Nucleotide sequence of a lysine tRNA from *Bacillus subtilis*

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ABSTRACT

A lysine tRNA ($\text{tRNA}_1^{\text{Lys}}$) was purified from *Bacillus subtilis* W168 by a consecutive use of several column chromatographic systems. The nucleotide sequence was determined to be pG-A-G-C-C-A-U-U-A-G-C-U-C-A-G-U-D-G-G-D-A-G-A-G-C-A-U-C-U-G-A-C-U-U(U*)-U-U-K-A-Ψ-C-A-G-A-G-G-m⁷G(G)-U-C-G-A-A-G-G-T-Ψ-C-G-A-G-U-C-C-U-U-C-A-U-G-G-C-U-C-A-C-A_{OH}, where K and U* are unidentified nucleosides. The nucleosides of U₃₄ and m⁷G₄₆ were partially substituted with U* and G, respectively. The binding ability of lysyl-tRNA₁^{Lys} to *Escherichia coli* ribosomes was stimulated with ApApA as well as ApApG.

INTRODUCTION

Bacillus subtilis changes in the percentage of isoaccepting species of tRNAs in different growth stages or in different growth media [1-11]. Several authors have shown that there are two major isoaccepting species of lysine tRNAs in *B. subtilis* and that percentage of these species varies in vegetative cells and spores [2, 7-11]. Using lysyl-tRNA-DNA hybridization technique, Chuang *et al.* demonstrated that these two isoaccepting lysine tRNAs are transcribed from at least two different sites on *B. subtilis* DNA [12].

Here, we report purification, total nucleotide sequence and coding properties of $\text{tRNA}_1^{\text{Lys}}$ which is a major species of lysine tRNAs present in *B. subtilis* in late logarithmic stage.

MATERIALS AND METHODS

Purification of $\text{tRNA}_1^{\text{Lys}}$. *B. subtilis* W168 grown in Penassay medium was harvested in late logarithmic stage. Crude tRNA was prepared as described previously [13]. DEAE-Sephadex A-50 column chromatography at pH 7.5 or pH 4.0 was carried out according to Nishimura [14] on a smaller scale. Sepharose 4B column chromatography was performed according to Holmes *et al.* [15]. BD-cellulose column chromatography was operated according to Gillam *et al.* [16] with a slight modification [17]. Crude

aminoacyl-tRNA synthetases from *B. subtilis* W168 and *E. coli* Q13 were prepared according to Nishimura *et al.* [18] except for lysozyme digestion instead of glass beads treatment in case of *B. subtilis*. Amino acid acceptor activity of tRNA was assayed as described previously [19].

Sequence analysis. The products of complete digestion with RNase T₁ and pancreatic RNase were separated by DEAE-Sephadex A-25 column chromatography at pH 7.5 [20], following the same chromatography at pH 2.7 [21] or pH 3.3, or AG 1 column chromatography at pH 3.3 [22], all in the presence of 7 M urea. The oligonucleotides thus obtained were desalted according to Rushizky and Sober [23]. The sequences of oligonucleotides were determined by further enzymatic digestion with silkworm nuclease, *E. coli* polynucleotide phosphorylase, RNase U₂, RNase T₁, pancreatic RNase, RNase T₂ and/or nuclease P₁ [24, 25]. After digestion, the products were separated by paper or thin-layer chromatography and quantitated by UV absorption after elution or extraction. Two dimensional chromatography for base analysis and for separation of short oligonucleotides was carried out according to Kimura-Harada *et al.* [26]. Overlapping sequences were constructed by isolating the products of limited digestion with nuclease S1 [27] or with alkali treatment at pH 12.5, at room temperature for 15 min. [28]. These products were separated by successive uses of DE-23 (pH 7.5) and DEAE-Sephadex A-25 (pH 2.7) column chromatography both in the presence of 7 M urea. Large oligonucleotides were analyzed after complete digestion with RNase T₁ or pancreatic RNase, by two dimensional fingerprinting technique developed for analysis of [³²P]-labeled RNA by Sanger *et al.* [29]. Spots on DEAE-cellulose paper were detected by UV absorption. In some cases, the fingerprint method was applied for separation of the complete digest of tRNA^{Lys} with RNase T₁ or pancreatic RNase. In this case, usually 5 A₂₆₀ units [30] of the tRNA sample were digested and applied to electrophoresis in one experiment. The application over 10 A₂₆₀ units of the sample to a cellulose acetate strip does not give a good separation.

