

METHOD

MALDI-TOF mass spectrometry methods for evaluation of in vitro aminoacyl tRNA production

E. JAMES PETERSSON,¹ MONA SHAHGOLI,¹ HENRY A. LESTER,²
and DENNIS A. DOUGHERTY¹

¹Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, USA

²Division of Biology, California Institute of Technology, Pasadena, California 91125, USA

ABSTRACT

Unnatural amino acid mutagenesis requires the in vitro production of aminoacyl tRNAs. Bacteriophage T4 RNA ligase is used to ligate α -amino-protected dCA amino acids to 74mer tRNA. Previously, there has been no facile method for evaluating the efficiency of this reaction prior to using the tRNA in translation. We report a novel use of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry in monitoring the formation of aminoacyl 76mer tRNA. This method is more efficient and precise than the traditional technique of gel electrophoresis. These MALDI conditions should also prove useful for analyzing aminoacyl tRNAs produced through aminoacyl tRNA synthetases and other methods.

Keywords: aminoacylation; gel electrophoresis; matrix-assisted laser desorption/ionization; nonsense suppression; T4 RNA ligase; T7 RNA polymerase; THG73 tRNA; unnatural amino acid

INTRODUCTION

Nonsense suppression incorporation of unnatural amino acids permits subtle and elegant site-specific manipulations of both side chain and backbone protein structure (Cornish et al., 1995; Dougherty, 2000). Using both in vitro and in vivo expression systems, well over 100 unnatural amino acids have been incorporated into dozens of different proteins. The methodology requires the in vitro production of an aminoacyl suppressor tRNA (Fig. 1) which will be used to deliver our unnatural amino acid at the site of a mutagenically introduced stop codon. Aminoacyl tRNAs (aa-tRNAs) are made by ligating a chemically synthesized aminoacyl dCA dinucleotide to the 3' end of a transcribed 74mer tRNA. There is no simple technique for evaluating the aminoacylation state of the tRNA prior to using it in translation. Current methods rely on gel electrophoresis, which infers mass from electrophoretic mobility. Radiolabeling can be used to ascertain aminoacylation information (Weygand-Durasevic et al., 1996), but this is impractical for the "everyday" production of tRNAs for suppression. Aminoacyl tRNAs can be observed on acid/urea gels, but

very long running times are required (Varshney et al., 1991; Wolfson et al., 1998; Kohrer et al., 2001).

We now report that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Kirpekar et al., 1994; Zenobi & Knochenmuss, 1998; Bakhtiar & Nelson, 2000; Bakhtiar & Tse, 2000) provides a simple and quick way of evaluating the products of the ligase reaction. It is also much more precise, enabling one to distinguish tRNA species down to the single-nucleotide level, and to verify the identity of the amino acid. The use of MALDI MS with tRNA is not new (Rubelj et al., 1990; Gruic-Sovulj et al., 1997, 2001; Wei & Lee, 1997; Sochacka et al., 2000), but we demonstrate its application to a novel problem. Here MALDI-TOF MS is used to monitor the production of aa-76mer tRNA by following the disappearance of 74mer starting material and the appearance of the desired aa-76mer and the 76mer hydrolysis product.

RESULTS AND DISCUSSION

The suppressor tRNA is produced through a combination of chemical and biological steps. The amino acid to be delivered is made as an α -amino-protected cyanomethyl ester that is coupled to chemically made pdCpA. The suppressor tRNA is transcribed from linearized

Reprint requests to: Dennis A. Dougherty, Department of Chemistry, Caltech 164-30, Pasadena, California 91125, USA; e-mail: dad@igor.caltech.edu.

