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Biochemistry

Thermally induced biosynthesis of 2′-*O*-methylguanosine in tRNA from an extreme thermophile, *Thermus thermophilus* HB27

[posttranscriptional modification/tRNA (guanosine-2′)-methyltransferase/tRNA (adenine-1)-methyltransferase/D loop]

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ABSTRACT **The contents of 2′-*O*-methylguanosine and 1-methyladenosine in unfractionated tRNA obtained from *Thermus thermophilus* HB27 were found to increase significantly when the bacterium was grown at a higher temperature (80°C). *S*-Adenosyl-L-methionine-dependent tRNA (guanosine-2′)-methyltransferase (EC 2.1.1.34) and tRNA (adenine-1)-methyltransferase (EC 2.1.1.36) were detected in a cell-free extract of the thermophile, and both of them were partially purified. tRNA (guanosine-2′)-methyltransferase specifically catalyzed the methylation of the guanylate residue at position 19 from the 5′ end of *Escherichia coli* $tRNA_f^{Met}$. The amounts of these methyltransferases in the cells and their thermal characteristics seemed to be independent of the growth temperature of the bacterial cells from which the enzymes were extracted. It was inferred that the temperature dependence of the methylation process *in vivo* is accounted for, not by temperature dependence of enzyme formation, but by that of the enzyme activity.**

The tRNAs obtained from three kinds of extremely thermophilic bacteria, *Thermus thermophilus* HB8, *T. thermophilus* HB27, and *T. aquaticus* YT-1, have been found to show higher melting temperatures than those from mesophilic bacteria (1–4). In the process of protein biosynthesis, the thermostable tRNA of *T. thermophilus* HB8 functions in a way equivalent to that of *Escherichia coli* (5). Sequence studies on $tRNA_f^{Met}$ from the thermophile revealed that its nucleotide sequence was very similar to that of *E. coli* $tRNA_f^{Met}$ except for the facts that U51 of *E. coli* was replaced by G51 of *T. thermophilus* and that G19, T55, and A59 of the former were modified, respectively, to 2′-*O*-methylguanosine (Gm19), 2-thioribothymidine (s^2T55), and 1-methyladenosine (m^1A59) of the latter (6). These modified nucleosides are present at the corresponding sites in the primary structure of $tRNA^{Phe}$ and were also found in $tRNA_m^{Met}$, $tRNA^{Ile}$, and $tRNA^{Tyr}$ (7), suggesting that these modifications are common to most tRNAs of the extreme thermophile. Of special interest was the finding that the thiolation of the T residue in unfractionated tRNA of *T. thermophilus* HB8 was significantly enhanced when the bacterium was grown at higher temperatures. Moreover, the modification of T to s^2T was positively correlated with the high melting temperature of the thermophile tRNA (8). On the basis of these findings, we have proposed that thiolation of the T residue in the TΨC region is mainly responsible for the thermostability of this thermophile tRNA (2, 7–9).

tRNA from *T. thermophilus* HB27 also showed the unique modifications found in that of *T. thermophilus* HB8. In *T. thermophilus* HB27, besides thiolation of T to s^2T (3), methylation of A and ribose methylation of G were significantly dependent on the growth temperature and approached the upper limits of their variability (one mole each of the modified nucleosides per mole of tRNA) when the thermophile was cultured at 80°C.

It is shown in this paper that from the cells of *T. thermophilus* HB27 two kinds of methyltransferases, namely tRNA (guanosine-2′)-methyltransferase (Gm-methyltransferase; EC 2.1.1.34) and tRNA (adenine-1)-methyltransferase (m^1A-methyltransferase; EC 2.1.1.36) could be obtained in a cell-free state. The site specificity of Gm-methyltransferase and the implication of the temperature dependence of these methyltransferase activities *in vitro* in relation to the thermally induced methylation of tRNA *in vivo* are also the subjects to be dealt with in the present report.

