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SEQUENCE OF $tRNA^{Leu}_{CmAA}$ FROM BACILLUS STEAROTHERMOPHILUS

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The primary structure of Bacillus stearothermophilus $tRNA^{Leu}$ was determined and found to be :pGCCGAUGs^{4}UGGCGGAAUDGGCAGm^{1}ACGCGCACGACUCmA-Ams^{2}i^{6}AAψCGUGUGGGCUUUGCCCGUGUGGGTψCGACUCCCACCAUCGGCACCA.

The molecule has a large extraloop and contains only 8 minor nucleotides. There is a G at position 21 like in all other sequenced bacterial $tRNAs^{Leu}$.m^{1}A is in position 22, just before the D stem like in several other procaryotic tRNAs. The anticodon is CmAA and is adjacent to a ms^{2}i^{6}A in the 3'-direction.

Although the primary structures of more than 250 tRNAs have been determined (for a compilation, see ref.1) only $tRNA^{Phe}$, $tRNA^{Tyr}$ and $tRNA^{Val}$ are originating from Bacillus stearothermophilus, a thermophilic organism growing at 65°C (2,3,4). We studied therefore the primary structure of a $tRNA^{Leu}$ from this organism. This sequence was thought to be interesting because of a special feature occuring in $tRNAs^{Leu}$. Most of the tRNAs have an invariant nucleotide A in position 21. Exceptions to this are the $tRNA^{Ile}$ from Halobacterium volcanii (5) and the bacteriophage T_4 (6) which have respectively U and G, the $tRNA^{Met}$ from Thermoplasma acidophilum (7) which has a G, the yeast and Neurospora crassa mitochondrial $tRNAs^{Tyr}$ (8,9) which have respectively U and G, and 13 of the 26 known $tRNAs^{Leu}$ from several organisms such as E.coli, bacteriophage T_5, Anacystis nidulans, chloroplasts and yeast or Neurospora crassa mitochondria (for a compilation see (1)) which all have a G in this position. When we started our structure determination, only G had been found at position 21 of bacterial $tRNAs^{Leu}$. It was therefore interesting to determine the structure of another bacterial $tRNA^{Leu}$ to check if this was a general feature.

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MATERIAL AND METHODS

Total **Bacillus stearothermophilus** tRNA was obtained from Dr H. GROSJEAN (Brussel, Belgium) and from Dr R.S. BROWN (Cambridge, U.K.). BD-cellulose was from Schwarz Mann and Sepharose 4B from Pharmacia. Chemicals were from BDH and Merck, enzymes from Worthington, thin layer cellulose plates from Schleicher and Schull or Macherey-Nagel. All other materials were as described previously (10,11) γ-[^{32}P]-ATP (3000 Ci·mmol^{-1}) was purchased from Amersham.

tRNA fractionation

BD-cellulose and Sepharose-4B column chromatography were done as previously described (11,12). Polyacrylamide gel electrophoresis was performed according to SANGER and COULSON (11). The tRNA was eluted from the gel by diffusion using 0.5M ammonium acetate ,0.01M magnesium acetate, 0.1% sodium dodecylsulfate and 1mM EDTA (14). After several hours diffusion, soluble polyacrylamide was removed from the diffusion buffer by phenol extraction and salt and phenol were removed from the aqueous supernatant by desalting on G25: 1 ml G25 in a disposable syringe is centrifuged at 1000 RPM to remove excess water. Sample is finally desalted by application in 0.3M NaCl,50 mM sodium acetate (10 to 15% of the G25 volume) and centrifugation as above. The eluting fractions showing leucine accepting activity were determined using [^{3}H]-leucine (318 mCi·mmol^{-1}, C.E.A./Saclay) and E.coli aminoacyl-tRNA synthetase which was a gift from Dr G. JEANNIN (16).

Sequencing techniques

For the sequence determination of tRNA^{Leu}, the following post-labelling methods were utilized:

(i) The procedure of STANLEY and VASSILENKO (17) but using different conditions of hydrolyses were employed: the sample (3 µg) is incubated for 3 min at 80°C in 10µl of either desionized dimethylsulfoxyde or formamide (18). The digestion products are 5'-[^{32}P] post-labelled with polynucleotide kinase and separated on two-dimensional polyacrylamide gel according to (19) and (20). The first dimension using a 10% polyacrylamide slab gel, at pH 3.5. The second dimension is performed on a 90 cm long 15% polyacrylamide gel. All spots were analysed either for their 5'-terminal nucleotides as indicated in fig. 3 or by partial hydrolysis with bidistilled water (2 h at 90°C) in presence of 20 µg carrier tRNA followed by read-off "wandering spot" technique (21).
(ii) Read-off sequencing gels (22) using 5'-[^{32}P]-labelled tRNA. To label the tRNA we used the exchange reaction as indicated in (23), which permits to introduce 5.10^{6} to 10^{7} Cerenkov counts into 5 to 10 µg of tRNA without removal of the terminal phosphate.