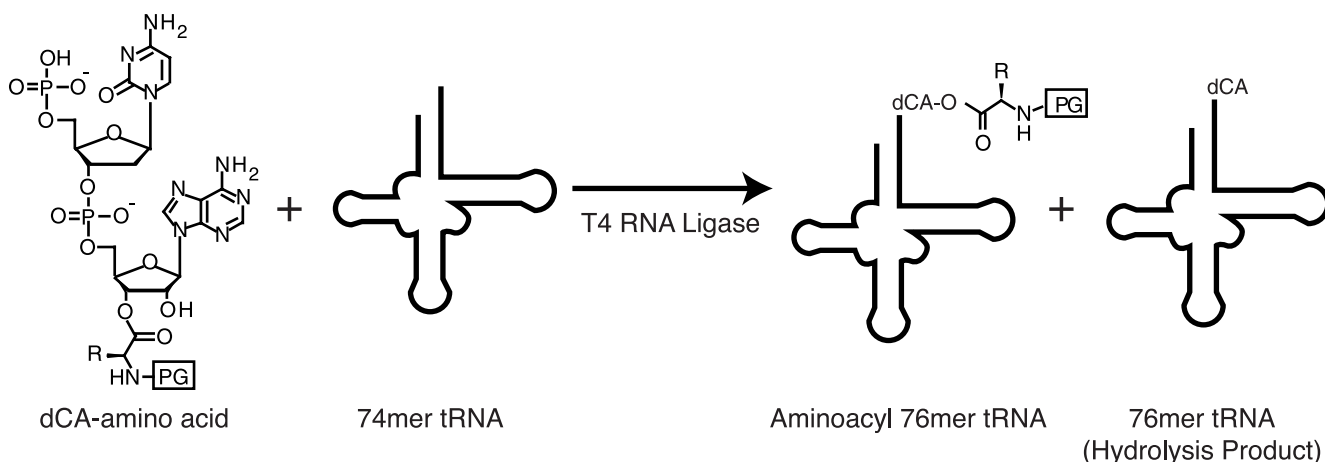


FIGURE 1. T4 RNA ligase reaction. The synthesized aminoacyl dinucleotide dCA is ligated to transcribed 74mer tRNA with T4 RNA ligase to give aminoacyl 76mer tRNA. Hydrolysis of the 3' ester bond gives 76mer tRNA. PG indicates that α -amine is protected as an amide functionality.

cDNA as a 74mer lacking its 3' CA. This transcript is ligated to the 5' phospho-dCA-amino acid (dCA-aa) with T4 RNA ligase (Fig. 1; Silber et al., 1972; Uhlenbeck & Gumpert, 1982; Uhlenbeck, 1983). The amino acid is then deprotected just prior to use in translation with an mRNA bearing the stop codon to which the tRNA will deliver its amino acid.

Traditionally, the reactions in the chemical steps have been monitored by thin-layer chromatography and high performance liquid chromatography, and the ligation step by gel electrophoresis (Fig. 2). One can easily differentiate unligated 74mer from 76mer produced either through transcription (76/77mer, some untemplated 77mer, see Materials and Methods) or ligation (dCA 76mer). One can also observe the aminoacyl

tRNAs Ala-76mer, Trp-76mer, and 5-cyano-tryptophan-76mer (5-CN-Trp-76mer). However, there are substantial amounts of the 76mer hydrolysis product present. Even under acid/urea gel conditions (see Materials and Methods), some of the aa-76mer may have hydrolyzed in the 36-h process of running the gel. This seems to be the case; mass spectra of the same tRNA samples (Fig. 3) shows them to be relatively free of 76mer hydrolysis product. In contrast, the gel shows nearly equivalent amounts of aa-76mer and 76mer hydrolysis product. This illustrates one of the several advantages of MALDI MS over gel electrophoresis as an analytical tool for our problem. A successful application of MALDI MS takes less time to run, uses less tRNA material, is more precise, and most importantly, provides aminoacylation information that cannot be obtained through standard gel techniques.

To identify a suitable matrix for analysis of the tRNAs, a variety of matrices were tested with 74mer transcript. These included 3-hydroxypicolinic acid (3-HPA; Tolson & Nicholson, 1998; Kirpekar et al., 2000), 6-aza-2-thiothymine (Gruic-Sovulj et al., 1997), 2, 4, 6-trihydroxyacetophenone (Patteson et al., 2001), and anthranilic/nicotinic acid (AA/NA) mixtures (Zhang & Gross, 2000). Among these, only 3-HPA and AA/NA (2.7 mg AA and 1.2 mg NA in 50 μ L CH₃CN and 60 μ L 50 mM diammonium citrate (DAC)) provided acceptable 74mer signal intensity. 3-HPA was chosen because it resulted in superior mass resolution and intensity. Ammonium-loaded cation-exchange beads improved signal intensities dramatically for the 74mer tRNA. More importantly, a 10-min treatment with the NH₄⁺-loaded beads (see Materials and Methods) was found to be essential to observing the aminoacyl tRNAs. The H⁺ and N(Bu)₄⁺ forms of the beads were found to produce inferior results.