Binding of [¹⁴C]-lysyl-tRNA^{Lys} to ribosomes. [¹⁴C]-lysyl-tRNA^{Lys} was prepared according to Nishimura and Novelli [31]. The triplet dependent binding to *E. coli* ribosomes was assayed according to Nirenberg and Leder [32]. ApApG was isolated from RNase T₁ digest of yeast RNA. ApApA was prepared by periodate treatment of ApApApG, a product of RNase T₁ digest of yeast RNA. Ribosomes were prepared from *E. coli* Q13 as described previously [19].

RESULTS

Purification of $\text{tRNA}_1^{\text{Lys}}$.

A single peak of lysine acceptor activity observed in DEAE-Sephadex A-50 column chromatography (pH 7.5) of crude tRNA preparation (Fig. 1) was separated into two peaks in subsequent column chromatography on Sepharose 4B (Fig. 2). The lysine tRNA eluted in the first peak part was designated as $\text{tRNA}_1^{\text{Lys}}$, the one eluted in the second as $\text{tRNA}_2^{\text{Lys}}$. The fraction containing $\text{tRNA}_1^{\text{Lys}}$ was then applied to BD-cellulose column chromatography (pH 7.5, with 5 mM MgCl_2). Finally, $\text{tRNA}_1^{\text{Lys}}$ was purified by DEAE-Sephadex A-50 column chromatography (pH 4.0). The peak of lysine acceptor activity showed complete accordance with that of UV absorption at 260 nm. The analytical data of this $\text{tRNA}_1^{\text{Lys}}$ preparation revealed the purity to be nearly 90%. This preparation can accept lysine with almost equal efficiency by crude aminoacyl-tRNA synthetase preparations from *B. subtilis* W168 and *E. coli* Q13.

Fig. 1 DEAE-Sephadex A-50 (pH 7.5) column chromatography of crude tRNA from *B. subtilis*.

Column size: 2.8×140 cm. Amounts charged: 15,000 A_{260} units. Linear gradient of NaCl and MgCl_2 . Mixing chamber (3 l): 0.375 M NaCl-8 mM MgCl_2 -20mM Tris-HCl(pH 7.5). Reservoir (3 l): 0.525 M NaCl-16 mM MgCl_2 -20 mM Tris-HCl(pH 7.5). Flow rate: 70 ml/hr. Temperature: room temp. Each fraction: 18 ml. Fractions indicated by arrows were collected.

Sequence analysis.

[Complete digestion with RNase T₁]

The products obtained by complete digestion with RNase T₁ of tRNA^{Lys}₁ were separated into 9 fractions from T-1 to T-9 on a DEAE-Sephadex A-25 column (pH 7.5). The fraction T-3 was further separated into 6 components, and T-4 into 2, by DEAE-Sephadex A-25 column chromatography (pH 2.7). Other fractions were eluted as single peaks by the same chromatography. The sequences of these fractions are listed in Table I with analytical data. T-4-1 (C-U-C-A-C-C-A_{OH}) is the oligonucleotide derived from 3'-terminus, and T-9 (A-C-U-U(U*)-U-U-K-A-Ψ-C-A-G-) from the anticodon part. Here, K (designated after one letter code for lysine) and U* are unidentified nucleosides. When the oligonucleotide (C-U-U(U*)-U-U-K-A-), an RNase U₂ product of T-9, was digested with silkworm nuclease, then treated with polynucleotide phosphorylase, C-U-U_{OH} was detected. At the same time, a small amount of C-U-U*_{OH} was also detected. U* seems to be a 2-thio-derivative of uridine, deduced from its absorption spectra. The content of U* altered from 0 to 0.3 mole per mole tRNA according to the preparations

Fig. 2 Sepharose 4B column chromatography of tRNA^{Lys}-rich fraction. Column size: 1.5 × 80 cm. Amounts charged: 4,200 A₂₆₀ units of tRNA^{Lys}-rich fraction from Fig. 1. Decreasing linear gradient of (NH₄)₂SO₄. Mixing chamber (940 ml): 10 mM sodium acetate(pH 4.5)-10 mM MgCl₂-6 mM β-mercaptoethanol-1 mM EDTA-1.3 M (NH₄)₂SO₄. Reservoir (1 l): the same except no (NH₄)₂SO₄. Flow rate: 50 ml/hr. Temperature: 4°. Each fraction: 5 ml. Fractions indicated by arrows were collected.

