Rheinberger, H. J., Sternbach, H., & Nierhaus, K. H. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5310-5314.
Sprinzl, M., & Cramer, F. (1973) *Nature (London), New Biol. 245*, 3-5.
Sprinzl, M., & Cramer, F. (1979) *Prog. Nucleic Acid Res. Mol. Biol. 22*, 1-69.
Sprinzl, M., & Sternbach, H. (1979) *Methods Enzymol. 59*, 182-190.
Sprinzl, M., von der Haar, F., Schlimme, E., Sternbach, H., & Cramer, F. (1972) *Eur. J. Biochem. 25*, 262-266.
Sprinzl, M., Scheit, K. H., Sternbach, H., von der Haar, F., & Cramer, F. (1973) *Biochem. Biophys. Res. Commun. 51*, 881-887.
Sprinzl, M., Sternbach, H., von der Haar, F., & Cramer, F. (1977) *Eur. J. Biochem. 81*, 579-589.
Sternbach, H., von der Haar, F., Schlimme, E., Gaertner, E., & Cramer, F. (1971) *Eur. J. Biochem. 22*, 166-172.
von der Haar, F. (1973) *Eur. J. Biochem. 34*, 84-90.
Wagner, T., & Sprinzl, M. (1979) *Methods Enzymol. 61*, 615-628.
Wagner, T., & Sprinzl, M. (1980) *Eur. J. Biochem. 108*, 213-221.
Wagner, T., Cramer, F., & Sprinzl, M. (1982) *Biochemistry 21*, 1521-1529.
Yoshikawa, M., Kato, T., & Takenishi, T. (1967) *Tetrahedron Lett.* 5065-5068.

Separation and Comparison of 2-Thioribothymidine-Containing Transfer Ribonucleic Acid and the Ribothymidine-Containing Counterpart from Cells of *Thermus thermophilus* HB 8†

Kimitsuna Watanabe,* Tairo Oshima, Fritz Hansske, and Takahisa Ohta

ABSTRACT: For the extreme thermophile *Thermus thermophilus* HB 8, a positive correlation was observed among the growth temperatures of the cells, the melting temperature, and the 2-thioribothymidine (s^2T) content of tRNA extracted from cells grown at various temperatures [Watanabe, K., Shinma, M., Oshima, T., & Nishimura, S. (1976) *Biochem. Biophys. Res. Commun. 72*, 1137-1144]. On the basis of these observations, studies were carried out from which the following results were obtained. (1) Both RNase T_1 and U_2 digestions of tRNA gave only four fragments containing s^2T or T: $s^2T\psi CGp$, $s^2T\psi CAp$, $T\psi CGp$, and $T\psi CAp$. For the different growth temperatures, the ratio of the content of $s^2T\psi CGp$ plus $s^2T\psi CAp$ to that of $T\psi CGp$ plus $T\psi CAp$ was almost the same as that of the s^2Tp to Tp content reported previously. (2) The midpoint of the s^2T-specific circular dichroism spectral change induced by heat was constant for all tRNAs extracted from cells grown at various temperatures, suggesting that the s^2T-containing tRNAs melt at about the same temperature, which is independent of the growth temperature of cells. (3) s^2T-containing tRNA was separated from the T-containing counterpart quantitatively by a specific modification of s^2T with bromoaceto-2,4-dinitroanilide followed by BD-cellulose column chromatography. The molar ratio of the s^2T- and T-containing tRNAs was also similar to that of s^2Tp to Tp as mentioned above. These results demonstrate that *T. thermophilus* cells have both s^2T- and T-containing tRNAs, whose relative content is controlled by the growth temperature. This phenomenon may be necessary to enable the thermophile to adapt to higher temperatures.

An extreme thermophile, *Thermus thermophilus* HB 8, is capable of growing over a wide temperature range between 48 and 85 °C (Oshima & Imahori, 1974). The melting temperatures of unfractionated tRNAs from cells cultured at various temperatures (50, 55, 60, 68, 75, and 80 °C) showed strong correlations to the different culture medium temperatures. In addition, the 2-thioribothymidine (s^2T)[1] content of the tRNAs was also proportional to the growth temperature of the cells (Watanabe et al., 1976a).

