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Specific cleavage of tRNA by nuclease S_1

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ABSTRACT

Nuclease S_1 specifically hydrolizes tRNAs in their anticodon loops, forming new 5' phosphate and 3' OH ends. Some single-stranded regions are not cut by nuclease S_1. The strong preference of nuclease S_1 for the anticodon region can be used for rapid identification of an anticodon-containing oligonucleotide and subsequent identification of the probable amino acid specificity of tRNA.

INTRODUCTION

Nuclease S_1 from Aspergillus hydrolizes single-stranded but not double-stranded nucleic acids (1) and has been widely used to assay the extent of annealing of DNA and RNA. Because of its sensitivity for nucleic acid structure, nuclease S_1 would be expected to hydrolize tRNA molecules only in exposed single-stranded regions. Thus it should serve as a probe of tRNA structure in solution.

In this communication we present experiments in which nuclease S_1 was used to digest several purified tRNAs whose nucleotide sequences were known. Under the proper conditions, the enzyme cleaves tRNA only at the anticodon loop and 3' terminus. Use of this digestion procedure can facilitate identification and sequence analysis of the anticodon regions of tRNAs. This technique permits rapid determination of the probable amino acid specificity of purified but uncharacterized tRNAs.

MATERIALS AND METHODS

Isolation of tRNAs and nuclease S_1

^{32}P-labeled chick embryo fibroblast $tRNA^{Trp}$ (2) and $tRNA^{Met}_4$ (3) and E. coli $tRNA^{Glu}_2$ (4) and $tRNA^{Leu}_1$ (5) were purified by two-dimensional polyacrylamide gel electrophoresis as described previously (6,7). Nuclease S_1 was a gift from Dr. Satoshi Mizutani of the University of Wisconsin and it was also purified in this laboratory. Both enzyme preparations were purified by the method of Vogt (8) through the DEAE-cellulose chromatography stage.

Both preparations were specific for single-stranded nucleic acid as assayed by digestion of heat-denatured but not native ^{32}P-labeled SV40 DNA at 45°C for 10 min.

Digestion conditions

The tRNA digestion mixture (0.25ml) contained 0.3 M NaCl, 0.03 M sodium acetate, pH 4.5, 0.001 M $ZnCl_2$, 5% glycerol, about 50,000 cpm of purified [^{32}P] tRNA, 50μg of carrier RNA, and 50 units of nuclease S_1. Incubation was carried out at 20° for an hour. After digestion, 25μg carrier RNA was added and precipitated with 2 volumes of ethanol. The precipitate was resuspended in water and the tRNA fragments were separated by two-dimensional polyacrylamide gel electrophoresis (6).

Characterization of oligonucleotides

The general methods used for isolation and identification of oligonucleotides, such as two-dimensional paper electrophoresis (fingerprinting) (9), and two-dimensional thin-layer chromatography were as described elsewhere (10).

RESULTS

Analysis of tRNA^{Trp}

After digestion of tRNA samples with nuclease S_1 at 20° the products obtained were fractionated by two-dimensional polyacrylamide gel electrophoresis. Figure 1 shows that the products obtained by digestion of chicken cell tRNA^{Trp}

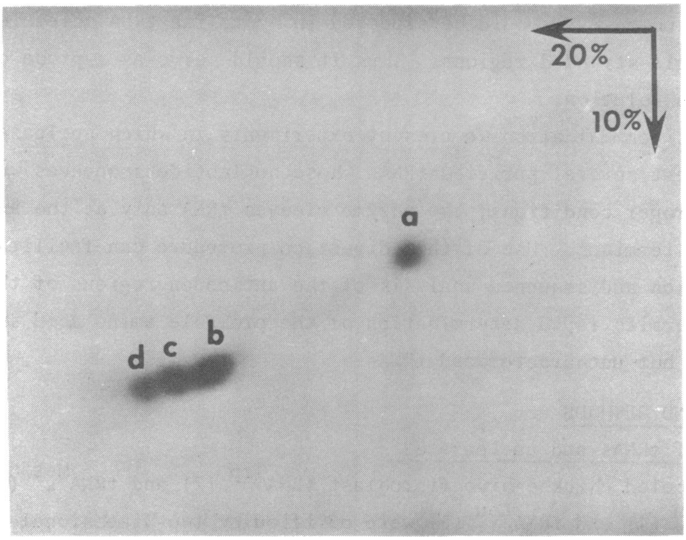

Fig. 1: **Autoradiograph of two-dimensional polyacrylamide gel electrophoresis** fractionation of products obtained by nuclease S_1 digestion of ^{32}P-labeled chicken cell tRNA^{Trp} at 20°. The first dimension (10% acrylamide at 400 V for 1 1/2 hrs.) was top to bottom and the second dimension (20% acrylamide at 200 V for 18 hrs.) was from right to left. The letters indicate products referred to in the text and Figure 2.

could be separated into four spots on the gel. The RNA of each spot was eluted and characterized by RNase T_1 fingerprinting (9) and subsequent modified nucleotide analysis.

