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Primary structure of an unusual glycine tRNA UGA suppressor†

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ABSTRACT

We have determined the nucleotide sequences of two UGA-suppressing glycine transfer RNAs. The suppressor tRNAs were previously shown to translate both UGA and UGG and to have arisen as a consequence of mutation in glyT, the gene for the GGA/G-reading glycine tRNA of Escherichia coli. In each mutant tRNA, the primary sequence change was the substitution of adenine for cytosine in the 3' position of the anticodon. In addition, a portion of the mutant glyT tRNA molecules contained N^6-(Δ^2-isopentenyl)-2-thiomethyl adenine adjacent to the 3' end of the anticodon (nucleotide 37). The presence or absence of this hypermodification may be a determinant in some of the biological properties of the mutant tRNA.

INTRODUCTION

The group of transfer RNAs that read codons related to the nonsense codons UAA and UAG by a single base change does not include any glycine isoacceptor. Nevertheless, a few years ago we were able to obtain ochre suppressor derivatives of $tRNA^{Gly}_3$, the GGU/C-reading glycine tRNA of Escherichia coli, through several mutational steps (1,2). One glycine codon, however, is related to a nonsense codon, UGA, by a single base change. It should be possible, therefore, to obtain UGA suppressors from a glycine tRNA.

In a recent report, we described the isolation and initial characterization of translational suppressors of a UGG missense mutation in E. coli (3). Some suppressors arose, spontaneously or after treatment with ethylmethanesulfonate, as a consequence of mutations in glyT, the gene for the GGA/G-reading glycine tRNA. Members of one class of glyT suppressor tRNA can translate the termination codon UGA as well as the tryptophan codon, UGG. In this respect, they are both nonsense and missense suppressors. They also represent the first UGA suppressors derived from glycine tRNA of any organism. In another study (4), members of this class were shown to survive in haploid cells, which have no other tRNA capable of translating

GGA. This implies that the suppressor tRNA retains some ability to read GGA and suggests the occurrence of wobble with respect to the first position of the codon. Finally, on a certain growth medium, this type of suppressor exhibits a "context effect", translating UGA at one position in trpA but not at another (5). In this paper we report the results of nucleotide sequence analysis of two of these unusual UGA/G-suppressing glycine tRNAs.

MATERIALS AND METHODS

Ribonuclease T2 was purchased from Sankyo Chemical Co. through Calbiochem, Inc. Phage T4 polynucleotide kinase was obtained from P-L Biochemicals, and PEI-cellulose thin layer plates from Brinkmann. [γ-^{32}P]ATP was prepared as described by Maxam and Gilbert (6) using carrier-free [^{32}P]orthophosphoric acid in water solution obtained from New England Nuclear Corporation. The bacterial strains examined were all E. coli K12. The isolation, genetic characterization, and RPC-5 column chromatographic analysis of the suppressors, designated SP-11 and EMS-9, were described previously (3-5). Each suppressor arose independently, SP-11 spontaneously and EMS-9 after treatment of the parental strain with ethylmethanesulfonate. Before further characterization, each was removed from the selective background by phage-mediated transduction. To obtain tRNA, cells were grown in 500 ml of glucose minimal medium (and any necessary supplements) and harvested in mid-log phase (absorbancy at 550 nm = 0.4). RNA was extracted from the cells with phenol solution (7). The upper phase was re-extracted once with phenol solution and twice with a phenol-chloroform mixture (1:1). The aqueous solution was passed through a DEAE-cellulose column and the tRNA eluted with NaCl and precipitated with ethanol (8). Each tRNA preparation (six A_{260} units) was fractionated in a two-dimensional polyacrylamide gel electrophoretic system similar to that of Garel et al. (9). The RNA pattern was visualized by staining with methylene blue (10). For nucleotide sequence analysis, each tRNA (nonradioactive) was eluted from an individual, stained "spot" in the two-dimensional gel. The procedure for RNA sequence analysis was the direct readout method of Gupta and Randerath (11), with minor modifications.

RESULTS

The nucleotide sequence of wild-type E. coli K12 glyT tRNA, determined by classical methods (12), has been confirmed (13) using the procedure of Gupta and Randerath (11). In the present study, mutational changes in tRNA

primary structure were determined by direct comparisons of the entire sequence of wild-type and mutant tRNAs. In this report, however, only the portion of the mutant tRNA that is different from the wild-type glyT tRNA will be shown and discussed. Other portions were found to be identical. The final step of the sequencing procedure was performed using both sulfate and formate chromatography systems (11).