MATERIALS AND METHODS

Materials. Unfractioned tRNAs of *E. coli* and *T. thermophilus* HB27 were prepared by Zubay's method (10). Yeast $tRNA^{Phe}$ was purchased from Boehringer Mannheim. *E. coli* $tRNA_f^{Met}$, $tRNA_m^{Met}$, $tRNA^{Phe}$, $tRNA^{Ile}$, and $tRNA^{Tyr}$ were kindly supplied by S. Nishimura of the National Cancer Research Institute. *T. thermophilus* HB8 $tRNA_{f1}^{Met}$, $tRNA_{f2}^{Met}$, $tRNA^{Phe}$, $tRNA_m^{Met}$, $tRNA^{Ile}$, and $tRNA^{Tyr}$ were prepared by procedures reported elsewhere (7). *S*-Adenosyl-L-[*methyl*-^{14}C]methionine was purchased from New England Nuclear. *E. coli* alkaline phosphomonoesterase was obtained from Boehringer Mannheim. Snake venom phosphodiesterase was from Worthington. RNases T1 and T2 were products of Sankyo (Tokyo). A prepacked column of μBondapak C_{18} for high-pressure liquid chromatography was purchased from Waters Associates. Thin-layer plates of Avicell SF-cellulose were purchased from Funakoshi Pharmaceutical (Tokyo).

Assay of tRNA Methyltransferase Activity. tRNA methyltransferase activity was assayed as described by Taya and Nishimura (11) with slight modifications. The reaction mixture contained 0.01 M Tris·HCl (pH 7.5), 0.01 M $MgCl_2$, 5 mM ATP, 6 mM 2-mercaptoethanol, 8.1 μM *S*-adenosyl[*methyl*-^{14}C]-methionine (62 Ci/mol; 1 Ci = 3.7×10^{10} becquerels), 0.4 A_{260} unit of *E. coli* tRNA, and 20 μl of the enzyme solution in a total volume of 100 μl. After incubation at 65°C for 30 min, 80 μl of the solution was applied to a Whatman 3MM paper disc. The discs were washed three times with cold 5% trichloroacetic acid and the radioactivity remaining in the disc was measured in a liquid scintillation counter. When necessary, the control values obtained in the absence of tRNA were subtracted from

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Abbreviations: s^2T, 2-thioribothymidine; m^1A, 1-methyladenosine; Gm, 2′-*O*-methylguanosine; Ψ, pseudouridine; m^7G, 7-methylguanosine; s^4U, 4-thiouridine; m^2G, 2-methylguanosine; Gm-methyltransferase, tRNA (guanosine-2′)-methyltransferase; m^1A-methyltransferase, tRNA (adenine-1)-methyltransferase.

experimental values. One enzyme unit was defined as the amount catalyzing the incorporation of 1 nmol of methyl group per hr under the assay conditions described above.

Isolation of Methyltransferases from *T. thermophilus* HB27 Cells. All operations were carried out at 4°C. The high-speed supernatants were extracted from both cells (160 g wet weight) of *T. thermophilus* HB27 grown at 80°C and 50°C as described (7). After precipitation of the protein fractions with 80% saturated ammonium sulfate followed by dialysis against standard buffer (0.01 M Tris·HCl, pH 7.5/0.01 M $MgCl_2$/0.05 M KCl/6 mM 2-mercaptoethanol), the dialysate (185 ml) was loaded on a column of DEAE-cellulose previously equilibrated with the standard buffer. Elution was carried out by applying a linear gradient of 0.05–0.4 M KCl. Fig. 1 shows the elution profiles of the 105,000 × *g* supernatants from *T. thermophilus* HB27 cells grown at 80°C and 50°C. The fractions containing methyltransferase activities were grouped into two portions, fractions I and II. Each fraction was then precipitated with 80% saturated ammonium sulfate and stored at −20°C. For routine enzyme assays, part of the precipitate was dissolved in a minimal volume of standard buffer, dialyzed against the same buffer, and mixed with glycerol at a final concentration of 50% (vol/vol). When stored at −20°C, this enzyme solution remained stable at least 4 months.