Nucleoside composition of unlabelled tRNA^{Leu} was done according to ROGG et al. (24). Study of U.V. spectrum of tRNA^{Leu} between 300 and 400 nm was used to search ofs^{4}U (2,25).

RESULTS

Purification of tRNA^{Leu}

Bacillus stearothermophilus tRNA^{Leu} was obtained after two chromatographic steps on BD-cellulose and Sepharose-4B (Fig. 1). The last step of purification was an one dimensional polyacrylamide gel electrophoresis in denaturing conditions which permits to obtain one tRNA^{Leu} spot free of contaminating tRNAs and fragments.

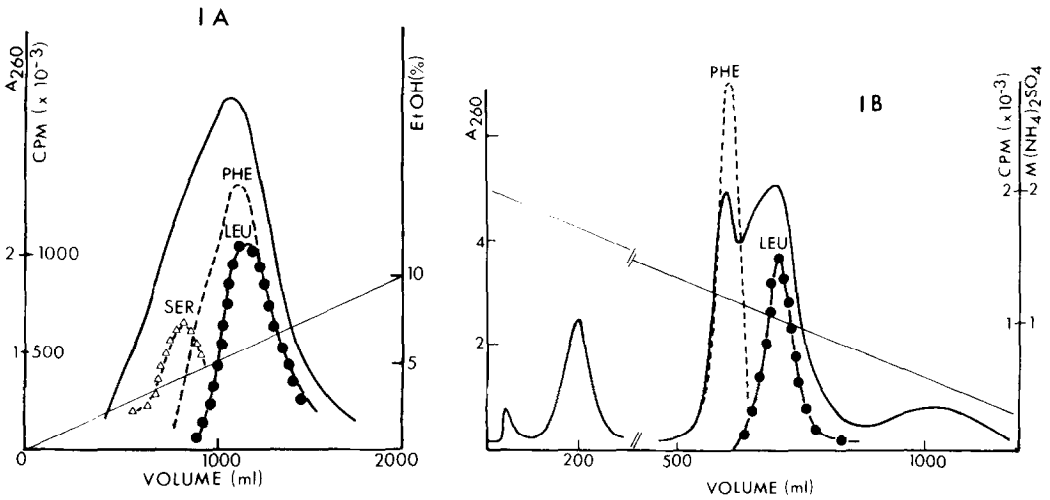


Figure 1: Purification of Bacillus stearothermophilus $tRNA^{Leu}$

1A : Separation on BD cellulose of the "hydrophobic" tRNA species. 120 mg of total tRNA were loaded on the column (100 x 2.5cm). A first elution was performed in 10 mM sodium acetate pH 4.5, 10 mM $MgCl_2$ and 1 M NaCl, then an ethanol gradient was applied (0 to 10% in the same buffer, total volume 2 liters).

1B : Separation on Sepharose 4B of the BD cellulose fraction containing $tRNA^{Leu}$ and $tRNA^{Phe}$. 65 mg were loaded on the column (100 x 1.5 cm). Elution was performed with a decreasing ammonium sulfate gradient from 2M to 1M (total volume 1,5l) in 10 mM sodium acetate pH 4.5, 10 mM $MgCl_2$, 1 mM EDTA and 6 mM ß-mercaptoethanol.
Accepting activities of $tRNA^{Leu}$ (●—●), $tRNA^{Phe}$ (- - -) and $tRNA^{Ser}$ (△—△). A 260 (——)

Sequence analysis

The primary structure of $tRNA^{Leu}$ was determined using the sequencing procedures listed in "Material and Methods" (Fig. 2,3,4,5).

The complete nucleotide sequence of Bacillus stearothermophilus $tRNA^{Leu}$ deduced from these analyses is shown in Fig. 6.

DISCUSSION

Bacillus stearothermophilus $tRNA^{Leu}$ is 86 nucleotides long. It has a Cm-A-A anticodon. It contains all the invariant or semi-invariant residues found in tRNAs active in elongation of protein synthesis (26). However like several $tRNAs^{Leu}$ (see "Introduction") it has a G in position 21*. This feature seems therefore typical for bacterial $tRNAs^{Leu}$.

