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Molecular mechanism of codon recognition by tRNA species with modified uridine in the first position of the anticodon

(post-transcriptional modification/base pair/conformation/NMR)

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ABSTRACT **Proton NMR analyses have been made to elucidate the conformational characteristics of modified nucleotides as found in the first position of the anticodon of tRNA [derivatives of 5-methyl-2-thiouridine 5′-monophosphate (pxm^5s^2U) and derivatives of 5-hydroxyuridine 5′-monophosphate (pxo^5U)]. In pxm^5s^2U, the C3′-endo form is extraordinarily more stable than the C2′-endo form for the ribose ring, because of the combined effects of the 2-thiocarbonyl group and the 5-substituent. By contrast, in pxo^5U, the C2′-endo form is much more stable than the C3′-endo form, because of the interaction between the 5-substituent and the 5′-phosphate group. The enthalpy differences between the C2′-endo form and the C3′-endo form have been obtained as 1.1, −0.7, and 0.1 kcal/mol (1 cal = 4.184 J) for pxm^5s^2U, pxo^5U, and unmodified uridine 5′-monophosphate, respectively. These findings lead to the conclusion that xm^5s^2U in the first position of the anticodon exclusively takes the C3′-endo form to recognize adenosine (but not uridine) as the third letter of the codon, whereas xo^5U takes the C2′-endo form as well as the C3′-endo form to recognize adenosine, guanosine, and uridine as the third letter of the codon on ribosome. Accordingly, the biological significance of such modifications of uridine to xm^5s^2U/xo^5U is in the regulation of the conformational rigidity/flexibility in the first position of the anticodon so as to guarantee the correct and efficient translation of codons in protein biosynthesis.**

In protein biosynthesis, certain tRNA species recognize more than one codon and, accordingly, the number of tRNA species required for translating genetic codes on mRNA is appreciably smaller than 61, the number of amino acid codons. In the wobble hypothesis proposed by Crick (1), wobble base pairs, as well as Watson–Crick A·U and G·C pairs, possibly play important roles in the recognition of the third letter of the codon by the first letter of the anticodon of tRNA. On examination of the structures of bases, Crick has pointed out that uridine may form base pairs with uridine, cytidine, and guanosine as well as adenosine. However, if uridine in the first position of the anticodon of $tRNA^{Gln}$, for example, should recognize uridine and cytidine in addition to adenosine and guanosine, the codons of histidine (CAU and CAC) would be incorrectly translated to glutamine (codon: CAA and CAG). Accordingly, Crick has suggested that, in the aminoacyl-tRNA binding site of the ribosome, the short U·U and U·C pairs should be generally prohibited while the U·A and U·G pairs may be formed (1).

Actually, however, uridine in the first position of the anticodon (position 34 of tRNA) is post-transcriptionally modified except for a few cases. As for tRNAs specific to glutamine (codon CAR, R = adenosine or guanosine), lysine (AAR), and glutamic acid (GAR), U(34) is always modified to the 5-methyl-2-thiouridine derivative (xm^5s^2U, Fig. 1) (2). In the triplet-dependent binding to the ribosome and in the *in vitro* protein synthesis, $xm^5s^2U(34)$ primarily recognizes adenosine as the third letter of the codon and the recognition of guanosine is much less efficient (3, 4).

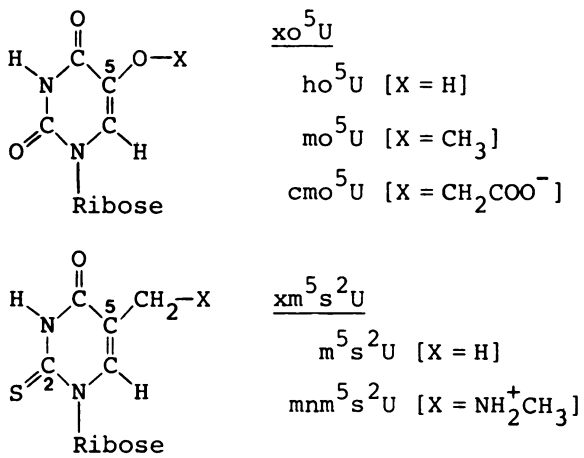

FIG. 1. Chemical structures of modified uridines.