These results imply the following possibilities: (1) the thermostability of the thermophile tRNA is mainly determined by the content of s^2T; (2) in the cells of *T. thermophilus* HB 8, two species of tRNA exist, one s^2T-containing tRNA and another ribothymidine- (T) containing tRNA. The ratio of these two species is determined by the growth temperatures of the cells.

The first possibility was confirmed by CD (Watanabe et al., 1976b) and NMR studies (Davanloo et al., 1979) and other biochemical approaches (Watanabe et al., 1980; Kumagai et al., 1982); however, there has been no evidence for the second possibility.

In this paper, we try to refine the previous observations by confirming the following points: First, the s^2T residue in fact replaces only the T residue in the $T\psi C$ sequence, by analysis of all the fragments containing T or s^2T, quantitatively. Second, we present evidence for the existence of the two tRNA species in *T. thermophilus* cells, by direct separation of s^2T-containing tRNA and its T-containing counterpart using a specific chemical modification on the s^2T residue. This process was performed by synthesizing a reagent that modifies thio-

† From the Department of Agricultural Chemistry, Faculty of Agriculture, The Unversity of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan (K.W. and T. Ohta), the Mitsubishi-Kasei Institute of Life Sciences, Minamiooya 11, Machida-shi, Tokyo 194, Japan (T. Oshima), and the Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada T6G 2G2 (F.H.). *Received July 12, 1982.*

[1] Abbreviations: s^2T, 2-thioribothymidine; BADA, bromoaceto-2,4-dinitroanilide; C=S, thiocarbonyl group; CDTA, *trans*-1,2-diaminocyclohexanetetraacetic acid; H_2O_2, hydrogen peroxide; LC, high-performance liquid chromatography; DCC, *N,N'*-dicyclohexylcarbodiimide; Me_2SO, dimethyl sulfoxide; Me_4Si, tetramethylsilane; CD, circular dichroism; NMR, nuclear magnetic resonance; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; BD, benzoylated diethylaminoethyl.

0006-2960/83/0422-0098$01.50/0

pyrimidine residues, preferentially, followed by BD-cellulose column chromatography. Finally, we discuss the molecular mechanism by which the thermophile adapts to higher temperature.

Materials and Methods

Materials. RNase T_1, RNase T_2, and RNase U_2 were purchased from Sankyo Co. *Escherichia coli* alkaline phosphomonoesterase (PMase) and snake venom phosphodiesterase (PDase) were obtained from Worthington Biochemical Co. Resins of DEAE-Sephadex A-25 and BD-cellulose were purchased from Pharmacia Fine Chemicals Co. and Boehringer Manheimm Co., respectively. Cellulose acetate strips (Separax) for the first dimensional paper electrophoresis were from Fuji Film Co., and DEAE-cellulose paper (Whatman DE-81) for the second dimension was from W. and R. Balston. ^{14}C-Labeled amino acids (sp act. 250–500 Ci/mol) were obtained from Amersham, England.

Thermus thermophilus HB 8 cells were cultured at 50, 55, 60, 68, 75, and 80 °C, respectively, and harvested at the middle log phase (Klett no. 100–200). tRNAs were extracted as described previously (Watanabe et al., 1976a).

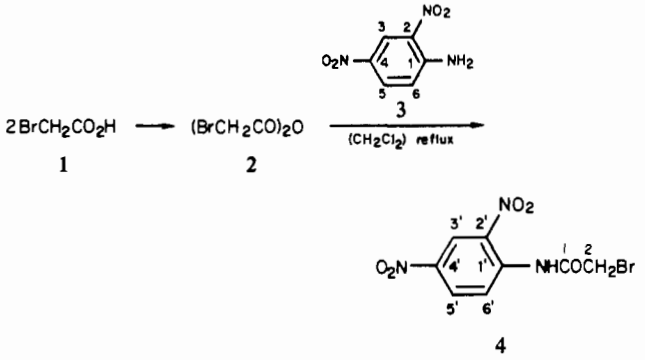
Analysis of Fragments Containing s^2T and T. A total of 200 A_{260} units of each tRNA from the cells cultured at 60 and 80 °C was digested first with 75 units of RNase T_1 in 0.02 M Tris-HCl, pH 7.5, in 1 mL of solution at 37 °C for 18 h and then with 2 units of RNase U_2 in 0.05 M KOAc, pH 4.6, under the same conditions. The reaction mixture was then adjusted to 0.01 N HCl and stored at 0 °C overnight to open the cyclic 2′,3′-phosphate of the 3′ end of the oligonucleotides produced by RNase U_2 digestion. Solid urea and NaOH were added to adjust the mixture to 7 M urea and neutral pH.