Figure 2a shows a fingerprint of intact chicken cell $tRNA^{Trp}$. The fingerprint of the fragment in spot a (Fig. 1) was the same as that shown in Fig. 2a except that the 3' terminal oligonucleotide was shorter (data not shown). The fragment in spot b had all of the oligonucleotides located in the 3' half of the molecule, except for the 3' end which, again, was shorter than normal. In addition, the spot b fragment had several new oligonucleotides which were not present in the intact tRNA molecule. As discussed below, these new oligonucleotides resulted from nuclease S_1 digestion of the anticodon oligonucleotide.

The fingerprint of the fragment in spot c (Fig. 2c) was almost identical to that of spot d (not shown). Both fragments contained all of the oligonucleotides which are derived from the 5' half of the tRNA; the only difference between fragments c and d was an oligonucleotide that came from the 5' side of the anticodon loop of the tRNA.

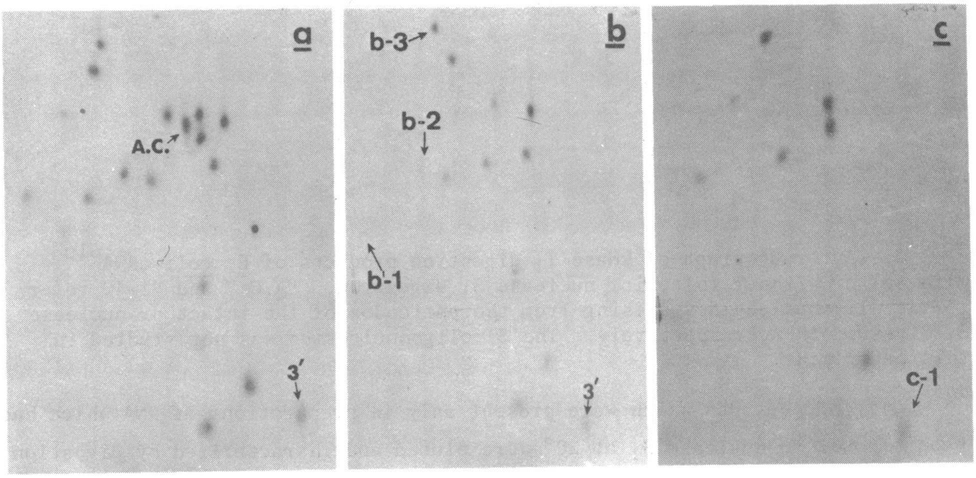

Fig. 2: Autoradiographs of RNase T_1 fingerprints of the nuclease S_1 digestion products of $tRNA^{Trp}$ fractionated in the experiment illustrated in Figure 1. a, $tRNA^{Trp}$ not treated with nuclease S_1, as control; b, spot b fragment of Figure 1, identified as coming from the 3' half of the intact molecule; c, spot c fragment of Figure 1, identified as coming from the 5' half of the intact molecule. Determination of fragments b and c coming from the 3' and 5' halves of the tRNA was made by comparison of the oligonucleotides in the fingerprints with the known sequence of the molecule. "A.C." and "3'" refer to oligonucleotides arising from the anticodon and 3' OH ends, respectively. "b-1-3" and "c-1" refer to oligonucleotides arising from nuclease S_1 digestion of the anticodon.

Analysis of other tRNAs

Analogous results were obtained after nuclease S_1 digestion of chicken cell

$tRNA^{Met}_{4}$, and E. coli $tRNA^{Glu}_{2}$ and $tRNA^{Leu}_{1}$. In the case of $tRNA^{Glu}_{2}$ the 3' and 5' halves were not separable by gel electrophoresis; the fingerprint of the nuclease S_1 digested fragments mixture (Fig. 3a) was the same as for the control, untreated $tRNA^{Glu}_{2}$ (Fig. 3b), except for the absence of the oligonucleotide which contained the anticodon and the appearance of several new oligonucleotides. No change was observed in the 3' OH end of $tRNA^{Met}_{4}$; this might be because an old and perhaps less active preparation of nuclease S_1 was used to digest that molecule.