Analysis of Methylated Products. For analysis of the methylated nucleotides, 4.0 A_{260} units of unfractionated *E. coli* tRNA was methylated with 0.6 unit of Gm-methyltransferase or m^1A-methyltransferase, separately, in the presence of *S*-adenosyl[*methyl*-^{14}C]methionine for 1 hr by the assay method described above. The modified tRNA was extracted with 88% (vol/vol) phenol and extensively dialyzed against distilled water. For nucleotide analysis, the tRNA recovered was hydrolyzed with RNase T2 and the hydrolysate was subjected to two-dimensional thin-layer chromatography by the methods of Nishimura (12).

For determination of the sites of methylation, 45 A_{260} units of *E. coli* $tRNA_f^{Met}$ was methylated with 81 nmol of *S*-adenosyl[*methyl*-^{14}C]methionine, by incubation with 2.35 units of partially purified Gm-methyltransferase for 1.5 hr at 65°C under standard assay conditions. The [^{14}C]methylated $tRNA_f^{Met}$ was reisolated and chromatographed on a reversed-phase RPC-5 column. Then, 6 A_{260} units of the purified [^{14}C]$tRNA_f^{Met}$ was digested extensively with RNase T1 and the digest was fractionated by DEAE-Sephadex A-25 column chromatography in the presence of 7 M urea (13). The radioactive oligonucleotide was treated with snake venom phosphodiesterase and *E. coli* alkaline phosphomonoesterase. The resulting nucleosides were analyzed by high-pressure liquid chromatography, on a reversed-phase column of μBondapak C_{18} under the conditions described by Gehrke *et al.* (14).

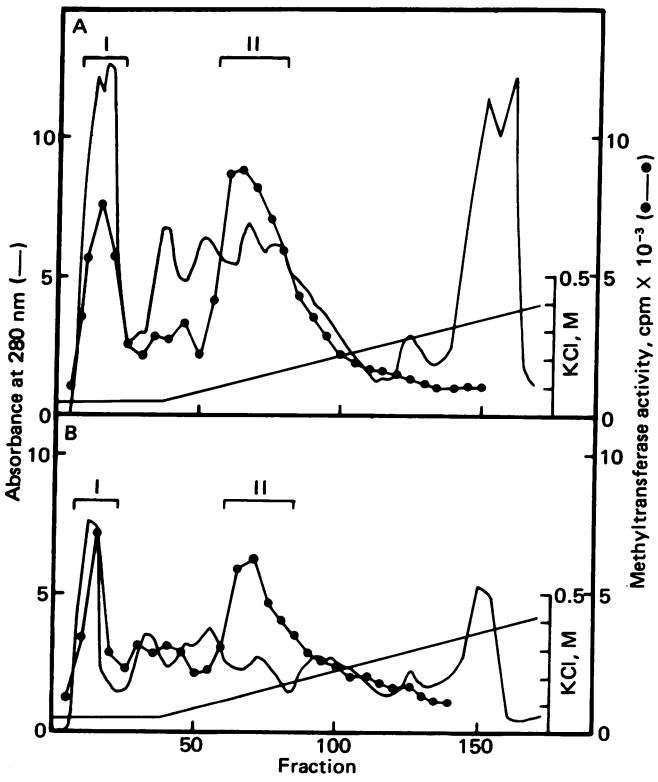

FIG. 1. DEAE-cellulose column chromatography of methyltransferases from *T. thermophilus* HB27. The 105,000 × *g* supernatant extracted from about 160 g of wet cells was applied to a DEAE-cellulose column (3.5 × 38 cm) equilibrated with standard buffer. The column was developed with 2.0 liters of a linear gradient of 0.05–0.4 M KCl in standard buffer. The flow rate was 42.7 ml/hr, and the fraction size was 15 ml. —, Absorbance at 280 nm; ●—●, radioactivity of [^{14}C]methyl group incorporated into *E. coli* tRNA by 20 μl of each fraction. The fractions indicated by brackets were pooled and designated as fractions I and II. (*A*) Elution pattern obtained with an extract of cells grown at 80°C. (*B*) Elution pattern with an extract of cells grown at 50°C.