Table I. Analysis of endproducts obtained by complete digestion with RNase T₁

Fragment Number	Outline of Sequence Determination	Molar Yield	Conclusion
T-0		14.0	4 { G-(cyclic)
T-1			4 { G-
T-2	T ₂ A-(1.0), G-(1)	4.0	4 A-G-
T-3-1	P ₁ A(1.0), -A(1.0), -G(1)	1.0	1 A-A-G-
T-3-2	T ₂ D-(+), A-(1.0), G-(1)	1.2	1 D-A-G-
	P D-(+), A-G-(+)		
	↳T ₂ A-(1.0), G-(1)		
T-3-3		0.8	1 -G-
T-3-4	P ₁ U(1.0), -D(+), -G(1)	1.0	1 U-D-G-
T-3-5	P ₁ m ⁷ G(+), -U(1.1), -C(0.9), -G(1)	0.8	1 m ⁷ G-U-C-G-
	P m ⁷ G-U-(+), C-(1.0), G-(1)		
T-3-6	P ₁ U(1.0), -C(1.0), -G(1)	0.2	1 U-C-G-
T-4-1	T ₂ A-(1.0), C-(3.8), U-(1.1), A(1)	1.0	1 C-U-C-A-C-C-A
	U ₂ C-U-C-A-(1), C-C-A-(1.2)		
	↳T ₂ C-(2.2), A(1)		
	↳P ₁ C(1.0), -U(1.0), -C(1.2), -A(1)		
	↳PM/PN C-U-C		
	↳T ₂ C-(1.0), U-(1.0), C(1)		
T-4-2	P ₁ T(0.8), -Ψ(0.9), -C(1.2), -G(1)	1.0	1 T-Ψ-C-G-
	PM/PN T-Ψ-C		
	↳T ₂ T-(1.1), Ψ-(0.9), C(1)		
T-5	P ₁ C(0.9), -U(1.1), -C(1.0), -A(1.2), -G(1)	1.0	1 C-U-C-A-G-
	PM/PN C-U-C		
	↳T ₂ C-(1.2), U-(1.0), C(1)		
T-6	P ₁ C(1.0), -A(1.0), -U(2.3), -C(1.1), -G(1)	1.1	1 C-A-U-C-U-G-
	SW C-A-U, -C-U-G-		
	↳T ₂ -C-(0.9), U-(1.0), G-(1)		
	↳T ₂ C-(0.9), A-(1.1), U(1)		
T-7	P ₁ C(0.9), -C(1.0), -A(2.0), -U(2.0), -G(1)	1.0	1 C-C-A-U-U-A-G-
	U ₂ C-C-A-(0.8), U-U-A-(0.8), G-(1)		
	↳T ₂ U-(2.2), A-(1)		
	↳T ₂ C-(2.1), A-(1)		
T-8	P ₁ U(1.4), -C(2.8), -U(3.0), -G(1)	0.9	1 U-C-C-U-U-C-A-U-G-
	PM/PN U-C-C		
	↳T ₂ U-(1.1), C-(1.0), C(1)		
	U ₂ U-C-C-U-U-C-A-(1.1), U-G-(1)		
	↳T ₂ U-(1.0), G-(1)		
	↳PM/PI/PM U-C-C-U-U-C		
	↳T ₂ U-(3.1), C-(2.3), C(1)		
T-9	P ₁ A(0.9), -U(3.5), -Ψ(1.0), -A(1.5), -C(2.1), -K(+), -U*(+), -G(1)	1.0	1 A-C-U-U(U*)-U-U-K-A-Ψ-C-A-G-
	U ₂ A-(1.3), G-(1), Ψ-C-A-(0.9), C-U-U(U*)-U-U-K-A-(0.9)		
	↳P ₁ Ψ(0.9), -C(1.0), -A(1)		
	↳P ₁ C(1.0), -U(3.5), -U*(+), -K(+), -A(1)		
	↳P C-(1), U-(3.6), U*(+), K-A-(+)		
	↳PM/T ₂ K-(+), A(1)		
	↳SW/PN C-U-U(U*)		
	↳T ₂ C-(1.0), U-(1), U(0.7), U*(+)		

T₂:RNase T₂ digestion. P₁:nuclease P₁ digestion. P:pancreatic RNase digestion.
 PM/PN:polynucleotide phosphorylase digestion after phosphatase treatment.
 SW:silkworm nuclease digestion. PM/PI/PM:periodate treatment after phosphatase treatment, then phosphatase treatment. PM/T₂:RNase T₂ digestion after phosphatase treatment. SW/PN:polynucleotide phosphorylase digestion after silkworm nuclease digestion.