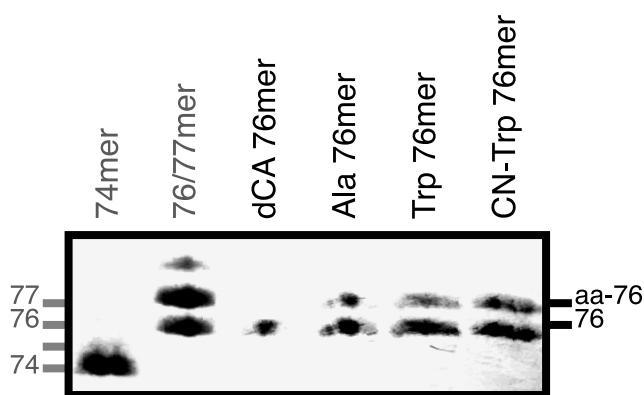


FIGURE 2. Gel separation of tRNA species. A 20% polyacrylamide acid/urea gel showing single-base resolution. 74mer and 76/77mer (in gray) are transcribed from cDNA. Corresponding markers are shown in gray at left. All others (in black) are produced through T4 RNA ligase reaction of either dCA or dCA-aa with transcribed 74mer. One can observe both the aa-76mer and 76mer hydrolysis product, marked at right in black.