RESULTS

Effects of Growth Temperature on Methylation of tRNA *In Vivo*. Unfractionated tRNA was extracted by Zubay's method from *T. thermophilus* HB27 cells grown at either 80°C or 50°C, and it was further purified by DEAE-cellulose column chromatography (15). The tRNA preparations were completely digested with RNase T2 for analysis of their nucleotide composition. The results shown in Table 1 indicate that the contents of m^1Ap and Gm-Gp, as well as s^2Tp, increased significantly with rise of growth temperature; a molar yield of as much as 1.0 was obtained when the thermophile was grown at 80°C. Other modifications, such as Ψp and m^7Gp, seemed to be independent of the growth temperature.

Characterization of Methyltransferases from *T. thermophilus* HB27. The high activity of *S*-adenosyl-L-methionine-

Table 1. Nucleotide composition of tRNA from the cells of *T. thermophilus* HB27 grown at different temperatures

Nucleotide	mol/tRNA At 50°C	mol/tRNA At 80°C
A_{OH}	1.0	1.0
Ap	14.1	12.0
Gp	24.7	23.1
Cp	27.1	26.8
Up	11.4	10.0
Ψp	1.64	1.9
pGp	0.92	0.93
s^4Up	0.65	0.90
m^7Gp	0.84	0.83
m^2Gp	0.31	0.25
m^1Ap	0.56	0.98
Gm-Gp	0.42	0.95
Tp	0.85	0.32
s^2Tp	0.15	0.68

tRNA preparations were digested with RNase T2 and the resulting nucleotides were analyzed by two-dimensional thin-layer chromatography (12). The m^1Ap content was estimated after conversion of m^1Ap to m^6Ap by treatment with 0.3 M NaOH, and the s^4Up content was determined spectrophotometrically. s^2Tp and Tp were analyzed as described (3, 8). It was assumed that the sum of s^2Tp and Tp was 1 mol per mol of tRNA (8).

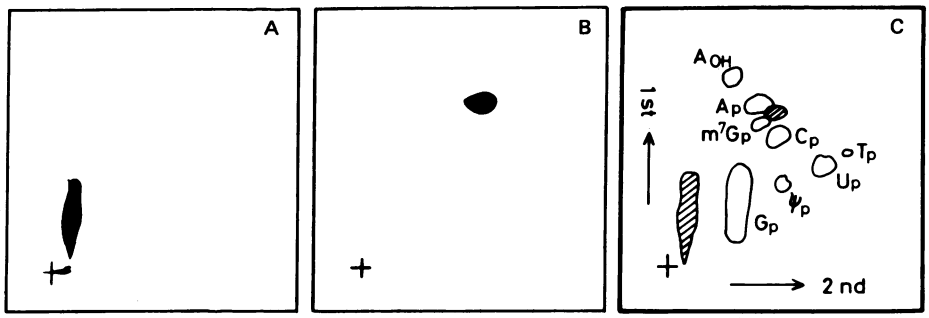

FIG. 2. Identification of methylated nucleotides in unfractionated *E. coli* tRNA obtained with Gm-methyltransferase and m¹A-methyltransferase, separately. RNase T2 digests of each [¹⁴C]methylated tRNA (2 A_{260} units each) were subjected to two-dimensional thin-layer chromatography. First dimension; isobutyric acid/0.5 M NH_4OH (5:3, vol/vol); second dimension; isopropyl alcohol/concentrated HCl/H_2O (70:15:15, vol/vol/vol). (*A*) autoradiogram obtained with the methylation by Gm-methyltransferase (fraction I); (*B*) autoradiogram with m¹A-methyltransferase (fraction II); (*C*) composite tracing of *A* and *B* in relation to the locations of spots with UV absorption.

dependent methyltransferase was demonstrated in a cell-free extract of *T. thermophilus* HB27 when unfractionated tRNA of *E. coli* was used as a methyl acceptor. As shown in Fig. 1, the methyltransferase activity in the 105,000 × *g* supernatant was separated into two major discrete peaks by DEAE-cellulose column chromatography. The elution profile of methyltransferase activity from the cells grown at 80°C was essentially identical to that of the activity from the cells grown at 50°C.

