A REVISED SEQUENCE FOR *BACILLUS STEAROTHERMOPHILUS* PHENYLALANINE tRNA

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1. Introduction

The primary structure of *Bacillus stearothermophilus* phenylalanine tRNA has been reinvestigated using non-radioactive tRNA. The results are different from those found previously with [^{32}P] phosphate in vivo labeled tRNA^{Phe} [1]. Among the minor nucleosides we now find s^4U and $ms^2\ i^6A$. We also show that the 5′ and the 3′ end sequences of tRNA^{Phe} from *B. stearothermophilus* are pG–G–C–U–C–G–G–s^4U and U–C–C–C–G–A–G–C–C–A–C–C–A, respectively.

2. Materials and methods

Phenylalanine specific tRNA from *B. stearothermophilus* (strain NCA 1518) is purified in two steps. First crude tRNA from *B. stearothermophilus* is chromatographed on a BD-cellulose column. No phenylalanine-tRNA containing fractions were eluted by 1.0 M NaCl in 10 mM sodium acetate buffer, pH 4.5, containing 10 mM $MgCl_2$. The tRNA^{Phe} was eluted in 6% ethanol using a linear gradient of NaCl (0.8–1 M) and of ethanol (0–15%) in the above-mentioned buffer. The purification is continued on a Sepharose 4B column at room temperature (adapted from [2]), using a reversed gradient of $(NH_4)_2SO_4$ (2–1 M) in 10 mM acetate buffer, pH 4.5, containing 10 mM $MgCl_2$, 6 mM β-mercaptoethanol and 1 mM EDTA.

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The conditions for complete T_1 RNAase, pancreatic RNAase or U_2 RNAase hydrolyses and primary sequences determination were as described [3].

3. Results and discussion

Phenylalanine tRNA from *B. stearothermophilus* as well as other tRNAs from this bacteria contain s^4U. We find approx. 1 s^4U for 1–2 tRNAs in total *B. stearothermophilus* tRNA as determined by ultraviolet spectrum (A_{max} 336 nm, εM 15 000, according [4]). The s^4U residue is almost completely destroyed after electrophoresis on DEAE-cellulose paper in 7% formic acid, elution with M TEABC and evaporation of this salt. We therefore used an indirect method to characterize the position of s^4U in the molecule. The tRNA digests were separated by DEAE-cellulose column chromatography and the ultraviolet spectra were done directly on individual fractions. Under these conditions, s^4U containing oligonucleotides eluted between tri- and tetranucleotides in pancreatic RNAase and T_1 RNAase digests. Since s^4U containing oligonucleotide elute slightly later than oligonucleotides of the same length because of the slight anionic charge of s^4U at pH 7.5, we therefore expect that s^4U is located in trinucleotides. Among the trinucleotides, G–G–Up or A–G–Up (pancreatic RNAase) and U–A–Gp or U–C–Gp (T_1 RNAase) were the possible candidates. G–G–Up and U–A–Gp were found in very low yields as compared to A–G–Up and U–C–Gp. Degradation products such as G–G–Np or N–A–Gp (see legend fig.1 and fig.2) were also found.

