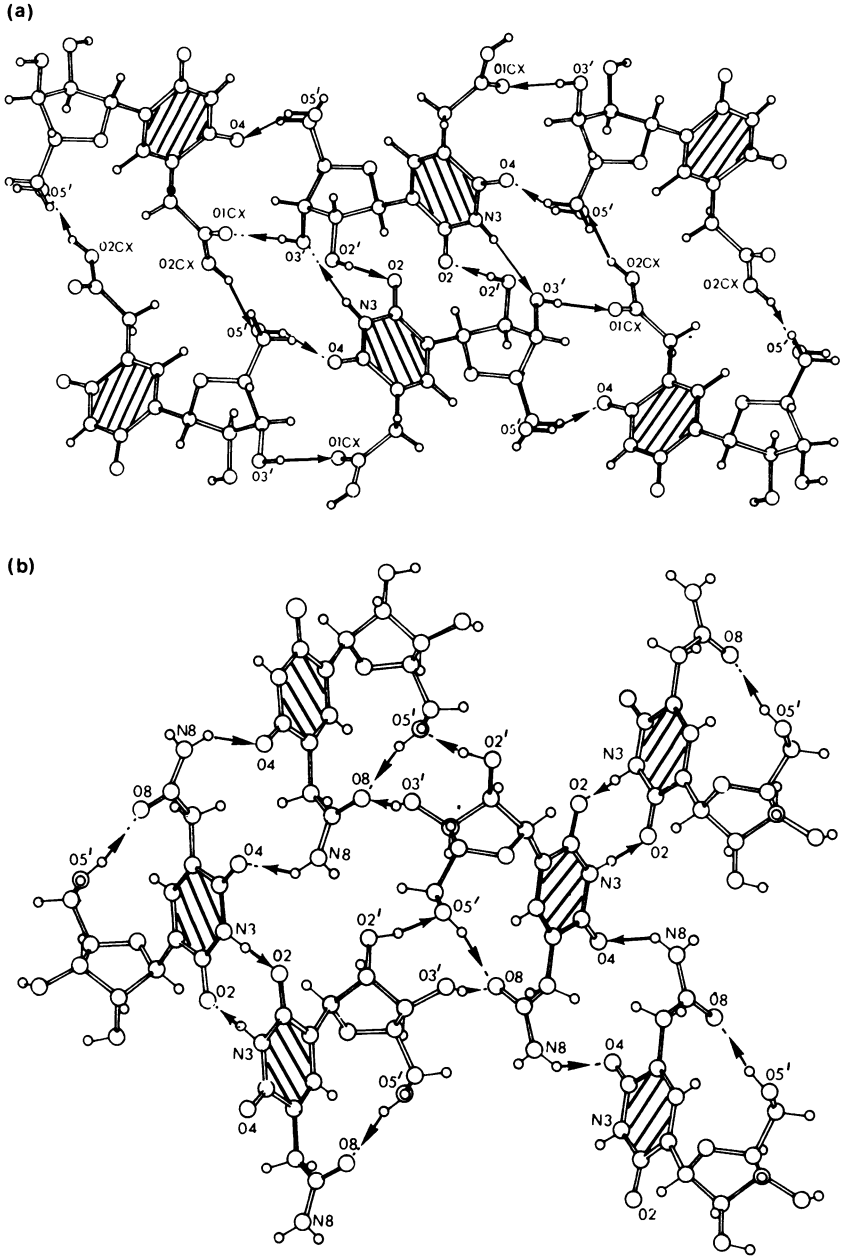

Fig. 2. The hydrogen bonding of (a) 5-carboxymethyl uridine and (b) 5-carbamoylmethyl uridine looking down the c axis. The atoms involved in hydrogen bonding are labelled. The arrows point from the donor to the acceptor atoms.

Table 4. Hydrogen Bonds

A. 5-carboxymethyl uridine

i	j	symmetry operation of j			Distance in Å
O2'	O2	1/2-X	1-Y	1/2+Z	2.786
O3'	O1CX	-X	-1/2+Y	1/2-Z	2.795
O5'	O4	-X	-1/2+Y	3/2-Z	2.705
O2CX	O5'	-1/2-X	1-Y	1/2+Z	2.638
N3	O3'	1/2-X	1-Y	1/2+Z	3.149
	O2'	1/2-X	1-Y	1/2+Z	2.970

B. 5-carbamoylmethyl uridine

O2'	O5'	-1/2+X	1/2-Y	-1-Z	2.765
O3'	O8	-1/2+X	1/2-Y	1-Z	2.726
O5'	O8	X	Y	-Z	2.904
N3	O2	3/2-X	-Y	1/2+Z	2.761
N8	O4	5/2-X	-Y	-1/2+Z	3.135

predicted pairing rules for the third position of the codon have been summarized by Weiss[22].