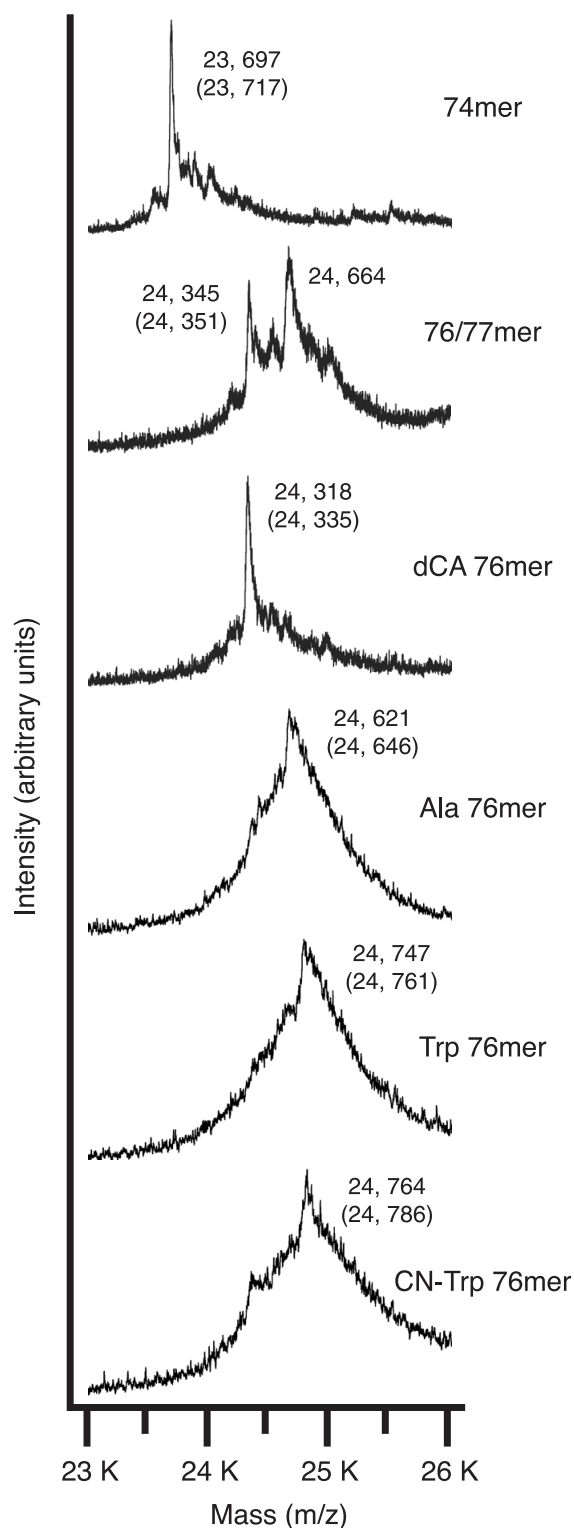


FIGURE 3. MALDI mass spectra of various tRNA species. The tRNA species shown are the same as those shown in the gel in Figure 2. Comparing the 74mer to the dCA-76mer shows that the addition of the dinucleotide can be clearly observed. The further increase in mass attributable to the amino acid is also clear in the Ala-76mer, Trp-76mer, and CN-Trp-76mer spectra. A small amount of 76mer hydrolysis product is apparent in the spectra of the aminoacyl tRNA. Observed masses (average of five spectra) are shown, with expected masses given in parentheses. The MS data confirms gel data indicating the presence of untemplated 77mer in the transcribed 76mer.

The final, optimized matrix sample preparation routinely resolves 74mer transcripts from 76mer and aa-76mer tRNAs (Fig. 3). Figure 3 depicts the same tRNA samples that were loaded onto the gel shown in Figure 2: transcribed 74mer, transcribed 76/77mer, ligated dCA 76mer, and Ala, Trp, and CN-Trp aa-76mers. One can see that hydrolysis of the amino acid has been minimal; the 76mer observed on the gel must have been produced in the process of running the gel.

The aa-76mer peaks are noticeably broader than the 74mer or even dCA 76mer peak. It is tempting to attribute the change in resolution to contaminants, as it seems surprising that aminoacylation, a relatively small change on the scale of a tRNA, would cause such a dramatic change in its behavior in the MALDI MS. The repeated phenol/chloroform/isoamyl alcohol extractions described in Materials and Methods were crucial to attaining the level of resolution shown in Figure 3. However, further extraction does not improve the aa-76mer signal, so it seems that residual ligation reagents are not limiting the resolution. This question could be addressed by gel purifying the aa-tRNAs prior to analyzing them, but this experiment has not been performed. The loss of resolution observed may be attributable to increased matrix-adduct formation by the aa-76mer relative to the dCA-76mer.

The resolution of aa-76mer from 76mer is aided by the fact that the amino acid is protected on its α -amine with a nitroveratryloxycarbonyl group (NVOC, 241 Da), increasing the mass shift. The inherent error of our system is about 0.1% (see Materials and Methods). This precludes resolving an alanyl tRNA from a glycyl tRNA, but one can still gain ample information about most ligation reactions.

It should be noted that the masses we observe are consistent with tRNAs lacking the 5' phosphorylation expected of a T7 RNA polymerase product. The 5' phosphate bond has been identified as one of the most labile RNA bonds in MALDI MS conditions, though RNAs are known to be generally stable in MALDI-TOF MS (Nordhoff et al., 1993; Kirpekar et al., 1994; Knochenmuss et al., 2000; Kirpekar & Krogh, 2001). While this may be cause for concern that any deaminoacylation observed is also a result of the MALDI process, the fact that we observe pure aa-76mer should assuage this concern.

In a valuable application of this methodology, monitoring the T4 ligation reaction by MALDI-TOF MS has shown that the usual 2-h incubation time (Nowak et al., 1998) leads to substantial hydrolysis of the amino acid (Fig. 4). In fact, the reaction is largely complete after 20 min, and incubation times longer than 30 min are unnecessary. This has held true for a wide variety of both natural and unnatural amino acids, including Ala, Trp, CN-Trp, and two positively charged tyrosine derivatives. Although one can get a clear impression of the degree of hydrolysis from mass spectra like those in