Oligomers thus produced were separated by DEAE-Sephadex A-25 column chromatography as described previously (Watanabe et al., 1974), and the base compositions of the resulting peak fractions were analyzed by high-performance liquid chromatography (LC, Wataers Limited) as reported previously (Watanabe, 1980). The fourth peak fraction contained T exclusively, whereas the fifth peak contained s^2T mainly and T to a small extent, but no other peak contained T or s^2T. Both peak fractions were joined together, and the mixture (10 A_{260} units) was applied on the two-dimensional paper electrophoresis (Watanabe & Cramer, 1978). The appropriate spots detected by a UV lamp were measured for their absorbance and analyzed for their base compositions by LC as described above.

Measurement of CD Spectra. CD spectra were recorded on a JASCO J-20 spectropolarimeter, as described previously (Watanabe et al., 1976b). The molecular ellipticity coefficient [θ] was calculated on the assumption that the molar extinction coefficient [ϵ] of each tRNA was the same as that of the cells cultured at 68 °C. The $[\epsilon]_{260}$ of this tRNA was found to be 6900 in 0.01 M Tris-HCl, pH 7.5, and 0.2 M NaCl by phosphorus assay (Ames & Dubin, 1960).

Chemical Synthesis of the Modification Reagent Bromoaceto-2,4-dinitroanilide (**4**) (*Scheme I*). To a cooled solution (0 °C) of 0.3 mol (41.69 g) of bromoacetic acid in 250 mL of anhydrous ether was added slowly a solution of 0.15 mol (30.95 g) of *N,N′*-dicyclohexylcarbodiimide (DCC) in 125 mL of anhydrous ether. After 30 min, the ice bath was removed, and the mixture was stirred at room temperature for 1 day. The precipitated *N,N′*-dicyclohexylurea was filtered off, and the solution was evaporated. After addition of 75 mmol (6.86 g) of 2,4-dinitroaniline, 2 mmol (244 mg) of 4-(dimethylamino)pyrimidine, and 400 mL of methylene chloride, the mixture was refluxed for 2 days. Thin-layer chromatography

Scheme I



(silica gel; pentane/ethyl acetate, 4:6) showed a clean conversion to the title compound. The mixture was evaporated and partitioned between 200 mL of chloroform and 100 mL of half-saturated sodium chloride solution. The organic phase was consecutively washed with 100 mL of half-saturated sodium chloride solution, 100 mL of saturated sodium bicarbonate solution, and 100 mL of water. After being dried with Na_2SO_4 and evaporation, the resulting solid was twice recrystallized from methanol with the addition of decolorizing charcoal: yield 13.7 g (60.9%) of light yellow needles; mp 104 °C; high-resolution mass spectrum (AE1 MS-50), *m/z* 304.9540 [11.8, M^+ (^{80}Br) = 304.9471], 302.9495 [9.4, M^+ (^{78}Br) = 302.9491], 258.9544 [12.3, M^+ (^{80}Br) − NO_2 = 258.9542], 256.9563 [12.3, M^+ (^{78}Br) − NO_2 = 256.9562], 183.0272 (100, $C_6H_5N_3O_4$ = 183.0280), 122.9271 (19.1, $^{80}BrCH_2CO$ = 122.9269), 120.9291 (19.5, $^{78}BrCH_2O$ = 120.9289); ^{1}H NMR (Varian HX-100) (using [$^2H_6$]Me_2SO and Me_4Si as internal standards) (ppm) 11.16 (br s, 1, NH), 9.79 (d, 1, $J_{3',5'}$ = 2.7 Hz, $H_{3'}$), 9.61 (quartet, 1, $J_{3',5'}$ = 2.7 Hz, $J_{5',6'}$ = 9.1 Hz, $H_{5'}$), 9.09 (d, 1, $J_{5',6'}$ = 9.1 Hz, $H_{6'}$), 4.24 (s, 2, CH_2); ^{13}C NMR (Brucker HFX-90 spectrometer) broad-band ^{13}C-decoupled spectra obtained at 22.6 MHz (using [$^2H_6$]Me_2SO and Me_4Si as internal standards). Anal. Calcd for $C_8H_6N_3O_5Br$ (304.06): C, 31.60; H, 1.99; N, 13.82; Br, 26.28. Found: C, 31.61; H, 2.01; N, 13.75; Br, 26.29. The assignment was made by increment calculations (Breitmaier & Bauer, 1976), line-width determinations of the signals, and comparison of the chemical shifts of **3** and **4** (data not shown). The ortho, meta, and para shifts [$\Delta(\delta_4 - \delta_3)$] are in good agreement with the literature (Breitmaier & Bauer, 1976).