* The numbering of nucleotides is that of yeast cytoplasmic $tRNA^{Phe}$.

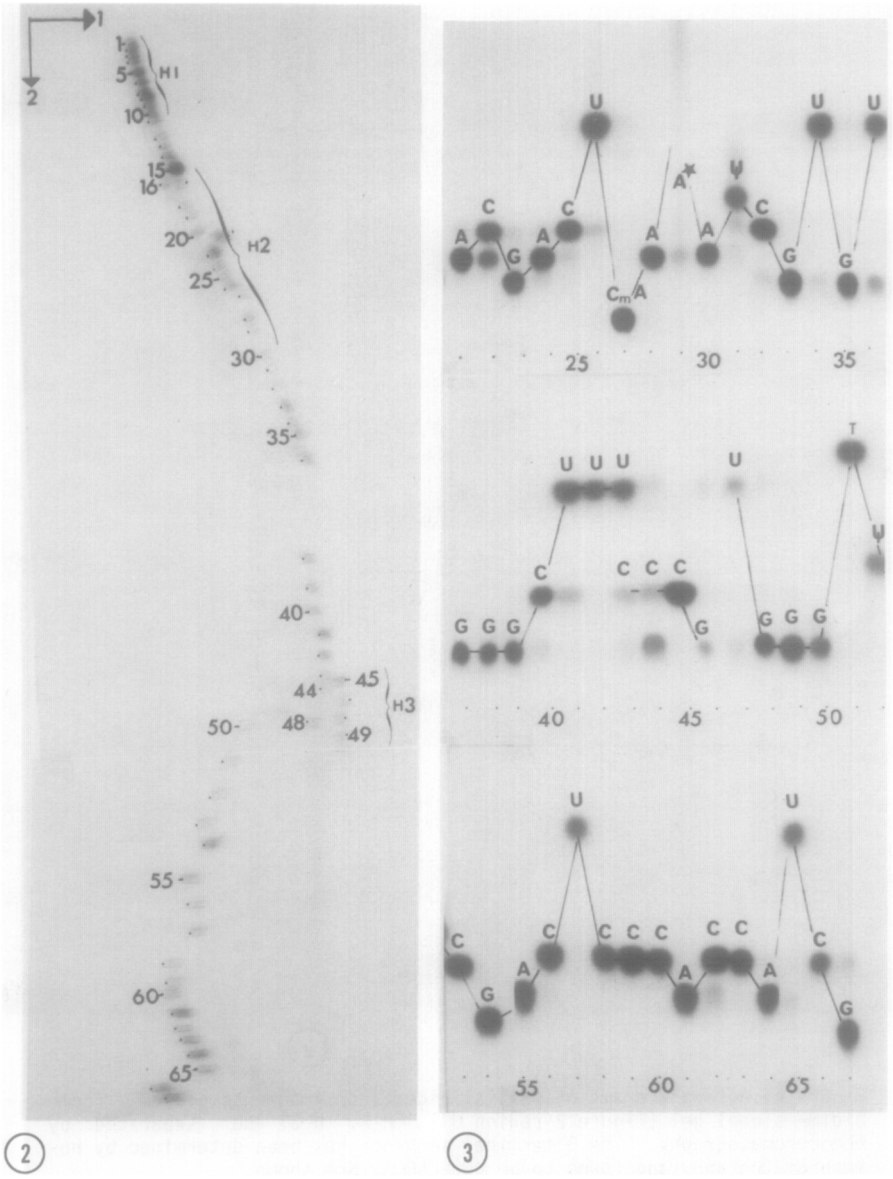
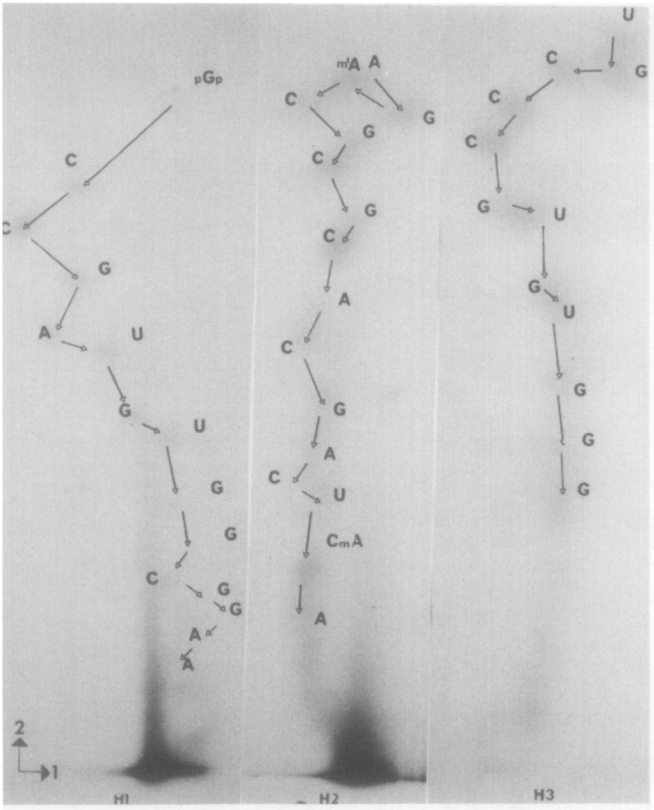