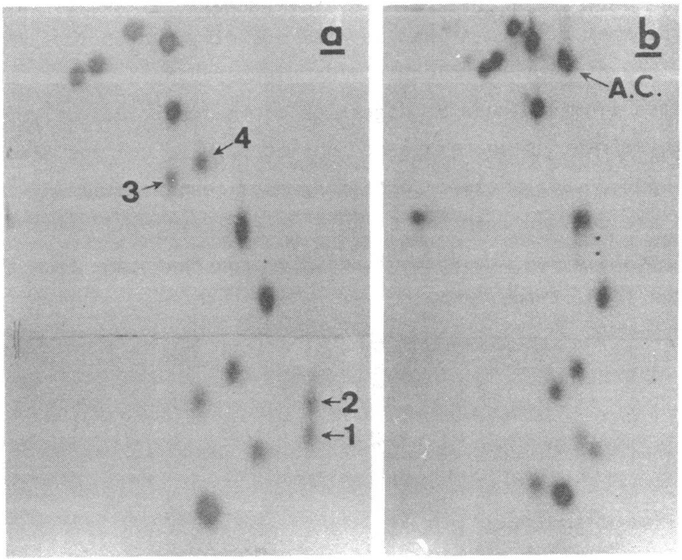

Fig. 3: Autoradiograph of RNase T_1 digestion products of E. coli $tRNA^{Glu}_{2}$ with (a) or without (b) prior nuclease S_1 digestion. "A.C." and "1-4" refer to the oligonucleotides arising from the anticodon of the intact or nuclease S_1 digested tRNA, respectively. The 3' oligonucleotide was not studied in this experiment.

Oligonucleotides which were present only in preparations of RNA which had been exposed to nuclease S_1 at 20° were eluted and characterized by digestion with pancreatic RNase, RNase T_2 or snake venom phosphodiesterase. The redigestion products were separated by DEAE cellulose paper electrophoresis (11) or two-dimensional thin-layer chromatography (10). Such analyses, when compared to analyses and published sequences of the tRNAs in question, permitted us to deduce the sites of cleavage of the molecules by nuclease S_1 (Table 1).

DISCUSSION

Figure 4 shows a summary of the cleavage sites in the tRNAs for nuclease S_1. At 20°, the enzyme cut only the anticodon loop and 3' end of the tRNAs. Loops I and IV (the so-called dihydro-U loop and T-ψ-C-G loop) were resistant to the enzyme. Loop III, whose length varied from four to fifteen nucleotides in the molecules studied here, was also resistant to the nuclease. Under these

TABLE 1

tRNA and Anticodon Sequence	Spot Number	Redigestion		Deduced Structure
		Enzyme	Products	
tRNA^{Trp}(chick embryo fibroblast)	b-1	T_2	pm^{1}Gp	pm^{1}Gp
-G-A-Cm-U-$\underline{\text{Cm-C-A}}$-m^{1}G-A-ψm-C-A-G-	b-2	T_2	pAp, m^{1}Gp	pA-m^{1}Gp
	b-3	T_2	pm^{1}Gp, 2Ap, ψm-Cp, Gp	pm^{1}G-A-ψm-C-A-Gp
	c-1	T_2	Ap, Cm-Up, Cm-C	A-Cm-U-Cm-C
		panc	A-Cm-Up, Cm-C	
	d-1	T_2	Ap, Cm-U	A-Cm-U
		panc	A-Cm-U	
tRNA$^{Met}_4$ (Chick embryo fibroblast)	1	T_2	ψp, Cp, Up, Cm-A	ψ-C-U-Cm-A
-G-ψ-C-U-$\underline{\text{Cm-A-U}}$-t^{6}A-A-ψ-C-U-G-		venom	pA, pCm, pU, pC	
	2	panc	pt^{6}A-A-ψp, Cp, Up, Gp	pt^{6}A-A-ψ-C-U-Gp
tRNA$^{Glu}_2$ (E. coli)				
-G-C-$\bar{\text{C}}$-C-$\bar{\text{U}}$-$\underline{\text{S-U-}\bar{\text{C}}}$-m^{2}A-C-G-	1	T_2	3Cp, Up, S-U	C-C-C-U-S-U
		venom	pU, undigested	
	2	T_2	3Cp, Up, S-Up	C-C-C-U-S-U-C
		venom	pU, pC, undigested	
	3	T_2	pm^{2}Ap, Cp, Gp	pm^{2}A-C-Gp
	4	T_2	pCp, m^{2}Ap, Cp, Gp	pC-m^{2}A-C-Gp
tRNA$^{Leu}_1$ (E. coli)	b-1	T_2	pGp	pGp
-G-C-U-U-$\underline{\bar{\text{C}}\text{-A-}\bar{\text{G}}}$-m^{1}G-ψ-G-	b-2	T_2	pm^{1}Gp, ψp, Gp	pm^{1}G-ψ-Gp
	c-1	T_2	Cp, Up	C-U-U
		venom	pU	
	c-2	T_2	Cp, 2Up	C-U-U-C
		venom	2pU, pC	
	c-3	T_2	2Cp, 2Up	C-U-U-C-A
		venom	2pU, pC, pA	