The other type of modified uridines, 5-hydroxyuridine derivatives (xo^5U, Fig. 1) has been found in position 34 of tRNAs specific to valine (codon GUN, N = uridine, cytidine, adenosine, or guanosine), serine (UCN), threonine (ACN), and alanine (GCN) (5–10). In the triplet-dependent binding to the ribosome (8, 10–13) and also in the *in vitro* synthesis of MS2 coat protein (14, 15), $xo^5U(34)$ recognizes uridine in addition to adenosine and guanosine as the third letter of the codon. The formation of such a stable xo^5U·U pair is important for efficient translation of codons in protein biosynthesis.

The sharp contrast between the codon recognition patterns of the two types of modified uridines, $xm^5s^2U(34)$ and $xo^5U(34)$, has prompted us to undertake the elucidation of the conformational aspects as involved in the molecular mechanism of codon recognition by tRNA species. Thus, in the present study, we have made proton NMR analyses of the conformational characteristics of modified uridines, including 5-methylaminomethyl-2-thiouridine (mnm^5s^2U) as found in *Escherichia coli* $tRNA^{Gln}$, $tRNA^{Lys}$, and $tRNA^{Glu}$ (2), 5-carboxymethoxyuridine (cmo^5U) in *E. coli* $tRNA^{Val}$ (5–7), and 5-methoxyuridine (mo^5U) in *Bacillus subtilis* $tRNA^{Val}$, $tRNA^{Thr}$, and $tRNA^{Ala}$ (9, 10) (Fig. 1). Surprisingly, the

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Abbreviations: xm^5s^2U, 5-methyl-2-thiouridine derivative; xo^5U, 5-hydroxyuridine derivative.

conformational characteristics of xo^{5}U nucleotides have now been found to be remarkably different from that of xm^{5}s^{2}U(34) nucleotides; xo^{5}U is much more "flexible," whereas xm^{5}s^{2}U is much more "rigid" than unmodified uridine. These findings are consistent with the molecular conformations of modified uridine residues in the codon recognition, as obtained by our model-building studies. The modified uridine xm^{5}s^{2}U(34) is retained in the usual C3'-endo form and is allowed to form a stable base pair with adenosine, but never with uridine, as the third letter of codons. By contrast, xo^{5}U(34) takes the unusual C2'-endo form as well as the usual C3'-endo form and thus forms base pairs with uridine and guanosine as the third letter of codons in addition to the standard base pair with adenosine. Accordingly, the post-transcriptional modifications of U(34) result in the regulation of rigidity/flexibility of the anticodon of tRNA species and allow the correct and efficient translations of codons in protein biosynthesis.

MATERIALS AND METHODS

Nucleosides and Nucleotides. 5-Methylaminomethyl-2-thiouridine 5'-monophosphate (pmnm^{5}s^{2}U) was prepared from *E. coli* tRNA as described (16). The synthetic sample of mnm^{5}s^{2}U was a generous gift from T. Ueda. 2-Thiouridine (s^{2}U) and 2-thiouridine 5'-monophosphate (ps^{2}U) were kindly provided by S. Higuchi. 5-Hydroxyuridine (ho^{5}U) and 5-hydroxyuridine 5'-monophosphate (pho^{5}U) were purchased from P-L Biochemicals. mo^{5}U and cmo^{5}U were synthesized by methylation and carboxymethylation, respectively, of ho^{5}U as described (6, 9). 5-Methoxyuridine 5'-monophosphate (pmo^{5}U) and 5-carboxymethoxyuridine 5'-monophosphate (pcmo^{5}U) were synthesized by the phosphorylation of mo^{5}U and cmo^{5}U, respectively. Uridine and uridine 5'-monophosphate (pU) were purchased from Yamasa Shoyu (Choshi, Japan). 5-Methyluridine (m^{5}U) and 5-methyluridine 5'-monophosphate (pm^{5}U) were purchased from P-L Biochemicals.

Proton NMR Spectroscopy. The 270-MHz proton NMR spectra of nucleosides and nucleotides in ^2H_2O solution were recorded with a Bruker WH270 spectrometer. The probe temperature was controlled within 1 degree. Chemical shifts were measured from the internal standard of sodium 2,2-dimethyl-2-silapentane-5-sulfonate. As for the ribose protons, chemical shifts and spin-coupling constants were determined within 0.1 Hz by the spectral simulation with computer program NMRSIM (17).