Uracil and its modifications, when occupying the "wobble" position are capable of responding to two different letters in the third place of a pair of otherwise identical codons. However, in each such case, the responses to different codons need not be identical in terms of the strength of the interaction or the ultimate rate of protein synthesis. To date, there are only three known tRNAs participating in protein synthesis that possess an unmodified U at the 5'-end of the anticodon, i.e., baker's yeast $tRNA^{Leu}_{CUA}$ (anticodon UAG)[23], $tRNA^{Gly3}_{ins,GGA/G}$ (anticodon UCC) derived from E. coli $tRNA^{Gly3}_{GGU/C}$ by a G → U base change in the "wobble" position[24], and a portion of the $tRNA^{Gly2}_{GGA/G}$ obtained from $glyT^{+}$ E. coli strains[25]. The remaining portion of the latter contains an unidentified modified uridine in the "wobble" position, but still has the same coding properties responding to GGA/G[26]. Triplet binding studies with these tRNAs[22,23,25,27] indicate that an unmodified U in the "wobble" position either recognizes A exclusively or responds to A better than to G. On the other hand, several modified uridines at the same position bearing aliphatic substituents at the position 5 show enhanced recognition for G relative to A during the decoding process. This is demonstrated by the E. coli $tRNA^{Val}_{1}$ and $tRNA^{Ser}_{1}$ containing uridine-5-oxyacetic acid and responding equally well to the codons GUA, GUG[28] and UCA, UCG[29], respectively.[30] Other

tRNAs likely to belong to this group are $tRNA_{2a}^{Val}$ from baker's yeast[31] and the $tRNA^{Pro}$ and $tRNA^{Ser}$ from bacteriophage T4[32] all of which contain a modified uridine at the "wobble" position.

The methyl ester of 5-carboxymethyluridine has been identified in the "wobble" position of $tRNA_{III}^{Arg}$ from brewer's yeast[12b] (anticodon mcm^5UCU); however, its coding preferences, if any, between A and G have not been studied. The existence of two different blocking substituents (methyl ester, amide) for the carboxylic group of the side chain has been considered in relation to a dependent function of cm^5U-containing tRNAs.[33]

There are several mechanisms by which 5-substitution of the "wobble" uracil base may be related to the expression of its coding specificity. With regard to its hydrogen-bonding properties, these mechanisms are based upon Crick's hypothesis that alternate pairing of a uracil to a guanine moiety involves reverse Watson-Crick hydrogen-bonding to the 2- and 3-positions of uracil. This requires a translation of only 2.5 Å of that pyrimidine.[21] Modification of a uridine moiety at position 34 of the anticodon would be expected to modulate its relative binding properties with adenosine and guanosine through a direct effect on its intrinsic electronic complementarity,[34] an indirect effect on its ability to undergo conformational changes, or by some combination of these.

In many instances, the effects of substitution upon the hydrogen bonding or stacking properties of nucleic acid components, as a result of inherent electronic and conformational changes, have been well documented. The replacement of the 2-keto group on the uracil base by a 2-thione group results in preferential if not exclusive coding for A[35] presumably as a result of the poor hydrogen bond accepting ability of sulfur. Similarly, a 5-substituent on a uracil might affect the relative hydrogen bond accepting ability of the 2- and 4-keto groups, as has been proposed for uridine-5-oxyacetic acid, methyl ester.[36] Such an effect is unlikely to be significant in the two compounds in this study as the inductive effect of the side chain functionality is insulated from the pyrimidine ring by a methylene group. The possibility of steric hindrance exerted upon the 4-keto group, however, cannot be discounted.

Indirectly, a 5-substituent may alter the conformation of the "wobble" nucleotide or modify the ability and means of adjacent bases to stack with it. Evidence has been obtained for conformational rigidity in a modified nucleotide due to electrostatic interaction of charged groups,[37] as might occur between an ionized carboxymethyl side chain and an adjacent phosphate. The

excision of the hypermodified Y base at position 37 decreases significantly the binding capacity of the adjacent anticodon in $tRNA^{Phe}$ [38] This effect has been attributed to a disruption of the rigid conformation that is stabilized by the side chain. Thus, 5-substitution may effect both stacking and hydrogen bonding abilities in the wobble base. In fact the effect would be expected to be synergistic, as Grosjean et al., have demonstrated the large role that stacking can play in stabilizing the H-bonding interaction of complementary trinucleotide sequences.[39]

The anticodon loop seems to be characterized by an unusual conformational agility. Independent studies of the orthorhombic[40] and monoclinic[41] crystal forms of $tRNA^{Phe}$ demonstrate a 3' stacked conformation for the anticodon of each; i.e., there is a sharp turn (π turn[42] or U-turn[43]) made by the phosphate backbone on passing from U33 to N34, and the three bases of the anticodon (N34, N35, N36) are arranged in an approximately 8-fold helical stack. Recently, Lake has proposed that the anticodon undergoes a conformational change from a 5'-stack to a 3'-stack while attached to mRNA during protein synthesis.[44] It is likely that modifications of the "wobble" base could also affect modulation by enhancing or retarding this process.

