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CD AND NMR STUDIES ON THE CONFORMATIONAL THERMOSTABILITY OF 2-THIORIBOTHYMIDINE FOUND IN THE TΨC LOOP OF THERMOPHILE tRNA

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SUMMARY The intensities of the main CD bands of 2-thiouridine derivatives are from five to ten times stronger than those of uridine derivatives, suggesting a conformational 'rigidity' of the nucleoside unit of 2-thiouridine derivatives. 270 MHz proton NMR spectra of 2-thioribothymidine and ribothymidine were measured and fractional populations of the 3'-*endo* and *gauche-gauche (gg)* forms at 23-93°C were obtained. The enthalpy and entropy differences between the 2'-*endo* and 3'-*endo* forms of 2-thioribothymidine were determined as 1.0 kcal/mole and 1.3 eu, respectively. This inherent stability of the 3'-*endo-gg-anti* form of 2-thioribothymidine residue possibly contributes to the thermostability of thermophile tRNA's.

In the course of studies on the structure-function relationship of tRNA's taken from an extreme thermophile, *Thermus thermophilus* HB8, we have found that ribothymidine (T) in the TΨC loop is replaced with 2-thioribothymidine (s^2T) in these thermophile tRNA's (1) (Fig. 1) and we have presented experimental evidence for the important role of the s^2T residue for maintaining the tertiary structure of tRNA's (2-4) and stabilizing the interaction of aa-tRNA with ribosomes at high temperatures (5). This important role of the s^2T residue is possibly related to the conformational characteristic of the s^2T residue, since only one base replacement (T to s^2T) appreciably enhances the thermostability of tRNA's (6,7). Thus, the ^1H NMR spectra of 2-thiocytidine, 5-methoxycarbonylmethyl-2-thiouridine and 5-methylaminomethyl-2-thiouridine

Abbreviations: s^2T, 2-thioribothymidine; T, ribothymidine; s^2U, 2-thiouridine, U, uridine; mnm^5s^2U, 5-methylaminomethyl-2-thiouridine; mnm^5U, 5-methylaminomethyluridine; CD, circular dichroism; NMR, nuclear magnetic resonance; ^3E, 3'-*endo* form of ribose ring; *gg*, *gauche-gauche* form about the exocyclic bond.

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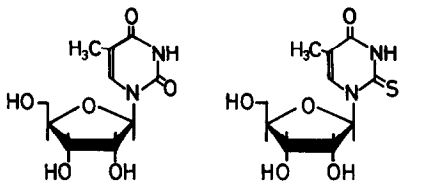

Fig. 1. The chemical structures of ribothymidine (T) and 2-thioribothymidine (s^2T).

(mnm^5s^2U) have been analyzed (8). 2-Thiopyrimidine nucleotides are almost exclusively in the ^3E-*gg*-*anti* form, while the fractional population of the ^3E-*gg*-*anti* form in unmodified pyrimidine nucleotides is less than 50% (8) (see Fig. 4 of Ref. 8 for the conformations of ribose moiety). In the present study, the temperature dependences of the CD and NMR spectra of 2-thiouridine derivatives were observed and compared with those of uridine derivatives. The results show that s^2T is predominantly in the ^3E-*gg*-*anti* form even at high temperatures and this characteristic of s^2T contributes to the thermal stability of thermophile tRNA's.

MATERIALS AND METHODS

A sample of s^2T was synthesized (9) and T was purchased from CALBIOCHEM. mnm^5s^2U was kindly provided by Dr. I. Ikeda of Hokkaido University. 5-Methylaminomethyluridine (mnm^5U) was prepared by the chemical desulfurization of mnm^5s^2U (10). s^2U was synthesized by the standard method (11,12).

CD spectra were measured by a JASCO J-20 spectropolarimeter as described previously (2). The sample concentration was determined by measuring the ultraviolet absorption with a Cary 17 spectrophotometer. The molar extinction coefficients (13) of 14.1×10^3 (at 272 nm), 13.6×10^3 (at 275 nm), 9.8×10^3 (at 267 nm), and 10.1×10^3 (at 262 nm) were used for s^2T, s^2U, T and U, respectively. The molar extinction coefficients of s^2T and T were used for mnm^5s^2U and mnm^5U, respectively. 270 MHz ^1H NMR spectra were recorded with a Bruker WH270 spectrometer. Spin-coupling constants were determined by the spectral simulation with the program NMRTRY/PLOT.