Figure 1 presents the anticodon loop region of the glyT tRNA of EMS-9, one of the two UGA suppressors analyzed in this study (the results with SP-11 were the same and are not presented). The region shown contains the anticodon loop, nucleotides 32 to 38. The loop sequence of wild-type glyT tRNA is CUU*CCAA (anticodon underscored). U* represents an unidentified nucleo-

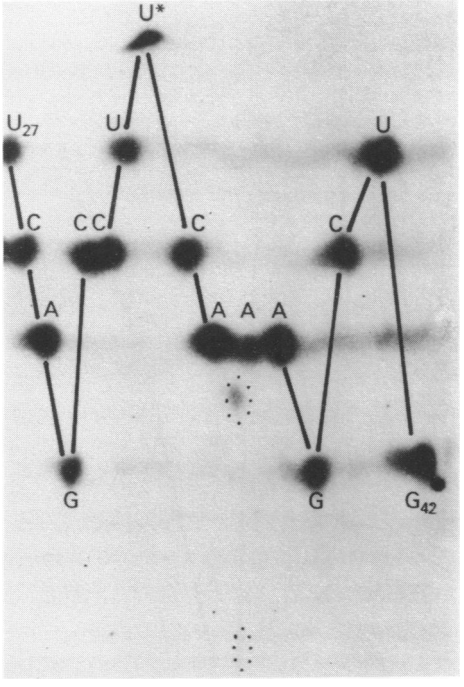

Figure 1. Analysis of the 5' terminus (11) of fragments from PEI-cellulose prints of gel ladders (not shown) obtained after hydrolysis, 5'-^{32}P-labeling with polynucleotide kinase and gel electrophoresis of EMS-9 glyT tRNA. After RNase T_2 treatment in situ, the released 5'-terminal nucleoside diphosphates were contact-transferred to a PEI-cellulose sheet for chromatographic analysis in 0.55 M ammonium sulfate. The sequence is displayed from 5' to 3', left to right. The dotted circles indicate spots that correspond to position 37 and show up more clearly on longer exposure.

side previously reported as a modified uridine (12). Figure 1 shows that the mutant tRNA is altered in two ways. First, adenine was substituted for cytosine in the 3' position of the anticodon. The second change involves the presence of multiple nucleotide species at position 37, adjacent to the 3' end of the anticodon. One species migrated in both chromatographic systems as an unmodified adenylate residue (pAp). In addition, its identity was confirmed in a two-dimensional chromatographic system (14). Two other species were evident (Fig. 1, dotted circles). The location of the more slowly migrating (lower) nucleotide, suggests that it is pms^{2}i^{6}Ap (11). The identity of the other, which migrates slightly more slowly than pAp, is unknown. As shown in Fig. 2, however, two nucleotides with similar mobilities are present in the sequence of E. coli cysteine tRNA, which is known to contain ms^{2}i^{6}A. Hence, it may be that the unknown nucleotide is a partially modified adeno-

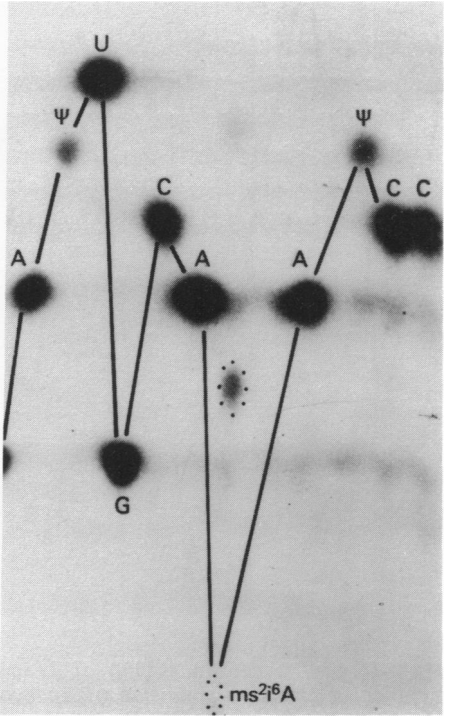

Figure 2. The anticodon loop region of E. coli cysteine tRNA obtained from the same two-dimensional gel as the UGA suppressor tRNA and analyzed in the sulfate chromatography system. Shown are nucleotides A31 to C41, which include the anticodon GCA.

sine diphosphate, for example pi^{6}Ap or perhaps ps^{2}i^{6}Ap (15). It is also possible, however, that the spot contains the dinucleotide pms^{2}i^{6}ApAp, perhaps due to incomplete hydrolysis of the phosphate bond between ms^{2}i^{6}A and the adjacent A. In contrast to the heterogeneity found at position 37 in the UGA suppressor tRNA, the A at that position in wild-type glyT tRNA is completely unmodified. This has been shown both by classical procedures of tRNA isolation and sequence analysis (12) and by more recent ones (13).

