HEAT-INDUCED STABILITY OF tRNA FROM AN EXTREME THERMOPHILE, *Thermus thermophilus*

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Summary: The melting temperature of tRNA from a thermophile, *Thermus thermophilus*, was raised concomitantly by increasing the growth temperature. The nucleotide analysis showed that the mole contents of two modified nucleotide residues, 5-methyl-2-thiouridylate and ribothymidylate, varied significantly with changes in growth temperature. The sum of the two nucleotide contents was one per tRNA molecule independent of the growth temperature. There is a linear relationship between the melting temperature and mole fraction of 5-methyl-2-thiouridylate of the tRNA, indicating that the thiolated nucleotide plays an important role in thermal stability. These results suggest that the unusual thermal stability of the thermophile tRNA is partly induced by the environmental temperature by modifying ribothymidine to its 2-thio derivative.

The molecular mechanism of thermophily has long interested biochemists, but it has not yet been completely clarified in terms of molecular biology. We have been studying the properties and structure of tRNA from an extreme thermophile, *Thermus thermophilus* strain HB8 (=ATCC 27634) (1,2). The thermo-

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Abbreviations: S, 5-methyl-2-thiouridine; ψ, pseudouridine; T, ribothymidine; s^4U, 4-thiouridine; m^1A, 1-methyladenosine; Gm, 2'-O-methylguanosine; m^7G, 7-methylguanosine; CD, circular dichorism; UV, ultraviolet; Tm, melting temperature.

phile tRNA is thought to be one of the best experimental materials for elucidation of the thermophily because the tRNA has a higher melting temperature than those of mesophilic and moderately thermophilic organisms (the melting temperature of *T. thermophilus* tRNA is 9°C higher than that of *Escherichia coli* (3)), and yet is equivalent to that of *E. coli* in its biological functions in protein biosynthesis *in vitro* (4).

Previous studies suggested that unusual thermostability of tRNA from *T. thermophilus* can be explained in part on the basis of its high G-C base pair content in the base-paired region (5) and in part from the presence of a minor component, 5-methyl-2-thiouridine(S), which specifically replaces ribothymidine (T) in TψC loop in the thermophile tRNA (6). In this communication, we report that the melting temperature of *T. thermophilus* tRNA is positively correlated to the growth temperature of the thermophile cells from which the tRNA had been extracted and the increment in melting temperature is parallel to the increment in S content of tRNA molecule.

Table 1. The relationship between melting temperature of tRNA and the growth temperature of T. thermophilus cells from which the tRNA was extracted. Melting temperatures and hyperchromicities at 260 nm upon the melting were measured in 0.01 M Tris-HCl, pH 7.5, containing 10 mM Mg^{2+}.

Growth temperature °C	Melting temperature °C	Hyperchromicity %
50	83.3	21.7
55	83.8	21.7
60	84.3	20.9
68	84.8	22.8
75	85.8	22.3
80	86.4	23.6

Table 2. Nucleotide compositions of tRNAs extracted from T. thermophilus cells grown at various temperatures.

Growth temperature °C	Nucleotide composition (moles/tRNA)												
	A_{OH}	A_p	C_p	G_p	U_p	m^7G_p	$G_{mp}G_p$	ψ_p	s^4U_p	m^1A_p	Sp + Tp	Sp*	Tp*
50	1.0±0.2	10.3±1.0	25.7±0.8	23.2±0.6	10.4±0.5	2.2±0.3	0.7±0.2	1.8±0.1	0.8±0.04	0.6±0.1	0.8±0.1	27.8	72.2
55	1.0	9.8	25.0	23.8	10.0	2.0	0.8	1.7	0.8	0.5	0.8	31.8	68.2
60	1.0	11.5	25.8	23.8	9.6	2.0	0.8	1.8	0.8	0.6	0.8	36.7	63.3
68	1.0	11.7	24.8	24.2	10.0	2.5	1.0	1.8	0.9	0.6	0.7	42.7	57.3
75	1.0	11.6	25.6	23.8	10.0	2.4	1.2	1.8	1.0	0.6	0.9	54.5	45.5
80	1.0	11.2	25.2	24.3	9.6	2.1	1.1	1.6	0.9	0.4	0.9	57.4	42.6

* mole fraction (%) of Sp + Tp.

Experimental and Results: tRNA was extracted from *T. thermophilus* HB8 cells, which had been grown at various temperatures and harvested at middle log phase. tRNA was purified using DEAE-cellulose column chromatography (7). Table 1 shows the melting temperature of the tRNA preparations in a buffer containing 10 mM Mg^{2+} ion. The higher the growth temperature of the cells, the higher the melting temperature of the extracted tRNA.