of $\text{tRNA}_1^{\text{Lys}}$. The content of m^7G ranged from 0.3 to 0.9 mole per mole tRNA. The sum of $\text{m}^7\text{G-U-C-G-}$ and U-C-G- was almost 1 mole per mole tRNA in the RNase T_1 digest of every $\text{tRNA}_1^{\text{Lys}}$ preparation. In some cases, the oligonucleotides obtained by complete digestion with RNase T_1 were separated by two dimensional electrophoresis. A fingerprint pattern is shown in Fig. 3a. [Complete digestion with pancreatic RNase]

The oligonucleotides obtained by complete digestion with pancreatic RNase of $\text{tRNA}_1^{\text{Lys}}$ were separated into 11 fractions from P-1 to P-11 by DEAE-Sephadex A-25 column chromatography (pH 7.5) operated at 65° . The fraction P-4, when applied to AG 1 column chromatography, was further separated into 2 components. P-5 gave 4 peaks by DEAE-Sephadex A-25 column chromatography (pH 3.3). Other fractions were composed of single components. Their sequences are listed in Table II with analytical data. A fingerprint pattern of the oligonucleotides obtained by complete digestion with pancreatic RNase is also shown in Fig. 3b. P-9 (pG-A-G-C-) is the oligonucleotide derived from 5'-terminus.

[Large fragments for overlapping and total nucleotide sequence]

Large oligonucleotide fragments were obtained by a partial digestion of the $\text{tRNA}_1^{\text{Lys}}$ preparation with RNase S1 or by chemical cleavage at m^7G . The products were fractionated by DE-23 column chromatography (pH 7.5) operated at 65° . Subsequently, these fractions (S-1 to S-6, and A-1 to

Fig. 3. Fingerprints of complete digests of $\text{tRNA}_1^{\text{Lys}}$ with RNases.
a. RNase T_1 digest. b. Pancreatic RNase digest.
1st dimension: 7M urea adjusted to pH 3.5 with glacial acetic acid.
2nd dimension: 7% formic acid.
Each spot number corresponds to the fragment in Table I or Table II.

Table II. Analysis of endproducts obtained by complete digestion with pancreatic RNase.

Fragment Number	Outline of sequence determination	Molar Yield	Conclusion
P-1		1.2	1 A
P-2		7.3	9 U-
			1 Ψ-
			1 D-
P-3		12.5	11 C-
P-4-1	P ₁ A(1.0), -C(1)	1.1	1 A-C-
P-4-2	P ₁ A(1.0), -U(1)	2.9	3 A-U-
P-5-1,	P ₁ A(1.0), G(0.9), -A(1.2), -G(1.0),	11.7	1 A-G-C-
P-5-2'	-C(2)		1 G-A-C-
	T ₁ A-G-(0.9), A-C-(1.1), C-(0.9), G-(1)		
	↳T ₂ A-(1.1), C-(1)		
	↳T ₂ A-(1.0), G-(1)		
P-5-3	P ₁ G(0.9), -G(0.9), -C(1)	1.1	1 G-G-C-
P-5-4	P ₁ A(0.9), -G(0.9), -U(1)	1.1	1 A-G-U-
P-5-5	P ₁ G(0.9), -G(1), -D(+)	1.2	1 G-G-D-
P-6	P ₁ K(+), -A(1.1), -Ψ(1)	0.9	1 K-A-Ψ-
P-7	P ₁ G(0.8), -A(1.1), -G(1.0), -U(1)	1.2	1 G-A-G-U-
	T ₁ G-(1.2), A-G-(0.9), U-(1)		
	↳T ₂ A-(1.0), G-(1)		
P-8	T ₂ A-(2.0), G-(2.1), C-(1)	1.0	1 A-G-A-G-C-
	T ₁ A-G-(2.1), C-(1)		
	↳T ₂ A-(1.1), G-(1)		
P-9	T ₂ -G-(1.0), A-(1.2), G-(1.0), C-(1)	0.9	1 -G-A-G-C-
	T ₁ -G-(1.0), A-G-(1.1), C-(1)		
	↳T ₂ A-(1.0), G-(1)		
P-10	T ₂ A-(2.0), G-(3.2), T-(1)	0.8	1 G-A-A-G-G-T-
	T ₁ A-A-G-(1.2), G-(1.9), T-(1)		
	↳T ₂ A-(2.1), G-(1)		
	SW G-A-A, -G-G-T-		
	↳T ₂ G-(1.0), G-(1.0), T-(1)		
	↳T ₂ G-(1.1), A-(1.0), A(1)		
P-11	T ₂ A-(2.0), G-(3.6), m ⁷ G-(+), U-(0.5)	0.8	1 A-G-A-G-G-m ⁷ G(G)-U-
	T ₁ A-G-(2.0), m ⁷ G-U-(+), G-(1.2), U-(1)		
	↳T ₂ m ⁷ G-(+), U-(1)		
	↳T ₂ A-(1.0), G-(1)		
	SW A-G-A, -G-G-m ⁷ G(G)-U-		
	↳T ₂ G-(1.0), G-(1.3),		
	m ⁷ G-(+), U-(1)		
	↳T ₂ A-(1.0), G-(1.0), A(1)		

