NMR analyses on the molecular mechanism of the conformational rigidity of 2-thioribothymidine, a modified nucleoside in extreme thermophile tRNAs

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^{1}H-NMR analyses have been made on the conformations of 2-thioribothymidine (s^2T), 2-thiodeoxyribothymidine (s^2dT), as well as ribothymidine (T) and deoxyribothymidine (dT). s^2T and s^2dT exclusively take the *anti* form rather than the *syn* form. The C3′-endo-gg form of the sugar moiety is remarkably stabilized on modification of T to s^2T, but not on modification of dT to s^2dT. The steric effects of the 2-thiocarbonyl group and the 2′-hydroxyl group cause the rigidity of the C3′-endo-gg form of s^2T. Such rigidity of s^2T probably contributes to the thermostability of 2-thiopyrimidine polyribonucleotides and extreme thermophile tRNAs.

tRNA *Extreme thermophile* *Thermostability* *NMR* *2-Thioribothymidine* *2-Thiopyrimidine polyribonucleotide*

1. INTRODUCTION

In extremely thermophilic eubacteria, tRNAs have 2-thioribothymidine (s^2T, fig.1) at the position 54 in the TψC-loop, in the place of ribothymidine (T) in eukaryotes and other eubacteria. This modification [T ⟶ s^2T] has been found to contribute to the thermostability of extreme thermophile tRNAs [1,2]. For elucidating the molecular mechanism of such thermostability of tRNAs, we have studied the conformational characteristics of s^2T in comparison with T [3]. By proton NMR analyses, we have found that the ribose moiety of s^2T predominantly takes the C3′-endo-gg form and the enthalpy difference between the C2′ and C3′-endo forms is much larger in s^2T (1.0 kcal.mol^{-1}) than in T (0.2 kcal.mol^{-1}) [3]. Here, the conformational characteristics of s^2T and the 2′-deoxy analog (s^2dT, fig.1) as well as T and thymidine (dT) have been analysed by nuclear Overhauser efffects (NOE) and vicinal coupling constants. The results suggest that the thermostability of extreme thermophile tRNAs and polyribonucleotides containing 2-thiopyrimidine is primarily due to the steric effects of the bulky 2-thiocarbonyl group and the 2′-hydroxyl group of the ribonucleoside moiety.

2. MATERIALS AND METHODS

s^2dT was synthesized from dT, by 5′-tosylation, 5′,2-cyclization, 3′-acylation, 2-thiolation (with H_2S), and 3′-deacylation. s^2T and T were prepared as in [3]. dT was purchased from Yamasa Shoyu. 270-MHz proton NMR spectra of nucleoside solutions (in ^2H_2O, 10 mM, 23°C) were recorded with a Bruker WH-270 spectrometer. Chemical shifts were measured from the internal standard of

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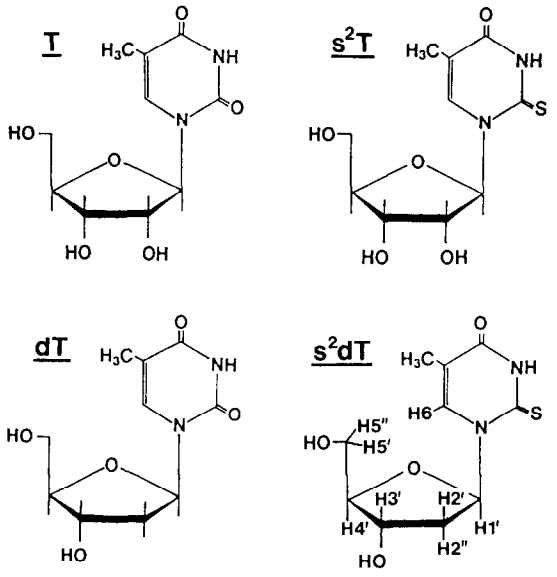

Fig.1. The chemical structures of ribothymidine [T], 2-thioribothymidine [s^2T], 2′-deoxyribothymidine [dT] and 2-thio-2′-deoxyribothymidine [s^2dT].