Figure 2 : Two dimensional gel electrophoresis of a partial digest of $tRNA^{Leu}$ postlabelled by [^{32}P]phosphate at the 5'termini.

First dimension was an acid gel 25 mM citric acid, 7M urea, pH 3.5 10% polyacrylamide. Second dimension was 90 mM Tris-borate pH 8.3, 7 M urea, 25 mM EDTA, 15% polyacrylamide (dimension of the gel 35 x 90 x 0.05 cm).
H 1, H 2 et H 3 correspond to regions which could not be sequenced by direct analysis of the 5' termini of the fragments corresponding to each spot. These sequences were determined using the wandering spot techniques (see Figure 4).

Figure 3 Thin layer chromatography of the 5'[^{32}P]-labelled termini corresponding to the spots in Figure 2.

The fragments were eluted from the gel and totally digested with nuclease P1. The resulting mononucleosides - 5'[^{32}P]-phosphate were identified by chromatography on cellulose thin layers developed by 2-propanol/con. HCl/H_2O 68/18/14 (by vol.).



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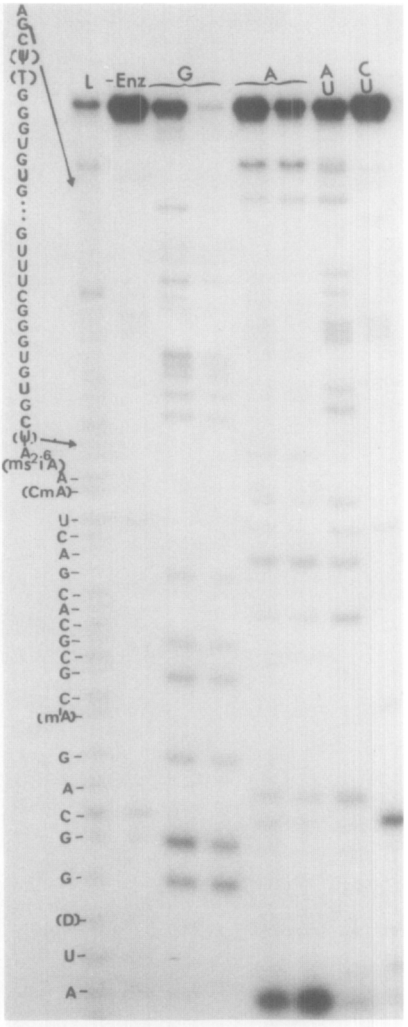

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Figure 4 :Autoradiograms of partial digests of fragments eluted from bidimensional gel (Figure 2 region H 1, H 2, H 3) and separated by homochromatography. The 3'terminal sequence has been determined by homochromatography and found to be UCGGCACCA. Not shown.

Figure 5 : Read-off sequencing gel using 5'-[^{32}P] labelled tRNA.

Partial digestions were carried out with RNAse T1(G), RNAse U2 (A) RNAse Phy M (A,U), RNAse from Bacillus cereus (C,U). L shows statistical degradation of the molecule. obtained by incubation in H_2O, at 100°C for 3min. -Enz is a control.

Like all tRNAs^{Leu} except mammalian mitochondrial tRNAs^{Leu} it has a long extra arm. However there are only 3 unpaired nucleotides in the loop of this extra arm. A similar feature is observed in mitochondrial tRNAs^{Leu} from Neurospora crassa (27) and in bean chloroplastic tRNA^{Leu} (28).