Modification and Separation of s^2T-Containing tRNA. The H_2O_2-treated tRNA was obtained by treating unfractionated tRNA with H_2O_2 at 20 °C for 3.5 h under the conditions described previously (Watanabe, 1980), and it was then dialyzed against CDTA to deprive the tightly bound Mg^{2+} from the tRNA (Watanabe et al., 1980). The denatured tRNA thus obtained was reacted with a 500-fold excess of BADA in 0.05 M phosphate buffer, pH 8.0, and 50% Me_2SO with stirring at room temperature for 3 days. The BADA-modified tRNA was recovered by ethanol precipitation 5 times and then subjected to BD-cellulose column chromatography (i.d. 0.8 × 6 cm). Elution was performed with 1 M NaCl in 0.02 M NaOAc, pH 6.0, and then with 20% ethanol added and finally with 50% ethanol added to the first elution buffer.

Results

Quantitative Analysis of s^2T- and T-Containing Oligonucleotides in tRNA Extracted from Cells Cultured at Different Temperatures. It was first necessary to demonstrate that the s^2T residue replaces only the T residue of the TψC

Table I: Molar Content of T- and s^2T-Containing Nucleotides and tRNAs from *T. thermophilus* HB 8 Expressed in Percent

	growth temp of cells (°C)			
	55	60	75	80
(1) T_2 digestion[a]				
Tp	68.2	63.3	45.5	42.6
s^2Tp	31.8	36.7	54.5	57.4
(2) T_1 and U_2 digestion[b]				
TψCGp + TψCAp		60.5		42.8
s^2TψCGp + s^2TψCAp		39.5		57.2
(3) tRNA				
T-containing tRNA	64.7	60.1	48.3	42.6
s^2T-containing tRNA	35.3	39.9	51.7	57.4

[a] Cited from the data of Watanabe et al. (1976a). [b] The molar extinction coefficient of each tetranucleotide was regarded as the sum of the extinction coefficients of mononucleosides, disregarding the hypochromic effect.

sequence. Unfractionated tRNA extracted from cells cultured at 60 and 80 °C, respectively, was digested with both RNases T_1 and U_2, and the resulting oligonucleotides were separated by DEAE-Sephadex A-25 and two-dimensional paper electrophoresis. Only four tetranucleotides were found to contain T or s^2T: TψCGp, TψCAp, s^2TψCGp, and s^2TψCAp. The molar ratio of the content of TψCGp plus TψCAp to that of s^2TψCGp plus s^2TψCAp was estimated from the absorbancy of each spot on the electrophoregram (Table I), and it coincided well with that of Tp to s^2Tp obtained by RNase T_2 digestion of each tRNA as reported previously (Watanabe et al., 1976a) in both cases of 60 and 80 °C. Thus it is strongly suggested that the conversion of T to s^2T takes place in the TψC sequence of the thermophile tRNA as the growth temperature of the cells is raised.