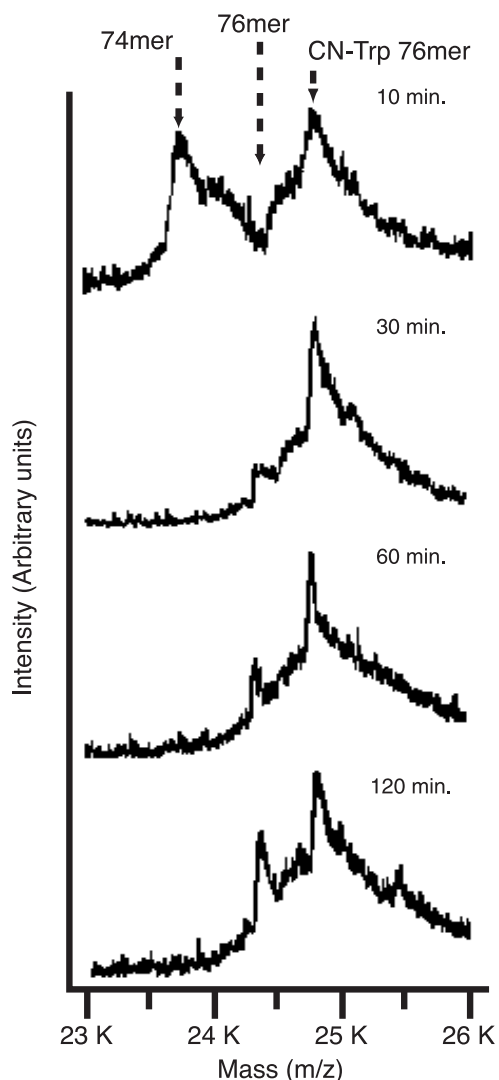


FIGURE 4. T4 RNA ligase reaction efficiency. MALDI mass spectra of aliquots of the ligation of 5-CN-Trp-dCA taken after various reaction times. At 10 min, starting material (74mer), hydrolysis product (dCA 76mer), and desired product (CN-Trp 76mer) are clearly seen. The reaction is complete after 20–30 min, and longer reaction times lead only to increased hydrolysis (giving dCA 76mer).

Figure 4, it is not possible to quantitate the relative amounts of 76mer and aa-76mer. The decrease in resolution (see above) for the aa-tRNAs indicates that they ionize differently than non-aminoacyl tRNAs making it unreasonable to compare peak intensities.

MALDI MS can also be used to observe the photocleavage of the NVOC protecting group from the aminoacyl tRNAs (data not shown). Removing the NVOC protecting group prior to using the tRNA in translation is essential, the α -amine must be exposed in order for it to be incorporated into the peptide backbone. Time course studies similar to those performed for the ligation reaction can be used to determine optimal photodeprotection conditions. This again, is information inaccessible through gel electrophoresis.

MALDI MS has proven useful in evaluating the dCA ligation reaction as well as in examining the deprotection of the α -amines of the aa-76mers. Not only is the MALDI analysis faster and more material efficient than gel electrophoresis, it can provide information about the aminoacylation state of the tRNA unobtainable through gels. We have applied the assay to a relatively specific problem, but the techniques should prove useful to those interested in tRNA aminoacylation, particularly those engineering synthetases.

MATERIALS AND METHODS

Materials

The synthesis of the pdCpA dinucleotide and its 3' aminoacylation have been described previously, as have the syntheses of the protected natural and unnatural amino acids that are coupled to the dCA for ligation (Ellman et al., 1991; Nowak et al., 1998). All water used in the enzymatic reactions below has been rendered RNase free by treatment with diethylpyrocarbonate (Sigma-Aldrich, St. Louis, Missouri). The chemicals used in matrix preparation, α -cyano-4-hydroxycinnamic acid (α -CN), 3-hydroxypicolinic acid (3-HPA), picolinic acid (PA), diammonium citrate (DAC), and DOWEX 50WX8-200 100–200 mesh size ion exchange resin, were also purchased from Sigma. The DOWEX beads were exchanged overnight with 1 M NH_4OAc , collected on a frit, and washed twice with 1 M NH_4OAc .