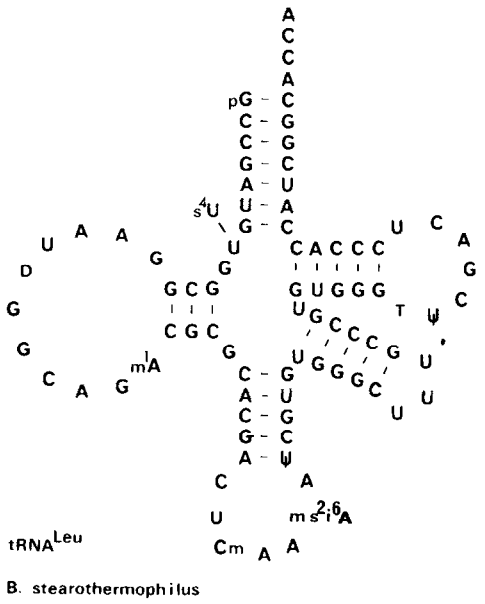

Figure 6 : Nucleotide sequence of $tRNA^{Leu}_{C_mAA}$ from Bacillus stearothermophilus in the cloverleaf model.

B.stearothermophilus $tRNA^{Leu}$ has 8 modified nucleosides: 2 ψ, 1 D, 1 T, 1 s^4U, 1 Cm, 1 m^1A and 1 ms^2i^6A. The position of s^4U was not determined. However we infer from all other published tRNA structures that it is at position 8. By calculation from the U.V. spectrum we find more than 0.5 mol of s^4U for 1 mol of $tRNA^{Leu}$. Like all other bacterial $tRNAs^{Leu}$ it contains a G at position 21. m^1A is situated at position 22 just before the D-stem. This is a characteristic of procaryotic tRNAs (29). ms^2i^6A is adjacent to the 5' terminal of the anticodon. This fits with the observation of NISHIMURA (30) that i^6A or its derivatives are almost always found in tRNAs that recognize codons starting with U. In fact **Bacillus stearothermophilus** $tRNA^{Leu}$ should recognize the leucine codons U-U-G and possibly U-U-A if there is a C·A wobble. We have compared all 27 known $tRNAs^{Leu}$ sequences. The number of structural similarities of the nucleotide backbone, including the minor nucleotides whose modifications were not taken into account, were calculated. Nor did we take into account the variable loop. **B. stearothermophilus** $tRNA^{Leu}_{CmAA}$ is very similar to **Anacystis nidulans** $tRNA^{Leu}_{CAA}$ (75% homology), bean chloroplast $tRNA^{Leu}_{UAA}$ (72% homology), **Rhodospirillum rubrum** $tRNA^{Leu}_{CAA}$ (70% homology), **Anacystis nidulans** $tRNA^{Leu}_{CAG}$ (69.5% homology) and **E. coli** $tRNA^{Leu}_{CAG}$ bacteriophage T_4 $tRNA^{Leu}_{GAA}$ and spirach chloroplast $tRNA^{Leu}_{UAG}$ (67% homology). It has still 65% homology

with Morris hepatoma $tRNA^{Leu}_{C*AA}$, bean chloroplast $tRNA^{Leu}_{CmAA}$ and E.coli $tRNA^{Leu}_{GAG}$. Finally with E.coli $tRNA^{Leu}_{A*AA}$ and Torulopsis utilis $tRNA^{Leu}_{CmAA}$ it has respectively 62 and 61% homology. Thus out of the 12 most similar tRNAs 10 are from procaryotes or chloroplasts. However high degree of homology between tRNAs from procaryotic origin is not a general feature, since Halobacterium volcalii $tRNA^{Leu}_{GAG}$ has only 51% homology. It must also be emphasized that six out of the seven known $tRNAs^{Leu}$ having a C-A-A or a Cm-A-A anticodon are among the 13 most similar ones. However yeast $tRNA^{Leu}_{m^5CAA}$ presents much less homology with it (51%).

Finally we calculated the G:C content in the secondary structure of B. stearothermophilus as compared to the 27 other $tRNAs^{Leu}$ structures. Bihelical regions of B. stearothermophilus $tRNA^{Leu}$ have 15 G:C base-pairs and 4 A:U base-pairs but this is not one of the highest content, indeed the 3 sequenced E.coli $tRNAs^{Leu}$ have 18-19 G:C versus 4-5 A:U. Thus there is no increased G.C base-pairing of the bihelical domain nor increased average length of the bihelical segments in that thermophile bacteria as compared to normal growing organisms. It seems that only extreme thermophile like Bacillus acidocaldarius or Thermus thermophilus RNAs exhibit such a property (31,32).

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