RESULT AND DISCUSSION

CD Spectra of 2-Thioribothymidine and Related Nucleosides

The CD spectra of 2-thiouridine derivatives and uridine derivatives in aqueous solution at various temperatures 24-88° are shown in Fig. 2. The

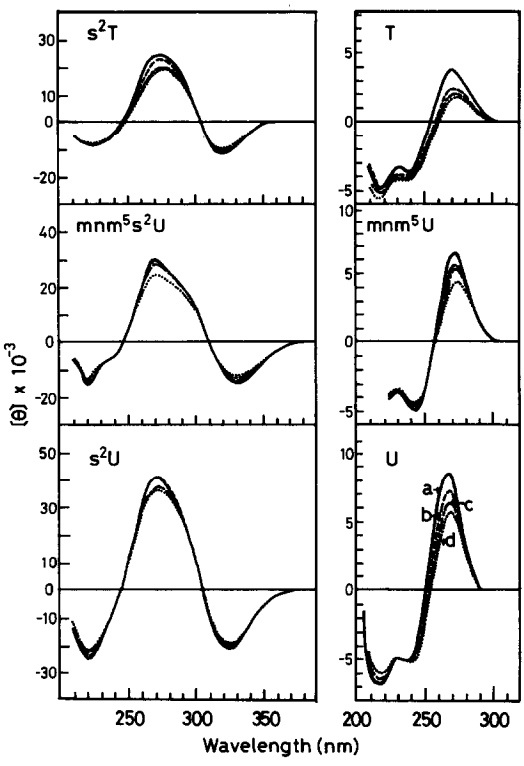

Fig. 2. The CD spectra of s^2T, mnm^5s^2U, s^2U, T, mnm^5U and U at 24° (a), 45° (b), 65° (c) and 88° (d). Sample concentration is 5-10 A_{260} units/ml in buffer solution (0.01 M Tris-HCl pH 7.5, 0.2 M NaCl, and 0.01 M magnesium acetate).

uridine derivatives show a positive band at about 270 nm while the 2-thiouridine derivatives exhibit a strong positive band at about 270 nm and an additional negative band at about 325 nm. This negative band is characteristic of 2-thiouridine derivatives (12,14) and is assigned to a n-π* transition derived from the C=S group of the base moiety (15).

The molar ellipticity of the positive peak for each 2-thiouridine derivative is, at 24°C, five times larger, and at 88°C, seven to ten times larger, respectively, than that of the corresponding non-thiolated nucleoside. These observations indicate, on the basis of the following reasons, the conformational 'rigidity' of the nucleoside unit of 2-thiouridine derivatives: first, there are some indications (16,17) that the molar ellipticity of mononucleoside is primarily reduced by the flexibility of the base-ribose

moiety; secondly, from the systematic NMR analyses on 2-thiopyrimidine nucleosides, it has been found that these nucleoside molecules predominantly take on the ^{3}E-*gg*-*anti* form in aqueous solution (8). For example, the fractional population of the ^{3}E-*gg*-*anti* form is as high as 80% for $mnm^{5}s^{2}U$ but is much smaller for U and 4-thiouridine, (the maximum [θ] value of 4-thiouridine is in fact smaller than 5×10^{3}). Thus, the large maximum [θ] value of $mnm^{5}s^{2}U$, as compared with those of non-thiolated nucleosides, is due to the predominance of one conformation, namely, the ^{3}E-*gg*-*anti* form.

Temperature Dependence of the Local Conformations of 2-Thioribothymidine and Ribothymidine

For certain qualitative analyses of the temperature dependences of the CD spectra of $s^{2}T$ and T, the vicinal spin-coupling constants ($J_{1'2'}$, $J_{3'4'}$, $J_{4'5'}$, and $J_{4'5''}$) of $s^{2}T$ and T at various temperatures 23°-93°C were measured as shown in Table I. The fractional population of the ^{3}E and *gg* forms were then calculated (Table I) from vicinal spin-coupling constants according to the well established methods (18).

For the ribothymidine molecule (T), the population ratio of 2'-*endo*/3'-*endo* changes little, from 0.89 at 23°C to 0.94 at 93°C. By the method of least squares, the enthalpy and entropy differences between the 2'-*endo* and 3'-*endo* forms are obtained at 0.16±0.02 kcal/mole and 0.35±0.04 eu, respectively. Thus, the 3'-*endo* form is only slightly more stable than the 2'-*endo* form. The entropy difference of 0.35 eu is due possibly to the correlation between the ring puckering and the internal rotation about the exocyclic bond; the ribose ring in the 3'-*endo* form exclusively takes on the *gg* form about the exocyclic bond, while the ribose ring in the 2'-*endo* form takes the *gauche-trans* and *trans-gauche* forms as well as the *gg* form (19).