For analysis of the methylated products, unfractionated *E. coli* tRNA was methylated separately with the enzyme fractions I and II isolated from cells grown at either 80°C or 50°C. The RNase T2 digests of both [¹⁴C]methylated tRNAs thus obtained were subjected separately to two-dimensional thin-layer chromatography and the radioactive spot was detected by autoradiography (Fig. 2). In both cases, only one radioactive spot was detected on the chromatogram. The spot obtained with the enzyme in fraction I was found in the position of Gm-Gp. The material extracted from this spot contained equivalent amounts of G and Gm, as analyzed by the method of high-pressure liquid chromatography (14) described below (see Fig. 3). On the other hand, the spot obtained with fraction II was located in the same position as that of m¹Ap.

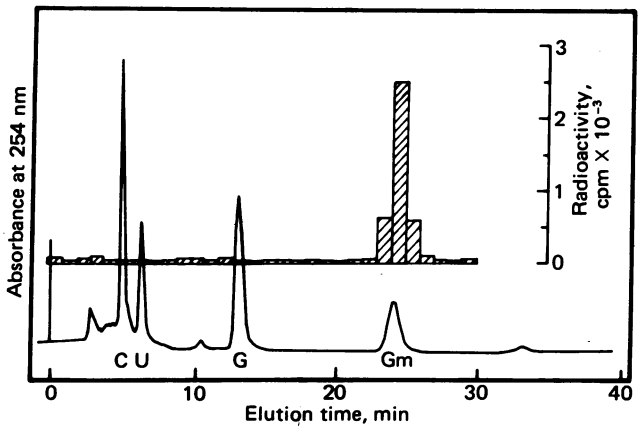

FIG. 3. Nucleoside analysis of the radioactive pentanucleotide derived from the RNase T1 digest of [¹⁴C]methylated *E. coli* $tRNA_f^{Met}$ by high-pressure liquid chromatography (14). [¹⁴C]Methylated $tRNA_f^{Met}$ was digested with RNase T1 and the products were separated on a DEAE-Sephadex A-25 column in the presence of 7 M urea (13). Fractions in the pentanucleotide region were pooled, desalted, and digested with snake venom phosphodiesterase and *E. coli* phosphomonoesterase. The resulting nucleosides were anlayzed on a reversed-phase column of μBondapak C_{18} (4 × 300 mm) at room temperature. Solvent, 0.05 M $NH_4H_2PO_4$ (pH 5.1) with 5% (vol/vol) methanol; flow rate, 1.0 ml/min; detector range, 0.02 absorbance unit full scale at 254 nm. Fractions (1.0 ml) of effluent were collected, lyophilized, and dissolved in 100 μl of distilled water. Then, 80 μl of the solution was applied to a Whatmann 3MM paper disc. After drying, the radioactivity in the discs was measured by a liquid scintillation counter (histogram).

Table 2 summarizes the relative contents of the two methyltransferase activities in the high-speed supernatants obtained from the cells grown at 80°C and 50°C.

Temperature Dependence of the Methyltransferase Activity Toward Homologous and Heterologous tRNAs. As shown in Table 2, the contents of the two methyltransferases per unit of protein extracted from cells seem to be independent of the growth temperature. Thus, the temperature dependence of methylation of tRNA observed *in vivo* was probably due, not to the change in amount of the enzymes, but to the change in the enzyme activity. To elucidate this point further in detail, the temperature dependences of methyltransferases obtained from the cells grown at different temperatures were examined.