Melting Profiles of CD Spectra Specific for s^2T Residue of Each tRNA. We reported previously that s^2T showed a unique CD signal in the near-UV region, thus making it possible to follow the melting profiles of the s^2T-specific local region of the tRNA, and also that the midpoint of the melting profiles of this region corresponds well to the melting temperature of the tRNA detected by UV hyperchromicity (Watanabe et al., 1976b). Therefore, if the thermophile tRNA consists of two species, s^2T- and T-containing tRNAs, the melting temperature of the s^2T-containing tRNA should be estimatable by the midpoint of the melting profiles of this s^2T-specific CD signal, independent of the coexisting T-containing tRNA. Figure 1 shows the temperature-dependent changes of CD spectra in this region of six species of tRNA extracted from the cells cultured at various temperatures. As shown in the figure, the midpoint of the s^2T-specific CD spectral change falls in between 86 (curve e) and 90 °C (curve f) in all cases of the tRNA used. These observations suggest that all the s^2T-containing tRNAs melt at about the same temperature (at about 88 °C as described below) independent of the growth temperature of the cells.

Separation of s^2T-Containing tRNA from T-Containing Counterpart. The above results suggest strongly the existence of two species of tRNA in the thermophile cells: s^2T-containing and T-containing tRNAs. For separation of these two species of tRNA, we tried to utilize a reagent capable of modifying the s^2T residue specificially. Since a reagent containing a bromoaceto group is known to react preferentially with thiocarbonyl groups of the modified nucleoside residues in tRNA (Hara et al., 1970), we synthesized BADA as described under Materials and Methods. In order to modify the s^2T residue selectively, it was necessary to first treat tRNA with hydrogen peroxide (H_2O_2). By this reagent, thio-

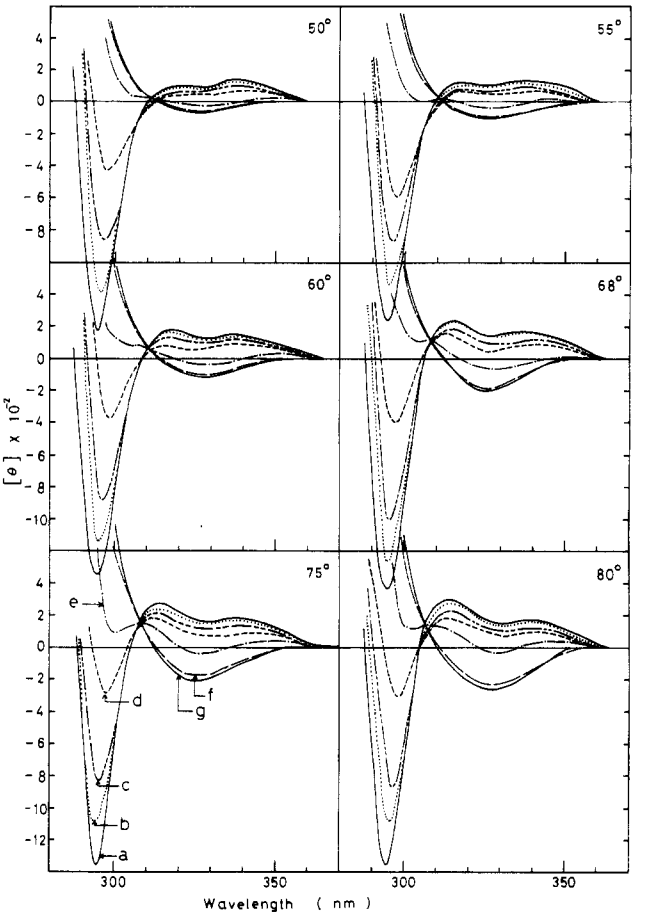


FIGURE 1: Temperature-dependent CD spectral change in the near-UV region of tRNAs from the cells cultured at various temperatures (shown at upper right in each panel). The temperatures (°C) for the CD measurements are 25 (a), 45 (b), 65 (c), 80 (d), 86 (e), 90 (f), and 96 (g).