The puckering equilibrium of the ribose ring moiety was analyzed by the use of vicinal spin-coupling constants; the fractional populations of the C2'-endo form and the C3'-endo form (Fig. 2) were calculated by the formulas $J_{1'2'}/(J_{1'2'} + J_{3'4'})$ and $J_{3'4'}/(J_{1'2'} + J_{3'4'})$, respectively. From the temperature dependencies of the equilibrium constants ([C2'-endo]/[C3'-endo]), the enthalpy and entropy differences between these two forms were obtained together with their standard deviations.

RESULTS AND DISCUSSION

The Puckering Equilibria of the Ribose Ring Are Significantly Affected by the Two Types of Modifications of Uridine. From the temperature dependences of equilibrium constants ([C2'-endo]/[C3'-endo]) of the ribose ring puckering, the enthalpy (and entropy) differences between the C2'-endo form and the C3'-endo form were obtained as shown in Table 1. The enthalpy difference for unmodified pU is as small as 0.1 kcal/mol (1 cal = 4.184 J), so that the C2'-endo form and C3'-endo form are nearly equally stable without the modification of the uracil base. On the other hand, for pmnm^{5}s^{2}U, the enthalpy difference is as large as 1.1 kcal/mol and the

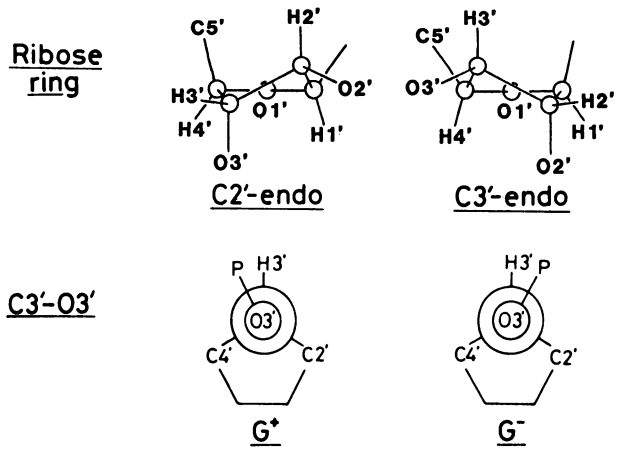

FIG. 2. Schematic representation of the local conformations of the ribose 3'-phosphate moiety of the nucleotide unit.

C3'-endo form is significantly more stable than the C2'-endo form. The C3'-endo form has also been found to be predominant (fractional population of 78%) in 5-methoxycarbonylmethyl-2-thiouridine (16), an xm^{5}s^{2}U-type nucleoside as found in tRNA species from yeast and mammals.

By contrast, for pmo^{5}U and pcmo^{5}U, the enthalpy differences are obtained as −0.7 kcal/mol (Table 1); the C2'-endo form is remarkably more stable than the C3'-endo form. These are the first examples of naturally occurring pyrimidine nucleotides that take the C2'-endo form as the predominant conformer. On the other hand, the rotamer equilibria about the C1'—N1 and C5'—C4' bonds are not significantly affected by these types of modifications (unpublished). Consequently, the two types of modification of uridine as found in the first position of the anticodon of tRNAs significantly affect the puckering equilibria of the ribose ring moiety. The enthalpy difference between the C2'-endo form and C3'-endo form (the relative stability of C3'-endo form) in pmnm^{5}s^{2}U is higher by 1.8 kcal/mol as compared to that in pmo^{5}U.