T :RNase T digestion. Other abbreviations are explained in the legend of Table I.

A-3, respectively) were purified by DEAE-Sephadex A-25 column chromatography (pH 2.7). One half of each fraction was digested completely with RNase T₁ and the other half with pancreatic RNase. The oligonucleotides produced were analyzed by two dimensional electrophoresis. The large fragments used for the derivation of total sequence are shown in Fig. 4 with the total nucleotide sequence thus determined. Although the following two possibilities are conceivable,

-A₅₀-A-G-G-T-Ψ-C-G-A-G-U-C-C-U-U-C-A-U-G₆₈-

and -A₅₀-G-U-C-C-U-U-C-A-U-G-A-A-G-G-T-Ψ-C-G₆₈-,

the latter possibility can be ruled out without much consideration, since T-Ψ-C- sequence should be in TΨC-loop. The clover-leaf secondary structure model is constructed like other tRNAs (Fig. 5). The total sequence consists of 76 nucleotides, containing D, T, Ψ, m⁷G, K and U* as minor nucleo-

Fig. 4. Nucleotide sequences of lysine tRNAs and large fragments. a) Oligonucleotides obtained by alkali treatment. b) Oligonucleotides obtained by partial digestion with nuclease S1. c) Sequences established by combining the data obtained from complete digestion with RNase T₁ and pancreatic RNase. d) Nucleotide sequence of tRNA^{Lys} from *B. subtilis*. e) Nucleotide sequences of lysine tRNAs from other sources. Nucleotides common to those of tRNA^{Lys} from *B. subtilis* were enclosed with brackets. Differences in the state of modification were disregarded for this comparison. Abbreviations. m⁷G:7-methylguanosine. m²G:N²-methylguanosine. m²G:N²,N²-dimethylguanosine. m¹A:1-methyladenosine. t⁶A:N⁶-(β-D-ribofuranosyl)-purin-6-ylcarbamoylthreonine. m⁵C:5-methylcytidine. mcm⁵U:5-methylaminomethyl-2-thiouridine. mcm⁵U:5-carboxymethyl-2-thiouridine methyl ester. nbt³U:3-(3-amino-3-carboxypropyl) uridine.

Fig. 5. Clover-leaf model of tRNA^{Lys}₁ from *B. subtilis*.

sides. The anticodon sequence is U-U-U or, in some cases, U*-U-U. The position adjacent to 3'-end of the anticodon is occupied by an unidentified nucleoside, K.

Characterization of K nucleoside.

When the oligonucleotide A-C-U-U(U*)-U-U-K-A-Ψ-C-G- was digested with RNase T₂ under the condition usually used for base analysis, 2',3'-cyclic-phosphate of K was obtained. However, a larger amount of RNase T₂ and prolonged incubation time hydrolyzed it to Kp. A two dimensional thin-layer chromatogram of the digest is shown in Fig. 6a. After the digest was treated with phosphatase, the mixture of nucleosides was chromatographed on a TLC aluminium roll cellulose sheet according to Rogg *et al.* [33] (Fig. 6b). On both chromatograms, Kp and K nucleoside, respectively, were detected as spots with strong bluish purple fluorescence under a UV lamp with a maximum wavelength at 253.7 nm. Fig. 7 shows UV spectra of K nucleoside at four pHs. These spectra and its fluorescence-emitting property suggest that K is a guanosine derivative. Mobility toward the anode of K nucleoside on thin-layer electrophoresis at pH 7.5 and behavior of K-containing oligonucleotides in column chromatography indicate one nega-

Fig. 6. Two dimensional thin-layer chromatography of Kp and K Nucleoside.
a. Chromatogram of Kp on Avicel SF plate.
b. Chromatogram of K nucleoside on aluminium roll cellulose plate.