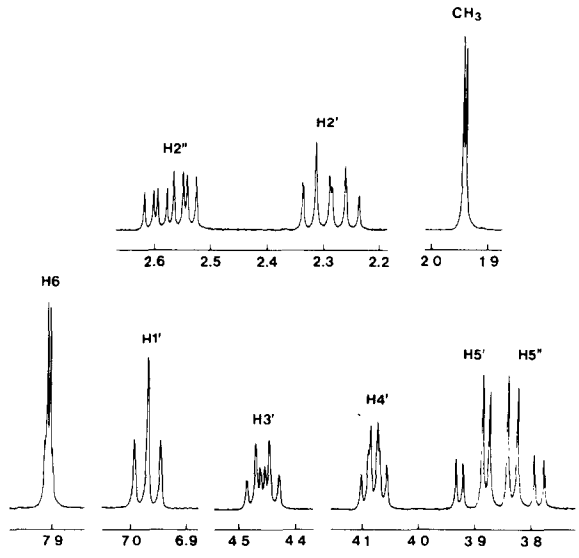

Fig.2. The 270-MHz proton NMR spectrum of s^2dT in ^2H_2O solution (10 mM, 23°C). The intensity scale for the methyl resonance is 1/4 of that for other resonances.

sodium 2,2-dimethyl-2-silapentane-5-sulfonate. Spin-coupling constants were determined within 0.1 Hz by the spectral simulation with a modified version of NMRTRY/PLOT program [4]. Selective spin-lattice relaxation rates were measured with the 180° pulse of about 10 ms and NOE were observed by the gated decoupling method.

3. RESULTS

3.1. *Assignments of proton NMR signals*

The proton resonances of s^2dT (fig.2) have been assigned by spin-decoupling and NOE experiments. On irradiation of the H1′ proton, NOE of 6% is observed for the resonance (2.563 ppm) of one of 2′-CH_2 protons but 0% for the resonance (2.289 ppm) of the other. Accordingly, the former is assigned to the H2′′ proton and the latter to the H2′ proton (fig.1), since the H1′ proton is much closer to the H2′′ proton than to the H2′ proton. The proton resonances of dT have also been assigned similarly.

3.2. *Conformation about the glycosidic band*

The conformation about the glycosidic band (*syn* and *anti*, fig.3a) has been analysed by the use of NOE and relaxation rates. For a pair of pro-

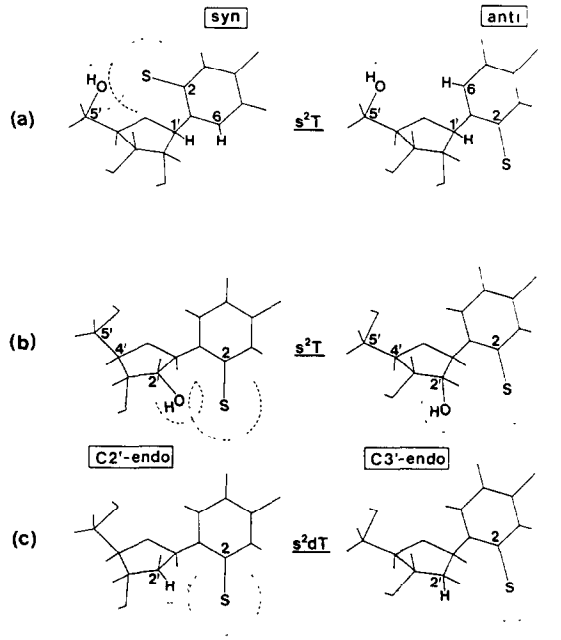

Fig.3. Schematic drawings of (a) the *syn* and *anti* forms of s^2T, (b) the C2′-endo and C3′-endo forms of s^2T, and (c) the C2′-endo and C3′-endo forms of s^2dT, where broken lines show the van der Waals radii.