Conformations of Modified U(34) as Base-Paired with the Third Letter of the Codons. We have already demonstrated that the conformational properties of RNA molecules may be discussed on the basis of the conformational characteristics of nucleotide units (19). We have taken this strategy in the present study on the modified uridines as involved in codon recognition. For the formation of non-Watson–Crick base

Table 1. Enthalpy differences (kcal/mol) and entropy differences (entropy units) between the C2'-endo form and the C3'-endo form

	Enthalpy difference	Entropy difference
Uridine	0.37 (0.03)	0.86 (0.08)
mo^{5}U	0.58 (0.02)	1.26 (0.06)
cmo^{5}U	0.43 (0.01)	1.03 (0.02)
ho^{5}U	−0.01 (0.04)	−0.01 (0.11)
mnm^{5}s^{2}U	1.32 (0.07)	1.70 (0.21)
m^{5}s^{2}U*	0.98 (0.02)	1.28 (0.05)
s^{2}U	1.12 (0.02)	1.61 (0.08)
m^{5}U*	0.16 (0.02)	0.35 (0.04)
pU	0.09 (0.02)	0.69 (0.07)
pmo^{5}U	−0.72 (0.02)	−1.27 (0.07)
pcmo^{5}U	−0.67 (0.04)	−1.36 (0.12)
pho^{5}U	−0.28 (0.06)	−0.81 (0.19)
pmnm^{5}s^{2}U	1.10 (0.05)	1.27 (0.17)
ps^{2}U	0.87 (0.03)	1.22 (0.08)
pm^{5}U	−0.11 (0.02)	0.31 (0.06)

Standard deviations are given in parentheses.
*From ref. 18.

pairs, conformational "flexibility" is required in the first position of tRNA anticodons; the first anticodon-base should be displaced from the location in the Watson–Crick base pair (1). We have now succeeded in obtaining, by the use of molecular models, the conformations of $xo^5U(34)$ as base-paired with adenosine, guanosine, and uridine as the third letter of the codon (Figs. 3 and 4). Note that the relative arrangements of the P atom of N(34), the C4′ atom of N(35), and the C1′ atom of the third letter of the codon are essentially the same for the four base pairs as shown in Figs. 3 and 4. Thus, the conformation of the other part of the anticodon loop and the locations of the second and third bases of the anticodon are not affected by the conformation changes around the first position of the anticodon.

Base-Pairing of $xo^5U(34)$ with Adenosine as the Third Letter of the Codon. The $xo^5U(34)$·A base pair is of the standard Watson–Crick type in A-RNA conformation as shown in Fig. 3*a*. The $xo^5U(34)$ residue is set in the same conformation as the 2′-*O*-methylguanosine(34) residue of $tRNA^{Phe}$ in the crystal (20–22). Thus, the ribose ring is in the C3′-endo form and the conformation about the C3′—O3′ bond is G^-. This combination of local conformations, C3′-endo–G^-, has been found to be the most stable one, from the analysis of the short-range conformational interrelations in uridine 3′-monophosphate in aqueous solution (19).

Base-Pairing of $xo^5U(34)$ with Uridine as the Third Letter of the Codon. The third letter of the codon is probably set in the A-RNA conformation on the ribosome even in non-Watson–Crick base pairs. Accordingly, for the formation of the xo^5U·U pair, the displacement of the base of $xo^5U(34)$ toward the codon is required, since the two C1′ atoms in this base pair are much closer to each other than in the standard U·A base pair. We have found that such a displacement is favored by the conversion from the G^- form to the G^+ form (Fig. 2) about the C3′—O3′ bond of $xo^5U(34)$. However, the C3′-endo–G^+ form is found to be practically prohibited, from the lanthanoid-probe NMR analyses on nucleoside 3′-monophosphate in aqueous solution (19, 23). Therefore, the conversion from the G^- form to the G^+ form about the C3′—O3′ bond will be accompanied by the conversion of the ribose ring from the C3′-endo form to the C2′-endo form. To our surprise, just this C2′-endo–G^+ form is suitable for the formation of short xo^5U·U pair (Fig. 3*b*). The local conformations of the other parts of $xo^5U(34)$ are also converted from the original forms (as shown in Fig. 3*a*), including the conformation change about the C5′—C4′ bond (from the gg form to the tg form), as proposed (24). It may also be remarked here that the other type of $xo^5U(34)$·U pair [with hydrogen bonding of the 4-carbonyl group of $xo^5U(34)$] and the $xo^5U(34)$·C pair are not stable because of the steric repulsion between the ribose moieties in positions 34 and 35.