Table 3 shows the results obtained with Gm-methyltransferases of the cells grown at 80°C and 50°C. As can be seen, tRNAs from *E. coli* and *T. thermophilus* HB27 grown at 50°C were effective acceptors, whereas that from *T. thermophilus* HB27 grown at 80°C was far less effective. On the other hand, the methyltransferase activities from cells grown at 80°C and at 50°C both increased 4 to 7 times when the incubation temperature was raised from 50°C to 71°C. Thus, the temperature dependence of the activities of Gm-methyltransferases was independent of the growth temperature of the cells from which the enzymes were extracted. The partially methylated tRNA obtained from *T. thermophilus* HB27 grown at 50°C could be further modified by incubation at a higher temperature with the enzyme(s) isolated from these cells. The results obtained with m¹A-methyltransferase were essentially the same as those described above.

Determination of the Site in *E. coli* $tRNA_f^{Met}$ Methylated by Gm-methyltransferase. The site specificity of Gm-methyltransferase was examined by using *E. coli* $tRNA_f^{Met}$, which

Table 2. Relative amounts of methyltransferases contained in the eluates obtained from DEAE-cellulose columns

Growth temperature of cells, °C	Relative amounts of enzymes, units/mg	
	Fraction I (Gm-methyltransferase)	Fraction II (m¹A-methyltransferase)
80	0.48	1.14
50	0.61	1.12

The relative amounts of the two methyltransferases are expressed as the enzyme activities of fractions I and II per total protein eluted from the DEAE-cellulose column (Fig. 1).

Table 3. Effect of incubation temperature on the activity of Gm-methyltransferase in homologous and heterologous systems

tRNAs	Enzyme source	Enzyme activity, pmol* At 50°C 50°C cells	At 50°C 80°C cells	At 71°C 50°C cells	At 71°C 80°C cells
T. thermophilus HB27					
80°C cells		1.8	2.2	5.8	5.7
50°C cells		9.4	12.2	41.0	60.6
E. coli		16.4	26.9	106.3	185.7

For assays of enzyme from cells grown at 50°C, 0.29 unit of methyltransferase was used. For cells grown at 80°C, 0.37 unit of methyltransferase was used.

* Expressed as pmol of [^{14}C]methyl group incorporated into 0.4 A_{260} unit of tRNAs per 30 min under standard assay conditions.

contains no Gm residue. The $tRNA_f^{Met}$ was treated with the methyltransferase in the presence of *S*-adenosyl[*methyl*-^{14}C]methionine, and the [^{14}C]methylated $tRNA_f^{Met}$ was completely digested with RNase T1. The digests were separated on a DEAE-Sephadex A-25 column in the presence of 7 M urea (13). There appeared only one radioactive oligonucleotide in the pentanucleotide fraction (data not shown), which was not detected in a control experiment using nonmethylated $tRNA_f^{Met}$ (16). Because the radioactive oligonucleotide should have the sequence Gm-Gp in the 3′-end as shown in Fig. 2A, the pentanucleotide in the RNase T1 digest of the methylated $tRNA_f^{Met}$ must be C-C-U-Gm-Gp, judging from the sequence of $tRNA_f^{Met}$ (see Fig. 4). The peak region was desalted and digested with snake venom phosphodiesterase and *E. coli* alkaline phosphomonoesterase, and the resulting nucleosides were determined by high-pressure liquid chromatography. As shown in Fig. 3, the nucleoside composition of the oligonucleotide was found to be C, U, Gm, and G in the molar ratio 2.1:0.78:0.75:1.0, and all of the radioactivity was recovered with the Gm.

Thus, the site of methylation of *E. coli* $tRNA_f^{Met}$ by the Gm-methyltransferase was identified as the ribose 2′-OH of guanosine located at position 19.