Table 1

Selective spin-lattice relaxation rates (R), relative intensity enhancements (F), and proximity factors (P) for the H1′ and H6 protons

	T	s^2T	dT	s^2dT
F_1(H1′)	0.13	0.02	0.10	0.01
F_1(H6)	−1.00	−0.99	−0.99	−1.00
R(H1′) [s^{-1}]	0.24	0.23	0.30	0.35
P(H1′,H6)	3.1	0.5	3.0	0.4
F_2(H6)	0.06	0.01	0.06	0.01
F_2(H1′)	−1.00	−1.00	−0.98	−1.00
R(H6) [s^{-1}]	0.55	0.48	0.45	0.40
P(H6,H1′)	3.3	0.5	2.8	0.4

Table 2

Vicinal spin-coupling constants (J) and fractional populations of local conformations of ribose moiety

	T	s^2T	dT	s^2dT
$J_{1'2'}$ (Hz)	4.9	2.7	6.8	6.5
$J_{3'4'}$ (Hz)	5.4	7.6	3.9	4.5
$J_{4'5'}$ (Hz)	3.0	2.7	3.5	3.3
$J_{4'5''}$ (Hz)	4.3	3.3	5.0	4.7
C3′-endo	52%	73%	36%	41%
C2′-endo	48%	27%	64%	59%
Ratio	1.1	2.7	0.6	0.7
gg	66%	80%	54%	59%
gt	24%	13%	31%	28%
tg	10%	7%	15%	13%

tons, Hi and Hj, the proximity factor P(Hi,Hj) is defined here as $100 \times R(\mathrm{H}i)F_k(\mathrm{H}i)/[-F_k(\mathrm{H}j)]$ on irradiation of the Hj proton in the k-th experiment; R(Hi) is the selective relaxation rate of Hi, F_k(Hi) and F_k(Hj) are the relative intensity enhancements of the Hi and Hj protons, respectively. The experimental data for the pair of H1′ and H6 protons (fig.1) are listed in table 1, with the irradiation of the H6 proton (k = 1) or the H1′ proton (k = 2). For each of T, s^2T, dT and s^2dT, the proximity factors P(H1′,H6) and P(H6,H1′) closely agree with each other, indicating that cross relaxation effects with other protons are negligible. Therefore, such proximity factors are approximately proportional to the product of the average value of the inverse sixth power of the distance (H1′–H6) and the correlation time (τ_c) [5]; the τ_c-values of the 4 nucleosides are expected to be nearly the same. The proximity factors for s^2T and s^2dT are as small as ~0.5 whereas those for T and dT are as large as ~3 (table 1). These results indicate that T and dT take the *syn* form together with the *anti* form while s^2T and s^2dT take exclusively the *anti* form, since the distance H1′–H6 is much longer in the *anti* form than in the *syn* form (detailed analyses of the conformational equilibria about the glycosidic bond will be reported elsewhere).

3.3. *Conformation of ribose moiety*

The vicinal coupling constants of s^2dT and dT are listed in table 2, together with those of s^2T and T as in [3]. The fractional populations of the C3′-endo and C2′-endo forms of the ribose ring may be obtained as $J_{3'4'}/(J_{1'2'} + J_{3'4'})$ and $J_{1'2'}/(J_{1'2'} + J_{3'4'})$, respectively, and the populations of the gg, gt and tg forms have been obtained from $J_{4'5'}$ and $J_{4'5''}$ by the standard method [6] (table 2). As for deoxyribonucleosides, s^2dT and dT, the conformations of deoxyribose ring (the populations of C3′-endo, C2′-endo, gg, gt and tg) are nearly the same as each other. However, for ribonucleosides, 2-thiolation drastically affects the conformational characteristics of ribose moiety. The population ratio [C3′-endo]/[C2′-endo] of s^2T (2.7) is much higher than that of T (1.1), and furthermore the population of the gg form is appreciably higher in s^2T (80%) than in T (66%).