Transcription of 74mer and 76mer tRNA

The transcription and ligations have been previously described; they were performed here with minor alterations (Saks et al., 1996). The tRNA used was THG73, *Tetrahymena thermophila* tRNA^{Gln}CUA having a G at position 73. This gene contains an upstream T7 RNA polymerase promoter and downstream restriction sites. *FokI* digestion provided the 74mer template and *BsaI* digestion gave the 76mer template. The in vitro transcription of linearized cDNA to produce THG73 74mer and 76mer tRNAs was performed with the Ambion T7-MEGAscript kit (Austin, Texas). Transcripts were isolated with a 25:24:1 phenol: CHCl_3 :isoamyl alcohol (PCI) extraction. The organic layer was reextracted with water and a 24:1 CHCl_3 :isoamyl alcohol (CI) was performed on the combined aqueous layers. The water layer was then mixed with an equal volume of isopropanol, precipitated overnight at -20°C , pelleted, dried, and redissolved in H_2O . The 76mer tRNA appears to contain a large amount of untemplated 77mer and will be referred to as 76/77mer. There is substantial precedent for the addition of untemplated nucleotides at both the 3' and 5' ends of T7 RNA polymerase transcription products (Milligan et al., 1987; Pleiss et al., 1998; Helm et al., 1999; Kao et al., 1999).

Ligation of dCA-aa to 74mer tRNA

Prior to ligation, the 74mer tRNAs were heated to 90°C in a 6.7 mM HEPES, pH 7.5, solution and allowed to cool to 37°C .

They were then incubated at 37°C in 40 μL of a ligation mixture containing 42 mM HEPES, pH 7.5, 10% dimethyl-sulfoxide (v/v), 4 mM dithiothreitol, 20 mM MgCl_2 , 0.2 mg/mL bovine serum albumin (Ambion), 150 μM ATP, 10 μM 74mer tRNA transcript, 300 μM protected dCA-aa, and 2,000 U/mL T4 RNA ligase (New England Biolabs, Beverly, Massachusetts). After incubation at 37°C for 10 to 120 min, the reaction mixtures were diluted to 100 μL by adding 8.3 μL 3.0 M NaOAc and 51.7 μL H_2O . They were then extracted against an equal volume of PCI (pH adjusted to 4.5 with NaOAc). The organic layer was reextracted with 4.2 μL 3.0 M NaOAc and 45.8 μL H_2O . Aqueous layers were combined and extracted again with 150 μL PCI. Two 150- μL CI extractions were performed on the water layer. Finally, the water layer was mixed with 450 μL EtOH and precipitated overnight at -20°C . The sample was pelleted, dried, and resuspended in 1 mM NaOAc to 1.0 $\mu\text{g}/\mu\text{L}$ (RNA quantified by UV absorption at 260 nm).

MALDI mass spectrometry

All tRNAs were analyzed on a PerSeptive Biosystems (Framingham, Massachusetts) Voyager DE PRO MALDI-TOF mass spectrometer operating in linear and positive ion modes. For all experiments, the accelerating voltage was held at +25 kV, grid voltage at 92.5%, and guide wire at 0.15%; delay was 500 ns. The nitrogen laser power was set to the minimum level necessary to generate a reasonable signal (except in those experiments in which we attempted to degrade the tRNA). Generally, a two-point external calibration was performed, using the $[\text{M} + 3\text{H}]^{3+}$ (22,144 Da) and $[\text{M} + 2\text{H}]^{2+}$ (33,216 Da) peaks of bovine serum albumin (BSA; PE Biosystems, Foster City, California) in an α -CN matrix (saturated in 2:1 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$). For tRNA analyses, the matrix solution consisted of 42 mg 3-HPA, 2 mg PA, and 2 mg DAC dissolved in 500 μL 9:1 $\text{H}_2\text{O}:\text{CH}_3\text{CN}$. A 1.0- μL aliquot of tRNA was exchanged with ~ 2 μL ammonium-loaded cation-exchange beads for 10 min prior to loading and mixed with 2.0 μL matrix. Of the resulting solution, 0.5 μL were spotted on the MALDI sample target and allowed to dry at room temperature. The mass accuracy with external calibration using BSA is estimated to be about 0.1%, or 25 Da for tRNAs of this size. Internal calibrations were performed to eliminate the possibility that mass accuracy was affected by the difference in crystal heights between the α -CN matrix used for calibration and the 3-HPA matrix used with the tRNAs. Apomyoglobin (16,953 Da) or DNA 40 and 88mers (12,111 and 27,210 Da) were used as standards.