Capacities of Other Amino Acid-Specific tRNAs for Being Methylated with Gm-Methyltransferase. Various tRNAs were tested for their capacities for being methylated with Gm-methyltransferase. The results obtained are summarized in Table 4. As may be seen, yeast $tRNA^{Phe}$, *E. coli* $tRNA_f^{Met}$, $tRNA^{Phe}$, $tRNA_m^{Met}$, and $tRNA^{Ile}$ were methylated to more or less appreciable degrees, whereas only poor results were obtained with *E. coli* $tRNA^{Tyr}$ and *T. thermophilus* HB8 $tRNA_{f1}^{Met}$, $tRNA_{f2}^{Met}$, and $tRNA^{Phe}$. Noteworthy is the fact that the tRNAs of the latter group are all known to contain the Gm-G sequence in the D loop. Also ineffective as methyl group acceptors were: *T. thermophilus* $tRNA^{Tyr}$, $tRNA^{Ile}$, and $tRNA_m^{Met}$. In the case of the most effective acceptor—i.e., yeast $tRNA^{Phe}$—it was calculated that 0.76 mol of methyl group per mol of tRNA was incorporated, assuming that 1 A_{260} unit of tRNA is equal to 1.66 nmol (17). The Gm content in the D loop of *E. coli* $tRNA_m^{Met}$ is known to differ from preparation to preparation (18), and the preparation used in the present study seems to be scarcely methylated in the G-G sequence of the D loop.

Table 4. Incorporation of methyl groups into various amino acid-specific tRNAs by Gm-methyltransferase from *T. thermophilus* HB27

Organism	tRNA	[^{14}C]Methyl group incorporated, nmol/A_{260} unit of tRNA
Yeast	$tRNA^{Phe}$	1.26
E. coli	$tRNA_f^{Met}$	1.11
	$tRNA_m^{Met}$	0.58
	$tRNA^{Phe}$	0.47
	$tRNA^{Ile}$	0.41
	$tRNA^{Tyr}$	0.073
T. thermophilus	$tRNA_{f1}^{Met}$	0.015
	$tRNA_{f2}^{Met}$	0.030
	$tRNA_m^{Met}$	0.060
	$tRNA^{Phe}$	0.066
	$tRNA^{Ile}$	0.028
	$tRNA^{Tyr}$	0.028

Between 0.10 and 0.14 A_{260} unit of individual tRNAs was methylated with 0.21 unit of Gm-methyltransferase under standard assay conditions.

The results obtained in the present study further support the idea that Gm-methyltransferase isolated from *T. thermophilus* HB27 catalyzes the specific methylation of guanosine 2′-OH in the "invariant" G-G sequence found in the D loop of most tRNAs whose sequences have been determined so far, and that most of the tRNAs obtained from the cells of *T. thermophilus* HB8 have the Gm-G sequence in the D loop.

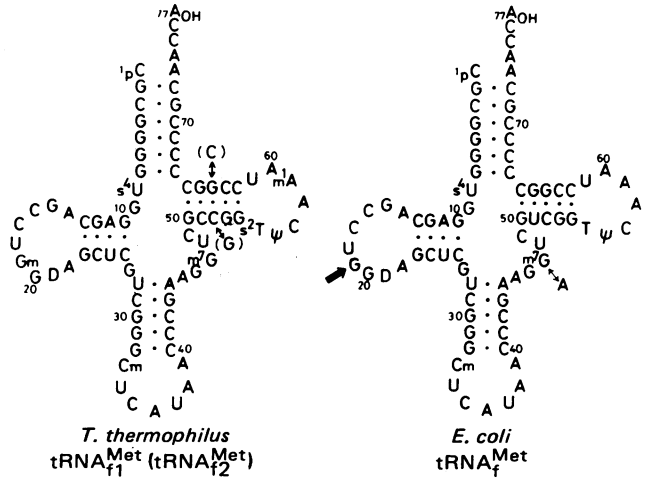

FIG. 4. Clover-leaf structures of *T. thermophilus* $tRNA_{f1(f2)}^{Met}$ and *E. coli* $tRNA_f^{Met}$, with an arrow indicating the site in *E. coli* $tRNA_f^{Met}$ methylated by Gm-methyltransferase.