4. DISCUSSION

4.1. *Steric effects of the 2-thiocarbonyl group and 2′-hydroxyl group in 2-thioribothymidine*

The C=S bond (0.17 nm) is longer than the C=O bond (0.12 nm) and furthermore the van der Waals radius of the sulfur atom (0.185 nm) is much larger than that of the oxygen atom (0.14 nm). The steric repulsion between this bulky thiocarbonyl group and the 5′-exocyclic group (CH_2OH) will not allow s^2dT or s^2T to take the *syn* form (fig.3a). In fact,these 2-thiolated nucleosides have been found, here, to take exclusively the *anti* form. Then the thiocarbonyl group in the *anti* form of nucleosides will possibly interact with the 2′-CHOH group of ribose moiety (fig.3b), but not appreciably with the 2′-CH_2 group of deoxyribose moiety (fig.3c). Here certainly, the 2-thiolation of dT has been found not to affect the conformation of deoxyribose moiety. By contrast, the conformational characteristics of the ribose ring of T is drastically affected by 2-thiolation, and s^2T predominantly takes the C3′-endo-gg form. As shown in fig.3b, the stabilization of the C3′-endo form rather than the C2′-endo form of s^2T is clearly due to the steric effects of the 2-thiocarbonyl group and 2′-hydroxyl group of the ribose ring. The stabilization of the gg form (about the C5′–C4′ bond) is probably due to the short range conformational interrelation; the C3′-endo ribose ring almost exclusively takes the gg form [7].

4.2. *Thermostability of polyribonucleotides containing 2-thiopyrimidine*

The melting temperatures of polyribonucleotides containing 2-thiopyrimidine are much higher than those of unmodified polyribonucleotides. For example, the duplex structure of homocopolymer of adenosine and 2-thiouridine, poly[r(A–s^2U):r(A–s^2U)] does not melt below 100°C while poly[r(A–U):r(A–U)] melts at about 45°C [8]. By contrast, the melting temperature of the homocopolymer of 2′-deoxyadenosine and s^2dT, poly[d(A–s^2T):d(A–s^2T)], is only about 18°C higher than that of poly[d(A–T):d(A–T)] [9]. These data are consistent with the present finding that the conformational characteristics are remarkably different between s^2T and T but not between s^2dT and dT. Accordingly, the extraordinary thermostability of polyribonucleotides containing 2-thiopyrimidine is primarily due to the conformational stability of 2-thiopyrimidine ribonucleoside moiety in the gg-C3′-endo-*anti* form, rather than to the effects of 2-thiolation, if any, on the base-stacking ability of the base moiety.

4.3. *Thermostability of tRNAs from extreme thermophiles*

In the molecule of yeast $tRNA^{Phe}$ [10], the T(54)·m^1 A(58) base pair is stacked between the G(53)·C(61) pair of TΨC-stem and the interloop Ψ(55)·G(18) pair. Thus, the invariant sequence of G(53)–T(54)–Ψ(55) forms an extension of the A-type duplex of TΨC-stem and participates in the association of the TΨC-loop and the D-loop [10]. A similar tertiary structure is probably formed around G(53)–s^2T(54)–Ψ(55) in tRNAs from extreme thermophile [11], and such tRNAs are much more thermostable than mesophile tRNAs [12].

Here, the modification of T to s^2T has been found to enhance the structural rigidity of nucleoside unit; because of the steric effects of the bulky 2-thiocarbonyl group and the 2′-hydroxyl group, s^2T predominantly takes the gg-C3′-endo-*anti* form. It should be remarked here that the T(54) residue of yeast $tRNA^{Phe}$ takes just this form in the tertiary structure [10]. Therefore, the inherent structural rigidity of s^2T at the position 54 of tRNA should significantly enhance the struc-

tural stability of the moiety including G(53)–s^2T(54)–Ψ(55), that is critical for maintaining the tertiary structure of tRNA as required for the function at high temperature around 80°C. Thus, the biological role of the post-transcriptional 2-thiolation of T(54) is to contribute to the thermostability of the tertiary structure of extreme thermophile tRNAs, through the steric effects of the 2-thiocarbonyl group and 2′-hydroxyl group.

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