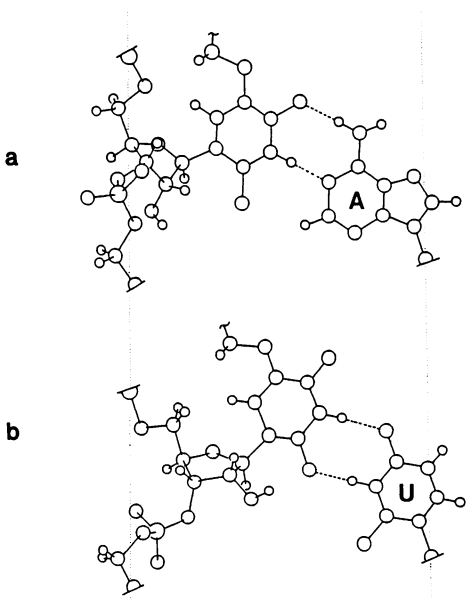

FIG. 3. Base pairs of $xo^5U(34)$ with adenosine (*a*) and uridine (*b*) as the third letter of the codons.

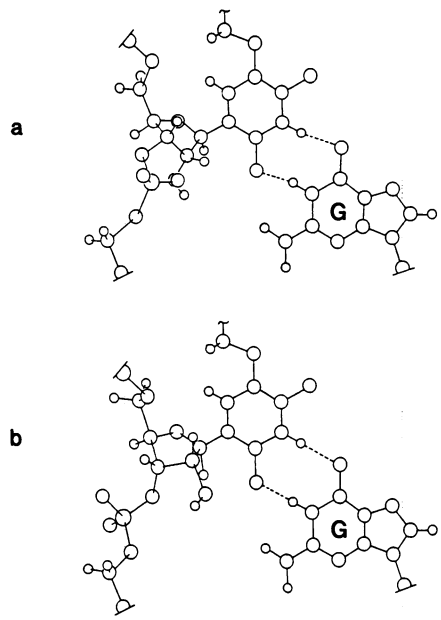

FIG. 4. Base pairs of $xo^5U(34)$ with guanosine as the third letter of the codons. The conformation of the ribose moiety of xo^5U is C3′-endo–G^- (*a*) or C2′-endo–G^+ (*b*).

Base-Pairing of $xo^5U(34)$ with Guanosine as the Third Letter of the Codon. A non-Watson–Crick base pair U(69)·G(4) has been found in the acceptor stem of yeast $tRNA^{Phe}$ (20–22), where the U(69) residue is in the C3′-endo form and adjacent nucleotide residues are somewhat distorted from the conformation of standard A-RNA duplex. Similarly, for the modified U(34) in the anticodon, we have constructed a model for the conformation of $xo^5U(34)$ as base-paired with guanosine in the third position of the codon (Fig. 4*a*), where the ribose moiety is retained in the C3′-endo–G^- form. In addition, we have also found another model for the conformation of $xo^5U(34)$ as base-paired with guanosine in the third position of the codon (Fig. 4*b*), with the C2′-endo–G^+ form of the ribose moiety. This model appears to be stable enough, since the local conformations of the other parts of ribose-phosphate chain are not appreciably distorted from the standard forms of stable rotamers.

Efficient and Correct Codon Recognition by Regulation of Rigidity/Flexibility of the Anticodon. The modification of U(34) to $xo^5U(34)$ stabilizes the C2′-endo–G^+ form as well as the C3′-endo–G^- form of the first letter of the anticodon, because of the remarkable stability of the C2′-endo form in the xo^5U unit itself. Then, the anticodon moiety is made flexible and the tRNA species with $xo^5U(34)$ recognize codons terminating in guanosine (Fig. 4) and uridine (Fig. 3*b*) as well as adenosine (Fig. 3*a*). Note that $xo^5U(34)$ is found in tRNA species specific to valine, threonine, serine, and alanine, which have four degenerate codons terminating in uridine, cytidine, adenosine, and guanosine. The modification to xo^5U allows recognition of codons terminating in uridine, adenosine, and guanosine and thus contributes to *efficient* translations of codons for these amino acids.