Gel electrophoresis

Four-microgram samples of various tRNA species were resolved on a 20% polyacrylamide (19:1 acrylamide:bis) gel in TBE (10 \times from BioRad, Hercules, California), 7 M urea, and 0.1 M NaOAc (solution also used to pour gel). The 1.6-mm thick gel was run for 48 h (1.25 times the amount of time required to run bromophenol blue dye off the gel) at 500 V and stained overnight with Stains-all (Sigma-Aldrich). The procedure was adapted from the acid/urea gel techniques used by Varshney et al. (1991).

ACKNOWLEDGMENTS

The authors thank Dr. John F. Leite for his valuable input. This work was supported by the National Institutes of Health (NS-34407 and NS-11756).

Received January 25, 2002; returned for revision February 1, 2002; revised manuscript received February 7, 2002

REFERENCES

- Bakhtiar R, Nelson RW. 2000. Electrospray ionization and matrix-assisted laser desorption ionization mass spectrometry—Emerging technologies in biomedical sciences. *Biochem Pharmacol* 59: 891–905.
- Bakhtiar R, Tse FLS. 2000. Biological mass spectrometry: A primer. *Mutagenesis* 15:415–430.
- Cornish VW, Mendel D, Schultz PG. 1995. Probing protein structure and function with an expanded genetic code. *Angew Chem Int Ed Engl* 34:621–633.
- Dougherty DA. 2000. Unnatural amino acids as probes of protein structure and function. *Curr Opin Chem Biol* 4:645–652.
- Ellman J, Mendel D, Anthony-Cahill S, Noren CJ, Schultz PG. 1991. Biosynthetic method for introducing unnatural amino-acids site-specifically into proteins. *Methods Enzymol* 202:301–336.
- Gruic-Sovulj I, Ludemann HC, Hillenkamp F, Weygand-Durasevic I, Kucan Z, Peter-Katalinic J. 1997. Detection of noncovalent tRNA-aminoacyl-tRNA synthetase complexes by matrix-assisted laser desorption/ionization mass spectrometry. *J Biol Chem* 272:32084–32091.
- Gruic-Sovulj I, Weygand-Durasevic I, Kucan Z. 2001. Influence of modified tRNA(Tyr) on the activation of tyrosine catalyzed by tyrosyl-tRNA synthetase from *Saccharomyces cerevisiae*. *Croatica Chemica Acta* 74:161–171.
- Helm M, Brule H, Giege R, Florentz C. 1999. More mistakes by T7 RNA polymerase at the 5' ends of in vitro-transcribed RNAs. *RNA* 5:618–621.
- Kao C, Zheng M, Rudisser S. 1999. A simple and efficient method to reduce nontemplated nucleotide addition at the 3' terminus of RNAs transcribed by T7 RNA polymerase. *RNA* 5:1268–1272.
- Kirpekar F, Douthwaite S, Roepstorff P. 2000. Mapping posttranscriptional modifications in 5S ribosomal RNA by MALDI mass spectrometry. *RNA* 6:296–306.
- Kirpekar F, Krogh TN. 2001. RNA fragmentation studied in a matrix-assisted laser desorption/ionization tandem quadrupole/orthogonal time-of-flight mass spectrometer. *Rapid Commun Mass Spectrom* 15:8–14.
- Kirpekar F, Nordhoff E, Kristiansen K, Roepstorff P, Lezius A, Hahner S, Karas M, Hillenkamp F. 1994. Matrix-assisted laser-desorption ionization mass-spectrometry of enzymatically synthesized RNA up to 150 kDa. *Nucleic Acids Res* 22:3866–3870.
- Knochenmuss R, Stortelder A, Breuker K, Zenobi R. 2000. Secondary ion-molecule reactions in matrix-assisted laser desorption/ionization. *J Mass Spectrom* 35:1237–1245.
- Kohrer C, Xie L, Kellerer S, Varshney U, Rajbhandary UL. 2001. Import of amber and ochre suppressor tRNAs into mammalian cells: A general approach to site-specific insertion of amino acid analogues into proteins. *Proc Natl Acad Sci USA* 98:14310–14315.
- Milligan JF, Groebe DR, Witherell GW, Uhlenbeck, OC. 1987. Oligoribonucleotide synthesis using T7 RNA-polymerase and synthetic DNA templates. *Nucleic Acids Res* 15:8783–8798.
- Nordhoff E, Cramer R, Karas M, Hillenkamp F, Kirpekar F, Kristiansen K, Roepstorff P. 1993. Ion stability of nucleic acids in infrared matrix-assisted laser-desorption ionization mass spectrometry. *Nucleic Acids Res* 21:3347–3357.
- Nowak MW, Gallivan JP, Silverman SK, Labarca CG, Dougherty DA, Lester HA. 1998. In vivo incorporation of unnatural amino acids into ion channels in *Xenopus oocyte* expression system. *Methods Enzymol* 293:504–529.
- Patterson KG, Rodicio LP, Limbach PA. 2001. Identification of the mass-silent post-transcriptionally modified nucleoside pseudouri-