DISCUSSION

The mutant nucleotide sequence reported in this paper represents the first demonstration of an opal (UGA) suppressor as a mutationally altered glycine transfer RNA. Previous suppressors of UGA were shown to be derived from tRNAs for tryptophan (16,17), arginine (18), leucine (19-21), and serine (21,22), representing E. coli, phage T4 and the fission yeast Schizosaccharomyces pombe. The suppressor tRNA arose from a cytosine to adenine change that converted the anticodon of glyT tRNA from U*CC to U*CA. An interesting feature of the primary structure of this type of UGA suppressor is the nature of the nucleotide adjacent to the anticodon on the 3' side (Fig. 3). Wild-type E. coli tRNAs that read codons beginning with uridine have ms^{2}i^{6}A next to the anticodon at position 37. Furthermore, a UGU suppressor tRNA derived from glyW, one of the genes that code for the GGU/C-reading species of glycine tRNA, was shown to have arisen from a cytosine to adenine change in the 3' position of the anticodon and to have gained an ms^{2}i^6 modification

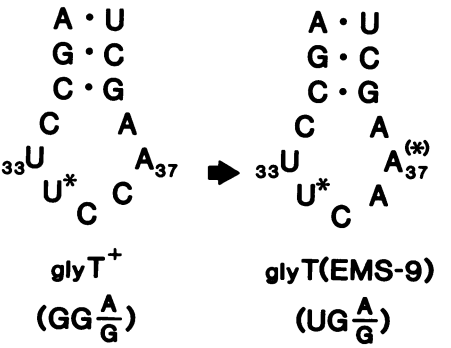

Figure 3. Comparison of the anticodon stem and loop region of wild-type glyT tRNA from E. coli with that of the glyT-derived UGA suppressor EMS-9. U* represents an unidentified modification of uridine. The parenthetical asterisk at A37 indicates that some of the suppressor tRNA molecules have ms^{2}i^{6}A at that position. See Fig. 1 and text for further details.

of the previously unmodified A37 (23). Nucleotide A37 of the glyT-derived UGA suppressor tRNA is partially modified. A portion of the molecules contains ms^2i^6A and a species that could be i^6A or s^2i^6A (15). Apparently the substitution of A for C in the 3' position of the anticodon is recognized by the enzymes responsible for the modifications in ms^2i^6A. But either the modifications are not complete, or the mutant tRNA, once modified, is subject to some extent to enzymatic demodification (24). Our finding of some unmodified A37 is not an artifact of the conditions of cell growth or of subsequent tRNA manipulations. First, for nucleotide sequence analysis, we obtained cysteine tRNA from the same two-dimensional gel as the glyT UGA suppressor tRNA. As is seen in Fig. 2, the cysteine tRNA has no unmodified adenine on the 3' side of the anticodon, but rather ms^2i^6A as expected (25) and a spot that appears to correspond with the unidentified spot found in the case of the glyT UGA suppressor tRNA. Second, we have found that tryptophan tRNA, obtained from similar gels, has no unmodified A at position 37 (data not shown).

An anticodon change was involved in the conversion of a phage T4 arginine tRNA to a UGA suppressor, and that suppressor does not have a modified A next to the anticodon (18). An unusual glyT UGG suppressor was found recently to employ the same anticodon (CCA) as tryptophan tRNA from wild-type E. coli but to have a completely unmodified A37 (13). These results suggest that not all tRNAs require ms^2i^6A for translation of codons beginning with U. The UGA-suppressing glycine tRNA sequenced in this study is the first E. coli UGA suppressor shown to be caused by an anticodon change and its A37 is partially modified. It will be of interest to determine whether in this case the ms^2i^6 modification is required for translation of UGA (or UGG).

Two biological properties were previously associated with the glyT UGA suppressor. The first is the display in certain genetic backgrounds of an unorthodox first position wobble (4). That is, besides having gained the new coding specificity (for UGA and UGG), the mutant tRNA retains some ability to read GGA (and GGG). The presence or absence of ms^2i^6A may play a role in this latitude in codon recognition. For nucleotide sequence analysis, the tRNAs of SP-11 and EMS-9 were obtained from cells of a genetic background in which that wobble does not occur, as was demonstrated in a previous study (4). Nucleotide A37 is partially modified. So it will be very interesting to determine the sequence of suppressor tRNA obtained from cells in which the apparent first-position wobble is permitted (such cells give rise to slowly-growing mucoid colonies on solid medium and to date we have

been unable to grow them in liquid medium). Although it is possible that in the permissive background the suppressor tRNA is completely unmodified at position 37, it might be found instead that the A is completely modified. There are data demonstrating that lack of a modification can restrict codon response (26), whereas presence of a modification can stabilize mismatched pairings (27). Alternatively, the background effects could be due to an unidentified mutation in some other kind of gene, such as one for a ribosomal protein or RNA.