By contrast, the other type of modification, the modification of U(34) to $xm^5s^2U(34)$, further stabilizes the C3′-endo–G^- form of the first letter of the anticodon (Fig. 3*a*) because of the intrinsic extreme stability of the C3′-endo form in the xm^5s^2U unit itself. Therefore, $xm^5s^2U(34)$ (in the C3′-endo form) certainly forms the standard Watson–Crick-type base pair with adenosine, but not with uridine where $xm^5s^2U(34)$ should take the C2′-endo form. In short, the anticodon moiety is made rigid and the tRNA species with $xm^5s^2U(34)$ never recognize codons terminating in uridine. Note that $xm^5s^2U(34)$ is found in tRNA species specific to glutamine, lysine, and glutamic acid only, which have two degenerate codons terminating in adenosine or guanosine. The modification to xm^5s^2U does not allow misrecognition of codons terminating in uridine and thus contributes to *correct* translation of codons. Thus, the biological significance of the two types of modifications of uridine in the first position of the anticodon is to contribute to the correct and efficient translation of codons, through the regulation of the rigidity/flexibility of the first letter of the anticodon.

Stabilization of the C2′-endo Form for $xo^5U(34)$·U and $xo^5U(34)$·G Pairs. Then, how do these modifications regulate rigidity/flexibility of anticodons? For investigating these regulation mechanisms, we have extensively compared the conformational properties of a variety of uracil nucleosides and nucleotides (Table 1). First, the mechanism of the stabilization of the C2′-endo form for the formation of $xo^5U(34)$·U and $xo^5U(34)$·G pairs will be discussed. As shown in Table 1, for pmo^5U and $pcmo^5U$, the C2′-endo form is much more stable than the C3′-endo form; the enthalpy difference between the C3′-endo form and C2′-endo form (the relative stability of the C2′-endo form) is about 0.8 kcal/mol higher than that for pU. This indicates that the $-OCH_2-$ moiety of the 5-substituent is important for the stabilization of the C2′-endo form.

The 5-Substituent of xo^5U Strongly Interacts with the 5′-Phosphate Group. We have also compared the conformational stabilities of nucleosides and nucleotides. As for pU, $pmnm^5s^2U$, ps^2U, and pm^5U (Table 1), the relative stabilities of the C3′-endo form (the enthalpy differences between the C2′-endo form and C3′-endo form) are slightly lower (by 0.22–0.28 kcal/mol) than those of corresponding nucleosides. By contrast, for pmo^5U and $pcmo^5U$, the relative stabilities of the C2′-endo form are remarkably higher (by 1.1–1.3 kcal/mol) than those of mo^5U and cmo^5U. This clearly indicates that there are significant interactions between the 5′-phosphate group and 5-substituent of pxo^5U. Further, in the case of pho^5U (an analog of pxo^5U with X = H, Fig. 1), the 5-hydroxyl group by itself does not appear to interact strongly with the 5′-phosphate group. Therefore, in pxo^5U, the $-OCH_2-$ group at least of the 5-substituent interacts with the 5′-phosphate group, so as to stabilize the C2′-endo form.

Conformation of the 5-Substituent of $xo^5U(34)$. The 5-substituents of $mo^5U(34)$ and $cmo^5U(34)$ probably lie in the same plane as the uracil base, as found for mo^5U and the methyl ester of cmo^5U in crystal (25, 26). Such a "coplanar" conformation may be ascribed to the partial double-bond character of the C5—O bond (25). In the coplanar orientation shown in Figs. 3 and 4, the 5-substituent is close to the 5′-phosphate group. To examine the possible interaction between the 5-substituent and 5′-phosphate group, we have observed the pH dependences of proton chemical shifts of cmo^5U and $pcmo^5U$ and obtained the pK_a values of the terminal carboxylate group as 2.9 and 3.3, respectively. Such a difference in the pK_a unit of 0.4 is due to an interaction between the phosphate and carboxylate groups. The interaction between the 5-substituent and 5′-phosphate group stabilizes the C2′-endo form and favors the formation of $xo^5U(34)$·U pair (Fig. 3*b*) and $xo^5U(34)$·G pair (Fig. 4*b*).

The Remarkable Stability of the C3′-endo Form in $xm^5s^2U(34)$ Is Due to the Steric Effect of the 2-Thiocarbonyl Group. We have already found that the C3′-endo form is predominant in 2-thiopyrimidine nucleosides and nucleotides (16, 18). The remarkable stability of the C3′-endo form is due to the steric interaction between the bulky 2-thiocarbonyl group and the 2′-hydroxyl group (17). As shown in Table 1, the relative stabilities of the C3′-endo form of 2-thiouridine derivatives (s^2U, ps^2U, and m^5s^2U) are higher, by 0.8 kcal/mol, than those of non-thiosubstituted derivatives (uridine, pU, and m^5U). Thus, the 2-thiosubstitution is certainly the major cause of the stability of the C3′-endo form in pxm^5s^2U.