Spot numbers refer to oligonucleotides obtained after RNase T_1 digestion of intact or nuclease S_1 treated tRNA. The sequences shown are the anticodon regions of the intact tRNAs and the anticodons are underlined. Redigestion with RNase T_2, pancreatic RNase or venom phosphodiesterase and analysis of the redigestion products was as described in Methods. S- is 5-methylaminomethyl-2-thiouridylic acid.

conditions, secondary and tertiary structure probably protects these loop regions from the nuclease. Such interactions are consistent with the three-dimensional crystal structure proposed for tRNA^{Phe} (12,13). We found that digestion at 37° or 50°, rather than 20°, led to degradation of the RNAs, indicating that between 20° and 37° some protective interactions are lost.

Not all sites in the anticodon loops were equally accessible to attack by nuclease S_1. The fragment from the 3' end of the tRNA always terminated with a 5' phosphate on the last nucleotide of the anticodon or the nucleotide immediately adjacent to the anticodon. Fragments from the 5' half of the tRNA did not have unique 3' OH ends. This result indicates that the most susceptable cleavage site is around the third nucleotide of the anticodon, and once the initial hydrolysis has occurred, the enzyme slowly degrades the new ends. This apparent exonucleolytic activity may result from a reduction in endonucleolytic activity near double-stranded regions. In addition, internucleotide bonds between modified nucleotides such as Cm-Cp or U-S-Up were resistant to cleavage.

Cleavage of tRNA in the anticodon loop is very useful for sequence analysis. A variety of methods have been developed in different laboratories for cleavage in that region. However, such methods were restricted by requirements for particular nucleotides in the loop and by low yields of products. The nuclease S_1 method described here is independent of nucleotide sequence and the products are obtained in high yield.

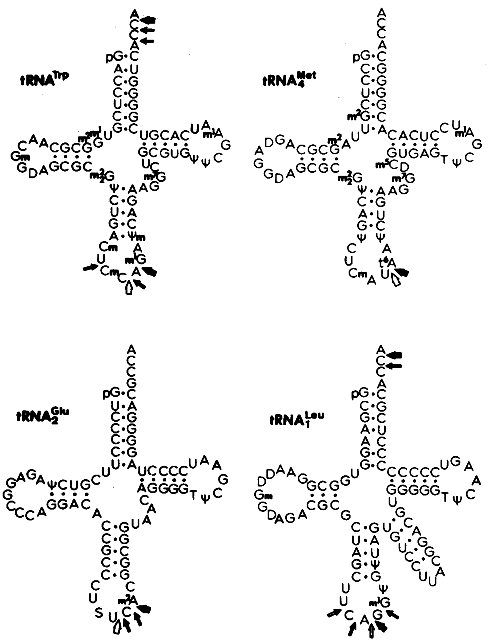

Fig. 4: Schematic summary of the preferred sites of cleavage in tRNA by nuclease S_1 at 20°. The filled and open arrows denote the ends of the 3' and 5' halves of the tRNA. The thickness of the arrows reflects relative yield of the various ends. The 3' OH oligonucleotides of tRNA$^{Glu}_2$ was not studied.

The selective hydrolysis of the anticodon region by nuclease S_1 suggests a powerful method for rapid determination of probable amino acid specificity of purified but uncharacterized tRNAs. Comparison of a fingerprint of an RNA that had been treated with nuclease S_1 (followed by removal of the nuclease with phenol before further digestion) with a fingerprint of the same RNA that had not been pre-treated should reveal oligonucleotides such as those seen in Figure 3 for tRNA$^{Glu}_2$. Oligonucleotides that disappear upon treatment would be those containing the 3' end and anticodon region. Oligonucleotides that appear after digestion would be useful partial digestion products for sequence analysis of the intact oligonucleotides. From the sequence of the anticodon region one could deduce the cognate codon and hence the probable amino acid, without the necessity of sequencing the entire tRNA. This technique would be especially useful for work on molecules that are obtainable in pure form but in only very small quantities such as tRNAs of RNA tumor virus virions.

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We have recently learned that Jacov Tal has obtained similar results using the single strand specific nuclease from *Neurospora crassa* (submitted to Nucleic Acids Research).

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