The tRNA preparations were digested by RNase T_2 and subjected to two-dimensional thin-layer chromatography to determine nucleotide composition (8). To estimate the mole fraction of Sp and Tp, the overlapped spot containing Sp, Tp and s^4Up was cut out and eluted with water. The eluate was heated (100°C, 1h) in 0.01N HCl to convert s^4Up to Up followed by desalting and treating with phosphomonoesterase. The resulting nucleosides were determined after separation by another thin-layer chromatography as described elsewhere (6). s^4Up content was determined spectrophotometrically and m^1Ap content was estimated after being converted to 6-methyladenylate by treating with NaOH. The results are shown in Table 2. The mole fractions of nucleotides components except Sp, Tp and GmpGp were fairly constant independent of the growth temperature. The sum of Sp and Tp contents was nearly one mol per tRNA independent of the growth temperature. However the ratio of Sp to Tp greatly differed with the change of growth temperature. The mole fraction of Sp was increased and that of Tp was reciprocally decreased with the raise of growth temperature. When the melting temperature was plotted as a function of Sp content, a linear correlation was obtained as shown in Fig. 1. The results suggest that the thermal stability of *T. thermophilus*

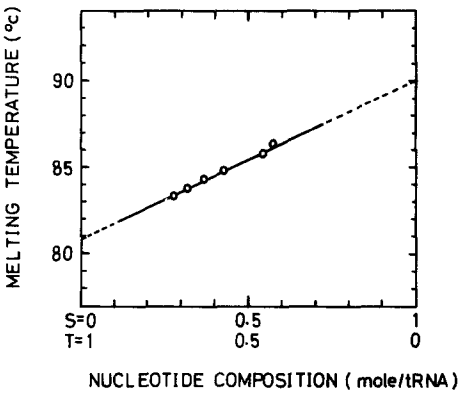

Fig. 1. The relationship between melting temperature of the thermophile tRNA and mole fraction of 5-mthyl-2-thiouridine and ribothymidine residues. The melting temperature was measured in a Tris-HCl buffer pH 7.5, containing 10 mM Mg^{2+}.

tRNA depends partly on the presence of Sp, and the Sp content in tRNA molecule which increased with the increment in environmental temperature provides a mechanism of adaptation of tRNA to heat.

The mole fraction of GmpGp also increased slightly with a rise in growth temperature as shown in Table 2, suggesting that the methylation of guanosine to 2'-O-methylguanosine was enchanced to some extent at higher temperatures. However the change in nucleotide composition was so small that it is not likely that the change significantly contributed in changes of melting temperature of tRNA preparations.

CD spectra of tRNAs from cells grown at various temperature did not show any significant difference in 300 to 200 nm region. In the near UV region, the thermophile tRNA has two CD bands, peaks at 313 nm and 340 nm which are assigned to S and s^4U, respectively (9), representing the contents and conformation of these thiolated components. The magnitude of CD band at 313 nm was increased when the growth temperature

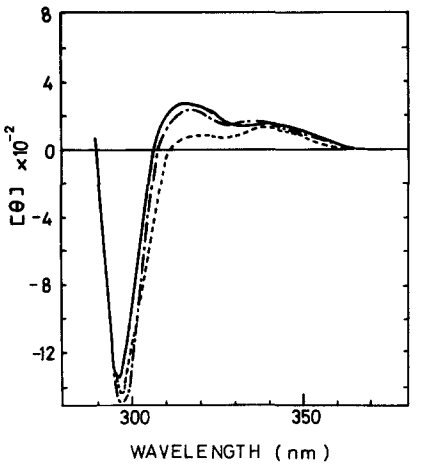

Fig. 2. CD spectra of tRNA in near UV region. The CD spectra were recorded on a JASCO J-20 Spectropolarimeter in the presence of 10 mM Mg^{2+}. ———: tRNA extracted from *T. thermophilus* cells grown at 80°C, —·—·—: at 68°C, -----: at 50°C.

was raised, whereas that at 340 nm remained mostly unchanged as shown in Fig. 2. These CD studies also suggest that the secondary structure of the thermophile did not change, but only the amount of S in a certain conformation was significantly increased by raising the growth temperature in correlation with the melting temperature of the tRNA.

Discussion: The present study clearly demonstrates that the thermal stability of tRNA from *T. thermophilus* is partly induced by heat. The stabilizing mechanism of the thermophile tRNA can be explained by combination of at least two mechanisms that is, intrinsic stability caused by high G-C pair content and heat induced mechanism caused by increment of S content accompanying decrease of equivalent amount of T.

Although at the present, it is hard to deny the possibility that there is two sets of genes for the thermophile tRNA, one

for tRNA which contains only T and one only S in TψC loop and their expression of these genes are regulated by the environmental temperature, it is most likely that biochemical thiolation of T to S was controlled by the growth temperature. Probably the action of enzyme which catalyze the thiolation of T was accelerated when the thermophile cells were grown at higher temperatures.

The present study, both the determination of chemical composition and CD measurement, provided further evidence for our previous consideration that the thermophile tRNA is stabilized in part by the presence of S as well as high G-C pair content (6). It seemed that S plays an essential role in the additional thermostability of the tRNA induced by high temperatures. When a three-dimensional structure of the thermophile $tRNA_f^{Met}$ was assumed based upon the data for yeast tRNA (10,11), S stacked with a nucleotide base (Gm) in DHU loop. Thus the stacking force seemed to play a role in the connecting of the TψC loop with the DHU loop. The connection between these two loops became more tighter in tRNA extracted from cells grown at higher temperatures resulting in higher melting temperature since 2-thiouridine derivatives are known to strengthen the stacking interaction with the neighboring bases (12,13).

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