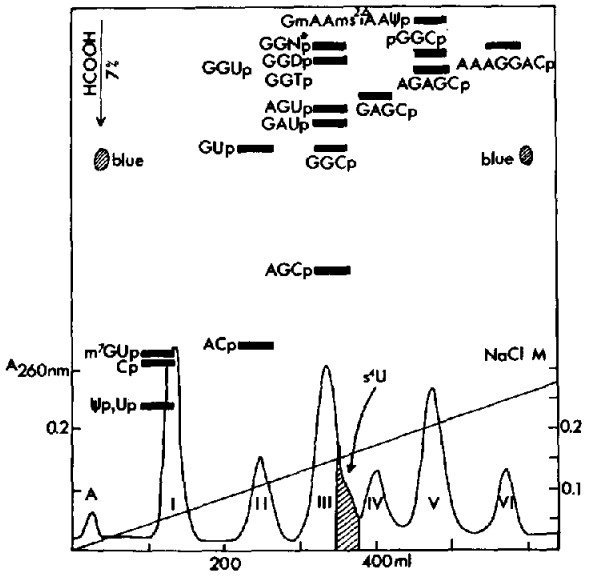

Fig.1. Chromatography of a pancreatic RNAase digest (4 mg *B. stearothermophilus* $tRNA^{Phe}$). Separation on a DEAE-cellulose column (0.8 × 50 cm) by a linear gradient of NaCl (0–0.4 M) in 7 M urea, 0.02 M Tris–HCl, pH 7.5 (vol. 1000 ml) (lower part of the figure) followed by high voltage electrophoresis of the oligonucleotide fractions of peaks I–VI on DEAE-cellulose paper (upper part of the figure). Most of the oligonucleotides were found in a one to one molar ratio. The only exceptions were: Cp, 14; Up, 6; G–Up, 2; G–G–N*p, 0.7 and G–G–Up, 0.2. The dashed area corresponds to the s^4U containing fractions. G–G–N*p is probably G–G–s^4U which has been converted while the paper was drying at daylight. Besides G only little quantities of U were found in that spot, meaning that most of the s^4U was destroyed by light. The little amounts of G–G–Up (20%/mol $tRNA^{Phe}$) found in spot G–G–Dp/G–G–Tp correspond probably to an early transformation during hydrolysis and column chromatography of G–G–s^4Up into G–G–Up.

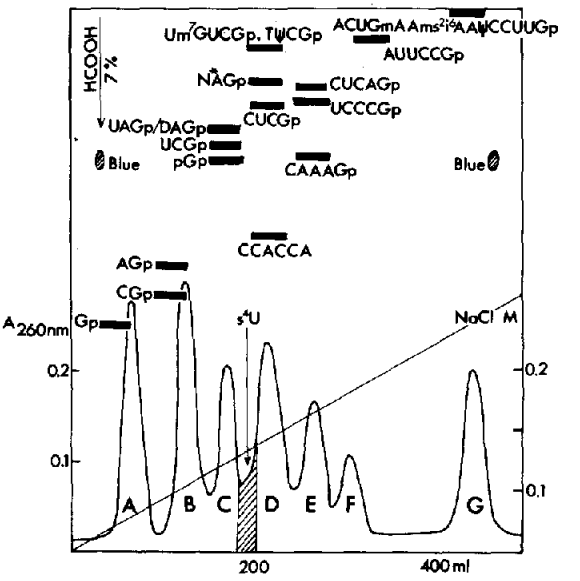

Fig.2. Chromatography of a T_1 RNAase digest (4 mg *B. stearothermophilus* $tRNA^{Phe}$). Separation on a DEAE-cellulose column (0.6 × 150 cm) by a linear gradient of NaCl (0- 0.4 M) in 7 M urea, 0.02 M Tris–HCl, pH 7.5 (vol. 500 ml) (lower part of the figure), followed by high-voltage electrophoresis of the oligonucleotides on DEAE-cellulose paper (upper part of the figure). Most of oligonucleotides were found in a one to one molar ratio. The only exceptions were: Gp, 6; A–Gp, 2; U–A–Gp, 0.2 and N*–A–Gp, 0.65. The dashed area corresponds to the s^4U containing fractions. N*–A–Gp is probably s^4U–A–Gp (see legend fig.1). Little amounts of U–A–Gp (20%/mol $tRNA^{Phe}$) found in spot D–A–Gp correspond probably to early transformation of s^4U–A–Gp into U–A–Gp.

Since s^4U is very sensitive to acid, alkali and light, we think that the s^4U containing oligonucleotides are G–G–s^4Up and s^4U–A–Gp. A further argument comes from a combined T_1 RNAase and U_2 RNAase hydrolysate (not shown here) which gave a characteristic spectrum at 336 nm between the di- and the trinucleotides. Among the dinucleotides only U-Ap was found in low yields and is the only U-containing dinucleotide which could contain s^4U in the native molecule.