- dine in RNA by matrix-assisted laser desorption/ionization mass spectrometry. *Nucleic Acids Res* 29:online.
- Pleiss JA, Derrick ML, Uhlenbeck OC. 1998. T7 RNA polymerase produces 5' end heterogeneity during in vitro transcription from certain templates. *RNA* 4:1313–1317.
- Rubelj I, Weygand-Durasevic I, Kucan Z. 1990. Evidence for 2 types of complexes formed by yeast tyrosyl-transfer RNA-synthetase with cognate and non-cognate transfer-RNA-effect of ribonucleoside triphosphates. *Eur J Biochem* 193:783–788.
- Saks ME, Sampson JR, Nowak MW, Kearney PC, Du F, Abelson JN, Lester HA, Dougherty DA. 1996. An engineered *Tetrahymena* tRNA^{Gln} for in vivo incorporation of unnatural amino acids into proteins by nonsense suppression. *J Biol Chem* 271:23169–23175.
- Silber R, Malathi VG, Hurwitz J. 1972. Purification and properties of bacteriophage T4-induced RNA ligase. *Proc Natl Acad Sci USA* 69:3009–3013.
- Sochacka E, Czerwinska G, Guenther R, Cain R, Agris PF, Mal-kiewicz A. 2000. Synthesis and properties of uniquely modified oligoribonucleotides: Yeast tRNA(Phe) fragments with 6-methyluridine and 5,6-dimethyluridine at site-specific positions. *Nucleosides Nucleotides Nucleic Acids* 19:515–531.
- Tolson DA, Nicholson NH. 1998. Sequencing RNA by a combination of exonuclease digestion and uridine specific chemical cleavage using MALDI-TOF. *Nucleic Acids Res* 26:446–451.
- Uhlenbeck OC. 1983. T4 RNA ligase. *Trends Biochem Sci* 8:94–96.
- Uhlenbeck OC, Gumport RI. 1982. T4 RNA Ligase. In: Boyer PD, ed. *The enzymes*. New York: Academic Press, Inc. pp 31–58.
- Varshney U, Lee CP, Rajbandary UL. 1991. Direct analysis of aminoacylation levels of transfer-RNAs in vivo application to studying recognition of *Escherichia coli* initiator transfer-RNA mutants by glutaminyl-transfer RNA-synthetase. *J Biol Chem* 266:24712–24718.
- Wei J, Lee CS. 1997. Polyacrylamide gel electrophoresis coupled with matrix-assisted laser desorption/ionization mass spectrometry for tRNA mutant analysis. *Anal Chem* 69:4899–4904.
- Weygand-Durasevic I, Lenhard D, Filipic S, Soll D. 1996. The C-terminal extension of yeast seryl-tRNA synthetase affects stability of the enzyme and its substrate affinity. *J Biol Chem* 271:2455–2461.
- Wolfson AD, Pleiss JA, Uhlenbeck OC. 1998. A new assay for tRNA aminoacylation kinetics. *RNA* 4:1019–1023.
- Zenobi R, Knochenmuss R. 1998. Ion formation in MALDI mass spectrometry. *Mass Spectrom Rev* 17:337–366.
- Zhang LK, Gross ML. 2000. Matrix-assisted laser desorption/ionization mass spectrometry methods for oligodeoxynucleotides: Improvements in matrix, detection limits, quantification, and sequencing. *J Am Soc Mass Spectrom* 11:854–865.