DISCUSSION

In our previous paper (8), we reported that the methylation of G to Gm in tRNA of *T. thermophilus* HB8 increased slightly at higher temperature, but that the contents of other methylated nucleosides remained unchanged at various growth temperatures. In the case of *T. thermophilus* HB27, higher growth temperatures caused enhancement of the methylation of adenine-1 and guanosine-2′-OH. In the latter strain, Gm-methyltransferase and m^{1}A-methyltransferase were detected in a cell-free extract. With both of these methyltransferases, the tRNA from the same bacterial cells grown at 80°C was scarcely methylated, indicating that these tRNAs are almost fully modified when the thermophile is grown at higher temperatures.

The quantity and thermal nature of the methyltransferase appeared to be independent of the growth temperature of the cells. The action of Gm-methyltransferases from the cells grown at either 80°C or 50°C increased 4 to 7 times when the incubation temperature was raised from 50°C to 71°C. On the other hand, the enzymes obtained from the cells grown at 80°C and 50°C showed practically the same thermal stability (unpublished data). These observations led us to conclude that tem-

perature dependence of the methylation process of tRNA in *T. thermophilus* HB27 *in vivo* should be primarily attributed to temperature dependence of the methyltransferase activities, although the possibilities that the methylation of tRNA is regulated by the substrate concentration or the presence of some effector(s) cannot be excluded.

A thermostable m^1A-methyltransferase was identified and partially purified from *T. thermophilus* HB8 by Y. Taya and S. Nishimura (personal communication). Gefter (19) reported that a cell-free extract of *E. coli* catalyzed the formation of Gm in a G-G sequence of methyl-deficient $tRNA^{Tyr}$. The advance in the present study is that the Gm-methyltransferase from *T. thermophilus* HB27 has been partially purified and characterized. The site of methylation in *E. coli* $tRNA_f^{Met}$ by this enzyme was shown to be the ribose 2′-OH of the guanylate residue at position 19 from the 5′ end, the same position that was found to be modified in $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$ from *T. thermophilus* HB8 (Fig. 4). That the tRNAs containing the Gm-G sequence in the D loop do not accept a methyl group in the presence of the Gm-methyltransferase is a matter worth noticing in connection with the fact that the enzyme catalyzing the methylation of *E. coli* $tRNA_f^{Met}$ shows a marked site specificity. The data available to date (20) do not allow us to say more about the recognition sites in the tRNA by Gm-methyltransferase; however, the tertiary structure of tRNA as well as the G-G sequence in the D loop may be of great importance in this respect.

Of the unique modified nucleosides (s^2T, m^1A, and Gm) found in the thermophile tRNAs, we have presented experimental evidence for an important role of s^2T in the thermal stability of the tRNA (2, 7, 8, 21). On the basis of data reported for yeast $tRNA^{Phe}$ (22, 23), it may be speculated that, in the tertiary structure of the thermophile tRNA, Gm19 is base paired with Ψ56 and stacks on a base pair between s^2T55 and m^1A59 (2, 6). In this respect, Gm19 and m^1A59 may also be responsible for reinforcing this stack. In fact, ribose methylation of guanosine is known to stabilize the stacking interaction in oligonucleotides (24). In addition, Agris *et al.* (25) reported that methylation of ribose in tRNA of a moderate thermophile, *Bacillus stearothermophilus*, increased three times when the growth temperature was shifted from 50°C to 70°C. The methylated nucleosides may contribute not only to make the tRNA more resistant to RNase attack but also to maintain a tighter conformation of the tRNA at higher temperature.

Both tRNA (guanosine-2′)- and tRNA (adenine-1)-methyltransferases described here will provide an experimental approach for elucidating the function of Gm19 and m^1A59 in the thermostability of the thermophile tRNAs.

We are indebted to Dr. S. Nishimura of National Cancer Center Research Institute for his kind supply of *E. coli* tRNAs and to Dr. Y. Taya of the same institute for his valuable suggestions and information in starting the investigation.

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