On the other hand, for the 2-thioribothymidine molecule ($s^{2}T$), the population ratio of 2'-*endo*/3'-*endo* changes appreciably, from 0.36 at 23°C to 0.51 at 93°C. By the method of least squares, the enthalpy and entropy differences between the 2'-*endo* and 3'-*endo* forms are obtained as 0.98±0.02

Table I. Spin Coupling Constants (Hz) and Fractional Populations (%) of the ^3E and *gg* Forms of 2-Thioribothymidine (s^2T) and Ribothymidine (T).

		23°	33°	43°	53°	63°	73°	83°	93°
s^2T	$J_{1'2'}$	2.7		2.9		3.1		3.3	3.3
	$J_{3'4'}$	7.6		7.3		7.0		6.8	6.7
	$J_{4'5'}$	2.7		2.8		2.8		2.9	2.9
	$J_{4'5''}$	3.3		3.6		3.7		3.9	3.9
	^3E	73		71		69		68	67
	gg	80		76		74		71	70
	$J_{1'2'}$	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
	$J_{3'4'}$	5.4	5.4	5.3	5.3	5.2	5.2	5.2	5.1
	$J_{4'5'}$	3.0	3.1	3.1	3.2	3.2	3.2	3.2	3.2
	$J_{4'5''}$	4.3	4.4	4.5	4.5	4.5	4.6	4.6	4.6
	^3E	52	52	52	52	51	51	51	51
	gg	65	64	63	62	62	61	60	60

The fractional populations of the ^3E and *gg* forms were calculated from $J_{3'4'}/(J_{1'2'}+J_{3'4'})$ and $(13.7-J_{4'5'}-J_{4'5''})/9.7$, respectively (18).

kcal/mole and 1.28±0.05 eu, respectively. The energy difference between these two forms is thus much larger in the s^2T molecule than in the T molecule. Because of this large energy difference, the fractional populations of the ^3E and *gg* forms of the ribose ring are much higher in the s^2T molecules than those in the T molecules, even at high temperatures and at 23°C as well, (Table I). The thiolation at the position 2 of the thymine base appreciably enhances the stability of the ^3E-*gg*-*anti* form of the s^2T molecule.

Such conformational characteristics of the s^2T and T molecules are consistent with the temperature dependences of the CD spectra of these molecules. Previously, the flexibility of pyrimidine nucleosides was suggested to be due to the fluctuation of the angle χ in conjunction with the ring puckering of the *anti* form, rather than with the *anti*-*syn* transformation

(16,17). In fact, the range of angle χ is different for the 3'-*endo* form ($\chi = \sim 20°$) and 2'-*endo* form ($\chi = \sim 50°$) (20). For the T molecule, the fractional populations of the 3'-*endo* and 2'-*endo* forms are nearly equal and accordingly the *anti* form takes the two χ values with equal probability and is thus 'flexible'. On the other hand, for the s^2T molecule, the fractional population of the 3'-*endo* form is appreciably higher than that of the 2'-*endo* form and accordingly the *anti* form takes the χ value of $\sim 20°$ with much higher probability and is thus 'rigid'.

Effect of 2-Thiolation on the Thermostability of the Conformation of Thermophile tRNA

In the present study on s^2T, the 3'-*endo* form was found to be more stable than the 2'-*endo* form, by as much as 1.0 kcal/mole. Thus, the 2-thiolation of the thymidine base will appreciably enhance the stability of the ^{3}*E-gg-anti* form around the s^2T residue in tRNA's. The T_{54} residue of yeast $tRNA^{Phe}$ has been found to be in the ^{3}*E-gg-anti* form by the x-ray analyses (21,22). Therefore, modification of the T residue to the s^2T residue in the thermophile tRNA's will contribute to the stability of the local conformation around the G_{53}-s^2T_{54}-Ψ_{55} sequence of tRNA's, owing to the inherent stability of the ^{3}*E-gg-anti* form of the s^2T residue.

The 2-thiolation of T_{54} in thermophile tNRA's is further expected to stabilize the association of the TΨC loop and the D loop, on the basis of the tertiary structure of yeast $tRNA^{Phe}$ (23). The s^2T_{54} residue of the thermophile $tRNA^{fMet}$ (6) is base-paired with the m^1A_{58} residue and this base pair is stacked with the base pair G_{53}-C_{61} and also with the interloop base pair Ψ_{55}-Gm_{18} [the numbering of residues conforms to the proposed rule (24)]. The inherent stability of the ^{3}*E-gg-anti* form of the s^2T residue will certainly stabilize this stacking and thus further contribute to the stability of the hydrogen-bonding between the Ψ_{55} residue of the TΨC loop and the Gm_{18} residue of the D loop.

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