Now the roles of the 2-thiosubstitution of U(34) in codon recognition may be described in terms of the steric interaction. In the base pair with adenosine (Fig. 3*a*), the xm^5s^2U residue takes the stable C3′-endo form where the 2-thiocarbonyl group and 2′-hydroxyl group are just in van der Waals contact with each other. By contrast, the formation of the base pairs with uridine (Fig. 3*b*) and guanosine (Fig. 4*b*) are practically prohibited because of the strong steric repulsion between the 2-thiocarbonyl group and 2′-hydroxyl group in the C2′-endo form involved.

The 5-Substituent Also Contributes to the Stability of the C3′-endo Form in $xm^5s^2U(34)$. We have also examined the role of 5-substitution of $xm^5s^2U(34)$ in codon recognition. As shown in Table 1, the relative stabilities of the C3′-endo form of mnm^5s^2U and $pmnm^5s^2U$ are higher, by 0.2 kcal/mol, than those of s^2U and ps^2U, respectively. This clearly indicates that, in addition to the 2-thiocarbonyl group, the 5-methylaminomethyl group also contributes to the stability of the C3′-endo form. On the other hand, the 5-methyl substitution stabilizes the C2′-endo form rather than the C3′-endo form by about 0.2 kcal/mol (Table 1). Thus, for the stabilization of the C3′-endo form in modified U(34), the 5-substituent is required to be as long as the methylaminomethyl and methoxycarbonylmethyl groups.

Effects of 5-Substituents Are Different Between $xm^5s^2U(34)$ and $xo^5U(34)$. We have found that the 5-substituents in $xm^5s^2U(34)$ further stabilize the C3′-endo form, whereas the 5-substituents in $xo^5U(34)$ stabilize the C2′-endo form. As we have discussed, the 5-substituent of $xo^5U(34)$ takes a coplanar orientation. By contrast, in the crystal of mnm^5s^2U, the methylaminomethyl group is extended in a plane perpendicular to the uracil ring plane (27, 28). In general, a substituent having a methylene group directly bonded to an aromatic ring is known to take such a "perpendicular" orientation (29). Accordingly, the contrast between the effects of these two types of 5-substituents is probably due to the difference in the orientation of 5-substituents relative to the uracil ring.

Molecular Mechanism of Codon Recognition by tRNA Species with Modified Uridine in the First Position of the Anticodon. The codon-recognition properties of tRNA species with $xm^5s^2U(34)$ are much the same, although there is wide variety in size and in charge among the 5-substituents ($-CH_2-X$ groups). The same is true for the codon-recognition properties of tRNA species with $xo^5U(34)$ (2). Therefore, the direct interactions between 5-substituents and ribosome do not appear to be essential as far as these two types of modified uridines are concerned. On the other hand, the two distinct types of the codon-recognition properties of tRNA are correlated with the conformational properties of modified uridine in the first position of anticodon. We have now shown that, in tRNA species with $xm^5s^2U(34)$, the steric effect between the 2-thiocarbonyl group and 2′-hydroxyl group remarkably stabilizes the C3′-endo–G^- form and enhances the "rigidity" of the anticodon moiety, so as to prohibit the misrecognition of codons terminating in uridine. By contrast, in tRNA species with $xo^5U(34)$, the interaction of the $-OCH_2-$ group of the 5-substituent with the 5′-phosphate group

stabilizes the C2′-endo–G^+ form as well as the C3′-endo–G^- form and thus brings about the flexibility of the anticodon moiety, so as to recognize codons terminating in uridine (Fig. 3*b*), adenosine (Fig. 3*a*), and guanosine (Fig. 4). Thus, the short-range intranucleotide interactions are as important as base-pairing and base-stacking. Accordingly, we conclude that the two types of post-transcriptional modifications of U(34) are essential for the regulation of the rigidity/flexibility of the anticodon moiety and contribute to the correct and efficient translations of codons in protein biosynthesis.

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