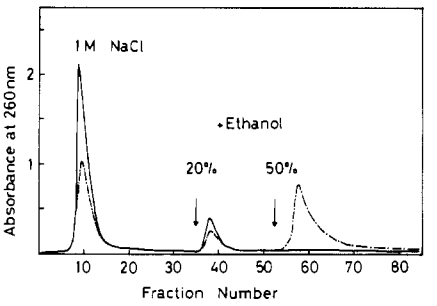


FIGURE 2: BD-cellulose column chromatography of intact (solid line) and BADA-modified tRNAs (dashed line). 30 A_{260} units of tRNA from the cells cultured at 75 °C were applied onto the column (i.d. 0.8 × 6 cm). The elution buffer used was 1 M NaCl and 0.02 M NaOAc, pH 6.0, into which ethanol was added stepwise to make 0%, 20%, and 50% solutions.

nucleosides in tRNA other than s^2T should be converted to their H_2O_2-altered forms in which the free thiocarbonyl (C═S) groups are modified. The s^2T residue is unreactive in the intact tRNA under these reaction conditions (Watanabe, 1980).

The modified tRNA was then denatured by dialyzing against CDTA so as to cause the s^2T residue to become exposed and be reactive with BADA, which attacks the C═S group of s^2T. The CDTA-treated tRNA was reacted with an excess amount of BADA for 3 days at room temperature under vigorous stirring.

The BADA-modified tRNA was finally fractionated by BD-cellulose column chromatography as shown in Figure 2.

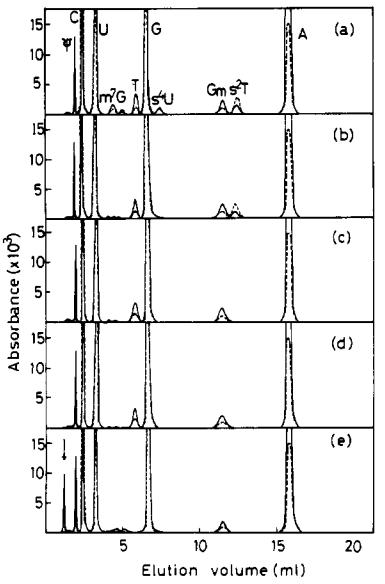


FIGURE 3: Elution patterns by LC of nucleosides derived from intact tRNA (a), H_2O_2-treated tRNA (b), and BADA-modified tRNA fractions on BD-cellulose column chromatography, eluted with 1 M NaCl (c), with 20% ethanol (d), and with 50% ethanol (e): (—) 254 nm; (---) 280 nm. The arrow in (e) shows the peak of BADA-modified s^2T.

Whereas most of the unmodified tRNA was eluted in the 1 M NaCl region and a minor portion in the 20% ethanol region, the BADA-modified tRNA was not eluted until the ethanol concentration was increased to as much as 50%.

The materials thus obtained for the three different concentrations of ethanol were analyzed by LC for the presence of T or s^2T. The first and second fractions eluted by 0% and 20% ethanol in 1 M NaCl buffer, respectively, contained only T but no s^2T (Figure 3c,d). On the other hand, the last eluent in the 50% ethanol region contained neither T nor s^2T but a new nucleoside peak in LC located at the beginning of the elution position (Figure 3e). The correspondence of the material of this peak fraction to the BADA-modified s^2T was confirmed by comparing its UV spectra as well as the elution position in LC with those of the authentic sample synthesized by reacting s^2T monomer with BADA (data not shown). Nucleotide content other than s^4U, m^7G, and s^2T appeared to remain unchanged throughout the modification. Therefore, it was concluded that the T-containing tRNA was recovered from the first and the second peaks of the BD-cellulose column chromatography, whereas the BADA-modified s^2T-containing tRNA was obtained from the last peak fraction eluted with 50% ethanol. Thus the separation of these two species of tRNA was successful.

The UV spectrum of the unmodified T-containing tRNA was almost the same as that of *E. coli* tRNA, the molar extinction coefficient ($[\epsilon]_{260}$ = 8100 in water) being the same as that of *E. coli* tRNA within the experimental error (Adler & Fasman, 1970; Willick & Kay, 1971; Blum et al., 1972). On the other hand, the BADA-modified s^2T-containing tRNA showed a new broad absorption band between 300 and 400 nm, although the 260-nm peak remained unshifted (data not shown). The molar extinction coefficient of this tRNA (in water) was determined to be 8600 at 260 nm by phosphorus assay (Ames & Dubin, 1960).