The second finding, associated with SP-11 and EMS-9 in vivo, is the display, on a certain medium, of a "context effect", that is, suppression of UGA at one position in trpA but not at another (5). This effect may be the result of differences in mRNA nucleotides adjacent to each codon. For example, some data have implicated the nucleotide following the nonsense codon (28,29). Nevertheless, no generalization can be made for all locations of UGA codons (5). The involvement of tRNA modification in context effects has clearly been shown for several types of modification (21,26,28, 30-31). It is not known, however, whether the presence or absence of ms^2i^6A has anything to do with the context effect seen with the glyT-derived UGA suppressor tRNA. Furthermore, it is clear that the interaction of a tRNA with the ribosome must be considered (26,32).

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REFERENCES

1. Murgola, E.J. and Jones, C.I. (1978) Mol. Gen. Genet. 159, 178-184.
2. Murgola, E.J., Prather, N.E. and Hadley, K.H. (1978). J. Bacteriol. 134, 801-807.
3. Murgola, E.J. and Childress, J.R. (1980) J. Bacteriol. 143, 285-292.

4. Murgola, E.J. and Pagel, F.T. (1980) J. Mol. Biol. 138, 833-844.
5. Murgola, E.J. (1981) J. Mol. Biol. 149,1-13.
6. Maxam, A.M. and Gilbert, W. (1977) Proc. Nat. Acad. Sci. U.S.A. 74, 560-564.
7. Kirby, K.S. (1965) Biochem. J. 96, 266-269.
8. Hill, C.W., Combriato, G., Steinhart, W., Riddle, D.L. and Carbon, J. (1973) J. Biol. Chem. 248, 4252-4262.
9. Garel, J.P., Garber, R.L. and Siddiqui, M.A.Q. (1977) Biochemistry 16, 3618-3624.
10. Chia, L.S.Y., Randerath, K. and Randerath, E. (1973) Anal. Biochem. 55, 102-113.
11. Gupta, R.C. and Randerath, K. (1979) Nucleic Acids Res. 6, 3443-3458.
12. Roberts, J.W. and Carbon, J. (1975) J. Biol. Chem. 250, 5530-5541.
13. Prather, N.E., Murgola, E.J. and Mims, B.H. (1981) Proc. Nat. Acad. Sci. U.S.A,. Vol. 78, December.
14. Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) Methods in Enzymology LIX, 58-109.
15. Agris, P. F., Armstrong, D.J., Schafer, K.P. and Söll, D. (1975) Nucleic Acids Res. 2, 691-698.
16. Hirsh, D. (1971) J. Mol. Biol. 58, 439-458.
17. Soll, L. (1974) J. Mol. Biol. 86, 233-243.
18. Kao, S.-H. and McClain, W.H. (1977) J. Biol. Chem. 252, 8254-8257.
19. Foss, K., Kao, S.-H. and McClain, W.H. (1979) J. Mol. Biol. 135, 1013-1021.
20. Wetzel, R., Kohli, J., Altruda, F. and Söll, D. (1979) Mol. Gen. Genet. 172, 221-228.
21. Kohli, J., Altruda, F., Kwong, T., Rafalski, A., Wetzel, R., Söll D., Wahl, G. and Leupold, U. (1980) in Transfer RNA: Biological Aspects, Söll, D., Abelson, J.N. and Schimmel, P.R., Eds. pp. 407-419, Cold Spring Harbor Laboratory, New York.
22. Rafalski, A., Kohli, J., Agris, P. and Söll, D. (1979) Nucleic Acids Res. 6, 2683-2695.
23. Carbon, J. and Fleck, E.W. (1974) J. Mol. Biol. 85, 371-391.
24. McLennon, B.D. (1975) Biochem. Biophys. Res. Comm. 65, 343-351.
25. Mazzara, G.P. and McClain, W.H. (1977) J. Mol. Biol. 117, 1061-1079.
26. Colby, D.S., Schedl, P. and Guthrie, C. (1976) Cell 9, 449-463.
27. Weissenbach, J. and Grosjean, H. (1981) European J. Biochem. 116, 207-213.
28. Bossi, L. and Roth, J.R. (1980) Nature 286, 123-127.
29. Engelberg-Kulka, H. (1981) Nucleic Acids Res. 9, 983-991.
30. Laten, H., Gorman, J. and Bock, R. (1980) in Transfer RNA: Biological Aspects, Söll, D., Abelson, J.N. and Schimmel, P.R., Eds. pp. 395-406, Cold Spring Harbor Laboratory, New York.
31. Janner, F., Vögeli, G. and Fluri, R. (1980) J. Mol. Biol. 139, 207-219.
32. Grosjean, H., de Henau, S., Houssier, C. and Buckingham, R. (1980) Arch. Internat. de Physiologie et de Biochimie 88, 168-169.