Fig. 7. Ultraviolet absorption spectra of K nucleoside.
(—····— pH 0, — · — pH 2, — pH 7.5, ····· pH 12)

tive charge in K nucleoside under neutral condition.

Binding of [^{14}C]-lysyl-tRNA $^{\text{Lys}}_1$ to ribosomes.

Binding of lysyl-tRNA $^{\text{Lys}}_1$ to ribosomes was stimulated with ApApA and ApApG with almost equal efficiency. This result consists with other studies showing that the major tRNA $^{\text{Lys}}$ from vegetative cells recognizes ApApA and ApApG [2, 11]. The predominant presence of U at the first position of anticodon explains this wobbling recognition.

DISCUSSION

When the sequence of tRNA $^{\text{Lys}}_1$ from *B. subtilis* is compared with those of lysine tRNAs from other sources [34-36] (Fig. 4), the regions common to all are seen particularly in D-region and T Ψ C-region. Complete accordance of the sequences in D-loop and D-stem (from U $_7$ to A $_{26}$) between lysine tRNAs from *B. subtilis* and *E. coli* suggests the participation of this region in recognition of tRNA $^{\text{Lys}}$ by prokaryotic lysyl-tRNA synthetases. Displacements of nucleosides between tRNA $^{\text{Lys}}_1$ from *B. subtilis* and those from *E. coli*, haploid *Saccharomyces cerevisiae* Sa288C and Baker's yeast are observed at 15, 29 and 28 sites, respectively, when differences in the state of modification are disregarded.

The first letter of anticodon is C in haploid yeast tRNA $^{\text{Lys}}$, which corresponds exclusively to codon AAG. The same positions are occupied by 5-methylaminomethyl-2-thiouridine and 5-carboxymethyl-2-thiouridine methyl ester in those from *E. coli* and baker's yeast, respectively. The function of 2-thiouridine derivatives is considered to achieve strict base-pairing with A, but not with G [37]. Sekiya *et al.* showed that in a cell-free system for hemoglobin synthesis, yeast tRNA $^{\text{Glu}}_3$, in which 5-carboxymethyl-2-thiouridine methyl ester occupies the first letter position of anticodon, recognizes only GAA [38]. The recognition of AAA and AAG with equal efficiency by tRNA $^{\text{Lys}}_1$ from *B. subtilis* is explained by the predominant presence of U at this position. The tRNAs containing unmodified U at this position so far reported are ochre suppressor tRNA $^{\text{UAA}}$, UAG [39] and tRNA $^{\text{Gly}}_{(\text{ins})}$ [40] both from *E. coli*, and tRNA $^{\text{Leu}}_{\text{CUA}}$ [41] from baker's yeast. The content of U* in tRNA $^{\text{Lys}}_1$ may depend on growth stage or growth condition of *B. subtilis*. Also, another possibility that U* was degraded to U during the preparation of tRNA cannot be ruled out.

Usually in tRNAs which recognize codons starting with A, the position adjacent to 3'-end of anticodon is occupied by *N*-[9-(β -D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine (t ^6A) or its derivative, except initiator tRNAs from prokaryote [37, 42, 43]. In fact, tRNA $^{\text{Thr}}$ from *B. subtilis*

contains t⁶A at the corresponding position [24]. The presence of K, a probable guanosine derivative, at this position of tRNA₁^{Lys} from *B. subtilis* clearly indicates that this nucleoside plays a similar role to t⁶A in the codon-anticodon interaction. Unidentified nucleosides similar to K were also detected in lysine tRNAs from rat and rabbit liver [Nishimura, S. and Gross, H. J.: personal communication]. These results suggest the possibility that guanosine may be modified to K, when it is located at the position adjacent to 3'-end of anticodon in the tRNAs which recognize codons starting with A.

Vold separated three species of lysine tRNAs from *B. subtilis* by RPC-5 column chromatography and named them tRNA₁^{Lys}, tRNA₂^{Lys} and tRNA₃^{Lys}, respectively, according to the order eluting from the column [11]. Identification of our tRNA₁^{Lys} with her tRNA₁^{Lys} was confirmed by cochromatography on an RPC-5 column of both samples and by cofingerprinting of their complete digests with RNase T₁ as well as with pancreatic RNase.

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