By using these extinction coefficients, we estimated the molar contents of T-containing and s^2T-containing tRNAs as shown in Table I. The molar contents coincided within experimental error with those of T and s^2T obtained from RNase T_2 digestion of the tRNAs (Watanabe et al., 1976a) as well as with those of T- and s^2T-containing tetranucleotides obtained above.

Amino Acid Acceptor Activities of Modified tRNA Preparations. The amino acid acceptor activities of various tRNA preparations used in the present study were investigated for four kinds of amino acid (Met, Ile, Phe, and Ser) by the manner reported earlier (Watanabe et al., 1980). The H_2O_2-treated bulk tRNA had almost the same acceptor activities as the intact tRNA, but the BADA-treatment seems to have caused a denaturation of tRNA to some extent. The 1 M NaCl eluent of the BADA-modified tRNA decreased the acceptor activities for each amino acid to half that of the intact tRNA, whereas both the 20% and 50% ethanol eluents of the BADA-modified tRNA completely lost the activities (data not shown). These results confirm the previous observations that the s^2T-modified tRNA no longer has the active conformation necessary for amino acid acceptance (Watanabe, 1980).

Discussion

Since we found that s^2T is located in the TψC region instead of T in tRNA from the extreme thermophile, the implication is that the thermophilic cells have two species of tRNA, T-containing and s^2T-containing tRNAs (Watanabe et al., 1974, 1976a). In order to verify this, we at first attempted to estimate the molar content of T- and s^2T-containing tetranucleotides by digestion of the thermophile tRNA with purine-specific RNases, followed by separation by column chromatography and paper electrophoresis. The results obtained were satisfactory as shown in Table I. However, there is the problem that the recovery of the total absorbancy from all the spots detected on the electrophoregram was no more than 50% of the original material subjected to electrophoresis. Attempts at estimating the molar ratio by 5′-^{32}P-labeling of the tetranucleotides were not successful, probably because of the problems in kination owing to the presence of modified nucleotides at their 5′ ends. Next, we made a direct separation of T-containing and s^2T-containing tRNAs by using BADA, which enabled a quantitative analysis with a good fit to the previous results (Table I).

It was not possible to calculate the melting temperatures of tRNAs from cells cultured at different temperatures (50–80 °C) by mixing the melting curves of T-containing and s^2T-containing tRNAs using the T to s^2T ratio at the defined temperature. The melting curves of T-containing tRNA were almost the same for all tRNAs obtained from cells grown at 55, 60, 75, and 80 °C. The melting temperature of s^2T-containing tRNA was estimated from the melting curve of native $tRNA_{f1}^{Met}$, since the s^2T-containing BADA-modified tRNA obtained in this study is considered to be considerably denatured, as judged from the complete loss of amino acid accepting activity. The relevance of using $tRNA_{f1}^{Met}$ is based on the following observations: (1) All the s^2T-containing native tRNAs showed similar transition temperatures between 86 and 90 °C, as indicated by the s^2T-specific CD melting profiles (Figure 1). (2) $tRNA_{f1}^{Met}$ containing 1 mol of s^2T has the transition temperature of 88 °C in both UV hyperchromicity and s^2T-specific CD melting profiles (Watanabe et al., 1976a, 1980).

When the melting curves of T-containing tRNA and $tRNA_{f1}^{Met}$ were mixed according to the molar ratios of Tp to s^2Tp (Table I), almost the same melting temperatures were obtained between calculated and measured values with the difference being within 0.3 °C, for each growth temperature (data not shown), although the calculated and measured melting curves differed significantly, probably because the isolated T-containing tRNAs are slightly denatured as judged

by amino acid acceptor activities. These results may in turn support the approximation that the melting temperature of the s^2T-containing tRNA can be estimated by the s^2T-specific CD spectral change.