This dinucleotide can only be placed in the G-G-U_8*–A–Gp sequence. Therefore s^4U is located in position 8 from the 5′ end.

The base, previously supposed to be a Y-like base [1], is now found to have ultraviolet spectra and chromatographic characteristics of ms^2i^6A [5,6]. We do not know yet if this compound has a supplementary hydroxyl group giving ms^2io^6A (ms^2 zeatin ribose). This nucleoside occurs in plants, and has ultraviolet spectrum similar to those of ms^2i^6A [7]. The fluorescence previously observed in $tRNA^{Phe}$-enriched fractions is easily removed from the tRNA by 3 reprecipitations of a solution of $tRNA^{Phe}$ with 9 vol. ethanol (final concentration 10 μg/ml). The origin of this fluorescence is unknown but probably due to contamination.

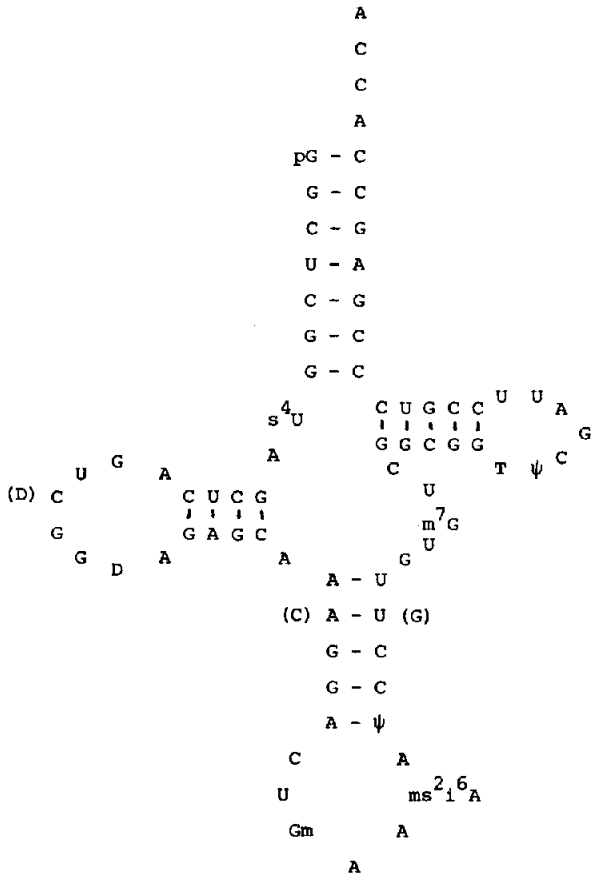

Fig.3. Cloverleaf model of *Bacillus stearothermophilus* $tRNA^{Phe}$. Between brackets the differences found in *Bacillus subtilis* $tRNA^{Phe}$ [8].

The presence of ms^2i^6A and s^4U is consistent with what is usually found in bacterial tRNAs.

The separation and the analyses of the nucleotides and oligonucleotides obtained by T_1 and pancreatic RNAase hydrolyses are shown in fig.1 and fig.2. These results support a revised cloverleaf model for *B. stearothermophilus* $tRNA^{Phe}$ as shown in fig.3 in which pG–G–Cp and C–C–A–C–C–A occupy the end positions and G–G–s^4Up and U–C–C–C–Gp are present. Therefore, the revised end sequences are pG–G–C–U–C–G–G–s^4U and U–C–C–C–G–A–G–C–C–A–C–C–A.

The primary sequence of *B. stearothermophilus* $tRNA^{Phe}$ from another strain (NCIB 8924) was found to be identical to the sequence shown in fig.3.

The $tRNA^{Phe}$ from *B. stearothermophilus* is very similar to $tRNA^{Phe}$ from *B. subtilis* (difference 3 nucleotides) [8] whereas both *B. stearothermophilus* and *B. subtilis* $tRNA^{Phe}$ differ substantially from *E. coli* $tRNA^{Phe}$ [9] (21 nucleotides and 22 nucleotides, respectively).

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