If one takes for a model the 3'-stacked conformation for $tRNA^{Phe}$ [45], and attempts to substitute 5-carbamoylmethyluridine into position 34, one finds that O8 is close enough to the 2'-hydroxyl of ribose 33 to form an H-bond (Figure 3). In an unmodified uracil, or one whose substituents are

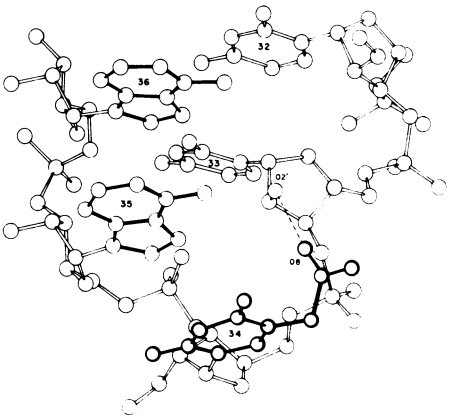

Fig. 3. The substitution of 5-carbamoylmethyl uridine at position 34 in the anticodon region of a tRNA. The three bases of the anticodon (34, 35, 36) are represented by solid black bonds. The possible hydrogen bond between O8 and O2' is shown.

coplanar with the base (such as the 5-oxyacetic acid derivative),[36] this phenomenon is absent. If it is assumed that such substitution may be made without significant conformational change for the nucleoside monomer or the tRNA, then the flexibility of the anticodon loop would be affected profoundly. Thus, 5-substitution of uracil can restrict the conformational freedom of the anticodon which may in turn modulate its H-bonding properties or ability to "wobble".

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REFERENCES AND FOOTNOTES

1 G. R. Wyatt and S. S. Cohen, Biochem. J. 55, 774 (1953)
2 R. G. Kallen, M. Simon and J. Marmur, J. Mol. Biol. 5, 248 (1962)
3 F. Tanaka, S. Takeuchi, N. Tanaka, H. Yonehara, H. Umezawa and Y. Sumiki, J. Antibiotics (Tokyo) Ser. A. 14, 161 (1961)
4 C. Brandon, P. M. Gallop, J. Marmur, H. Hayashi and K. Nakanishi, Nature, New Biol. 239, 70 (1972)
5 R. L. Lipnick and J. D. Fissekis, Bioorg. Chem. 6, 103 (1977) (see for leading refs)
6 M. W. Gray and B. G. Lane, Biochemistry 7, 3441 (1968)
7 T. D. Tumaitis and B. G. Lane, Biochim. Biophys. Acta 224, 391 (1970)
8 D. B. Dunn and M. D. M. Trigg, Biochem. Soc. Trans. 3, 656 (1975)
9 a. M. W. Gray, Canad. J. Biochem. 53, 735 (1975); b. M. W. Gray, Biochemistry 15, 3046 (1976)
10 L. Baczynskyj, K. Biemann and R. H. Hall, Science 159, 1481 (1968)
11 T. D. Kennedy and B. G. Lane, Canad. J. Biochem 53, 1 (1975) It is likely that in native tRNAs the carboxylic group of the acetate side chain exists, perhaps exclusively as the methyl ester or the amide.
12 a. M. Yoshida, K. Takeishi and T. Ukita, Biochem. Biophys. Res. Commun. 39, 852 (1970); b. B. Kuntzel, J. Weissenback, R. E. Wolff, T. D. Tumaitis-Kennedy, B. G. Lane and G. Dirheimer, Biochimie 57, 61 (1975); c. T. Kobayashi, T. Irie, M. Yoshida, K. Takeishi and T. Ukita, Biochim. Biophys. Acta 366, 168 (1974)
13 Abbreviations used are: cm^5U for 5-carboxymethyluridine, mcm^5U for 5-carboxymethoxymethyluridine, ncm^5U for 5-carbamoylmethyluridine, U for uridylate, C for cytidylate, A for adenylate, G for guanylate, N for any unspecified nucleotide
14 P. Main, M. M. Woolfson and G. Germain, Acta Cryst, Sec. A27, 368 (1971)
15 International Tables for X-Ray Crystallography, Vol. III, Birmingham, Kynoch Press (1962)
16 R. F. Stewart, E. R. Davidson and W. T. Simpson, J. Chem. Phys. 42, 3175 (1965)
17 D. Voet and A. Rich, Prog. in Nucleic Acid Research and Molecular Biol. 10, 183-265 (1970)
18 K. Morikawa, K. Torii, Y. Iitaka and M. Tsuboi, Acta Cryst B31, 1004 (1975)