There is evidence that the relative content of the two species of tRNA is determined only by the growth temperature and not by the growth stages of the bacterial cells. The cells were cultured at 75 °C and were harvested at different growth stages of Klett no. 50, 100, 200, and 400, from which unfractionated tRNAs were extracted and their base compositions were analyzed. All nucleotide compositions except for Tp and s^2Tp were constant as reported previously (Watanabe et al., 1976a), independent of the growth stage. The s^2T content was almost constant for tRNA from cells harvested at Klett no. between 50 and 200; however, it was slightly higher for tRNA from cells at Klett no. 400, which are at the stationary phase (data not shown). Since all the bacterial cells used in the present study were harvested at middle log phase with Klett no. between 100 and 200, it can be said that the molar content of Tp and s^2Tp, and consequently the relative amounts of T-containing and s^2T-containing tRNAs in the cells, is determined only by the growth temperature of the cells.

Thus it may be concluded that there exists a certain mechanism by which the thermophile tRNA acquires the s^2T residue in accordance with the environmental temperature. Lipsett & Peterkofsky (1966) reported an enzymatic activity in *E. coli* that thiolates uridine at position 8 of tRNA into 4-thiouridine. It is expected that there is probably a similar sulfur transferase to thiolate T to s^2T of the tRNA in the cells of *T. thermophilus* HB 8, whose activity may be regulated by the environmental temperature.

The existence of the two species of tRNA, T- and s^2T-containing tRNAs, may be important for the growth of thermophilic cells at extreme environmental temperatures. We have already purified two kinds of $tRNA^{Phe}$, one T-containing and another s^2T-containing $tRNA^{Phe}$ from cells cultured at 50 and 80 °C, respectively. In in vitro poly(U)-dependent poly(Phe) synthesis, below 65 °C, T-containing $tRNA^{Phe}$ and, above 65 °C, s^2T-containing $tRNA^{Phe}$ were more active than their counterparts, independent of ribosomes and S-100 fractions prepared from cells cultured at either 50 or 80 °C (unpublished results). These results lead to a speculation that the thermophile tRNA requires a certain flexibility mediated by the T to s^2T ratio, when functioning. Experiments are under way to examine this speculation.

Acknowledgments

We are indebted to M. Kato of Mitsubishi-Kasei Institute of Life Sciences for her kind technical assistance.

Registry No. **1**, 79-08-3; **3**, 97-02-9; **4**, 23543-24-0.

References

Adler, A. J., & Fasman, G. D. (1970) *Biochim. Biophys. Acta 204*, 183–190.

Ames, B. N., & Dubin, D. T. (1960) *J. Biol. Chem. 235*, 769–775.

Blum, A. D., Uhlenbeck, O. C., & Tinoco, I., Jr. (1972) *Biochemistry 11*, 3248–3256.

Breitmaier, E., & Bauer, G. (1976) *Pharm. Unserer Zeit 5*, 97–123.

Davanloo, P., Sprinzl, M., Watanabe, K., Albani, M., & Kersten, H. (1979) *Nucleic Acids Res. 6*, 1571–1581.

Hara, H., Horiuchi, T., Saneyoshi, M., & Nishimura, S. (1970) *Biochem. Biophys. Res. Commun. 3*, 305–310.

Kumagai, I., Watanabe, K., & Oshima, T. (1982) *J. Biol. Chem. 257*, 7388–7395.

Lipsett, M. N., & Peterkofsky, A. (1966) *Proc. Natl. Acad. Sci. U.S.A. 55*, 1169–1174.

Oshima, T., & Imahori, K. (1974) *Int. J. Syst. Bacteriol. 24*, 102–112.

Watanabe, K. (1980) *Biochemistry 19*, 5542–5549.

Watanabe, K., & Cramer, F. (1978) *Eur. J. Biochem. 89*, 425–432.

Watanabe, K., Oshima, T., Saneyoshi, M., & Nishimura, S. (1974) *FEBS Lett. 43*, 59–63.

Watanabe, K., Shinma, M., Oshima, T., & Nishimura, S. (1976a) *Biochem. Biophys. Res. Commun. 72*, 1137–1144.

Watanabe, K., Oshima, T., & Nishimura, S. (1976b) *Nucleic Acids Res. 3*, 1703–1713.

Watanabe, K., Oshima, T., Iijima, K., Yamaizumi, Z., & Nishimura, S. (1980) *J. Biochem. (Tokyo) 87*, 1–13.

Willick, G. E., & Kay, C. M. (1971) *Biochemistry 10*, 2216–2222.