19 E. A. Green, R. D. Rosenstein, R. Shiono, D. Abraham, B. L. Trus and R. E. Marsh, Acta Cryst B31, 102 (1975)
20 C. E. Bugg, J. M. Thomas, M. Sundaralingam and S. T. Rao, Biopolymers 10, 175 (1971)
21 F. H. C. Crick, J. Mol. Biol. 19, 548 (1966)
22 G. B. Weiss, J. Mol. Evolution 2, 199 (1973)
23 K. Randerath, L. S. Y. Chia, R. C. Gupta, E. Randerath, E. R. Hawkins, C. K. Brum and S. H. Chang, Biochem. Biophys. Res. Commun. 63, 157 (1975)
24 C. Squires and J. Carbon, Nature, New Biol. 233, 274 (1971)
25 a. J. W. Roberts and J. Carbon, Nature 250, 412 (1974); b. J. W. Roberts and J. Carbon, J. Biol. Chem. 250, 5530 (1975)
26 The anticodon of an ochre-suppressing derivative of E. coli $tRNA_1^{Tyr}$ previously identified as UUA, can contain a modified uridine in the first position.[6] This anticodon can suppress both UAA and UAG but suppresses the former more efficiently.[7]
27 a. S. Altman, Nucl. Acid Res. 3, 341 (1976); b. S. Altman, S. Brenner and J. D. Smith, J. Mol. Biol 56, 195 (1971); c. J. Carbon and C. Squires, J. Mol. Biol. 52, 571 (1970)
28 T. Takemoto, K. Takeishi, S. Nishimura and T. Ukita, Eur. J. Biochem. 38, 489 (1973)
29 H. Ishikura, Y. Yamada and S. Nishimura, Biochim. Biophys. Acta 228, 471 (1971)
30 For a recent NMR study in an aprotic system of the specific interactions of uridine-5-oxyacetic acid with other nucleosides, see H. Iwahashi and Y. Kyogoku, Nucl. Acid Res. Spec. Publ. No. 2, p. s41, Inform. Retrieval Ltd., London, 1977.
31 V. D. Axelrod, V. M. Kryukov, S. N. Isaenko and A. A. Bayev, FEBS Lett. 45, 333 (1974)
32 B. G. Barrell, J. D. Seidman, C. Guthrie and W. H. McClain, Proc. Nat. Acad. Sci. (USA) 71, 413 (1974)
33 M. W. Gray, Canad. J. Biochem. 53, 735 (1975)
34 Y. Kyogoku, R. C. Lord and A. Rich, Proc. Natl. Acad. Sci. (USA), 57, 250 (1967)
35 S. Nishimura, Prog. Nucleic Acid. Res. Mol. Biol., 12, 49 (1972)
36 K. Morikawa, K. Torii, Y. Iitaka, M. Tsuboi and S. Nishimura, FEBS Lett. 48, 279 (1974)
37 E. D. Hickey, L. A. Weber, C. Baglioni, C. H. Kim and R. H. Sarma, J. Mol. Biol., 109, 173 (1977)
38 V. Cameron and O. C. Uhlenbeck, Biochem. Biophys. Res. Commun., 50, 635 (1973); O. Pongs and E. Reinwald, ibid., 50, 357 (1973)
39 H. Grosjean, D. G. Söll and D. M. Crothers, J. Mol. Biol., 103, 499 (1976)
40 J. Sussman and S. H. Kim, Biochem. Biophys. Res. Comm., 68, 89 (1976); G. J. Quigley, N. C. Seeman, A. H. J. Wang, F. L. Suddath and A. Rich, Nucl. Acid Res., 2, 2329 (1975)
41 J. E. Ladner, A. Jack, J. D. Robertus, R. S. Brown, D. Rhodes, B. F. C. Clark and A. Klug, Nucl. Acid Res., 2, 1629 (1975); C. D. Stout, H. Mizuno, J. Rubin, T. Brennan, S. T. Rao and M. Sundaralingam, Nucl. Acid Res., 3, 1111 (1976)
42 S. H. Kim and J. L. Sussman, Nature 260, 645 (1976)
43 G. J. Quigley and A. Rich, Science, 194, 796 (1976)
44 J. A. Lake, Proc. Nat. Acad. Sci. (USA), 74, 1903 (1977)
45 The tRNA coordinates used were obtained from S. Holbrook and S. H. Kim.
46 M. Sundaralingam, Biopolymers, 7, 821 (1969)