Rapid Communication *J. Biochem.* 119, 832-834 (1996)

Detection of the Peptidyltransferase Activity of a Dipeptide, Alanylhistidine, in the Absence of Ribosomes

Mikio Shimizu

Institute of Space and Astronautical Science, Yoshinodai, Sagamihara, Kanagawa 229

Received for publication, December 18, 1995

It was shown that a dipeptide, alanylhistidine, can act as a catalyst for the peptidyl transfer reaction in the absence of ribosomes between the amino acid moieties of phenylalanyl, lysyl, prolyl, and glycyl tRNAs depending on their model templates, poly U, poly A, poly C, and poly G, respectively. A template effect was observed: The peptidyl transfer reaction between $tRNA^{Gly}$ molecules (anticodon GCC) occurred in the presence of poly G but not in the presence of poly C. The reaction was most efficient for the best stacked poly A ($tRNA^{Lys}$) and least efficient for the worst stacked poly U ($tRNA^{Phe}$).

Key words: dipeptide catalyst, peptidyl transfer, ribosomal protein L2, template effect, tRNA.

A protein synthetic system involves two major sequential processes: specific aminoacylation and the peptidyl transfer reaction. In the contemporary biosystem, specific aminoacylation is conducted with aminoacyl tRNA synthetase, which synthesizes the cognate aminoacyl adenylate at its active center and attaches the amino acid to the terminal adenosine of the cognate tRNA, by recognizing the identity determinants in the tRNA molecule with high precision. At the next step, the active center of the ribosome transfers the polypeptide moiety of the peptidyl tRNA at its P site to the amino acid moiety of the aminoacyl tRNA at its A site, thus elongating the polypeptide to form a protein eventually.

In a previous study, I found that an oligonucleotide (a hairpin RNA with three anticodon nucleotides at its 5′ end and a discriminator base, the 4th base from the 3′ end of tRNA, plus a CCA sequence at its 3′ end) can be specifically aminoacylated with its cognate aminoacyl adenylate in the presence of a dipeptide, valyl-aspartate. The dipeptide played the role of a catalyst interacting with the aminoacyl adenylate to transfer its aminoacyl group to the hydoxyl group of the terminal adenosine, as suggested by the structure of the active center of the contemporary aminoacyl tRNA synthetase (*1*). Consequently, this experimental result implies that an oligopeptide suggested by the structure of the contemporary enzyme can act as a specific catalyst similarly to the enzyme, although its activity is inevitably weak.

Nitta, Ueda, and Watanabe found that messenger RNA-directed oligo- and poly-peptide formation from a peptidyl tRNA and an aminoacyl tRNA on ribosomes was promoted by pyridine (*2*). In this reaction, chemical energy sources and a soluble protein fraction are not required. Pyridine, a tertiary amine, appears to accelerate the peptidyl transfer reaction by means of its catalytic character for this nucleophilic substitution reaction.

Judging from the series of evidence discussed above, it may be possible to find a dipeptide with peptidyl transferase activity. Furthermore, one of the proteineous amino acids, histidine, is a tertiary amine. This is a report that a dipeptide, alanylhistidine, can act as an efficient catalyst for a certain peptidyl transfer reaction in the absence of ribosomes.

I selected four aminoacyl tRNAs ($tRNA^{Phe}$, $tRNA^{Pro}$, $tRNA^{Lys}$, and $tRNA^{Gly}$) having the nucleotide sequences of the wild-type *Escherichia coli* tRNAs, because poly U, poly C, poly A, and poly G could be the corresponding mRNAs, respectively. The anticodons of the tRNAs are GAA, CGG, UUU, and GCC, respectively. The native tRNAs other than $tRNA^{Pro}$ were purchased from Subriden, ^{14}C-labeled amino acids from Amersham, and homopolynucleotides from Sigma. The $tRNA^{Pro}$ transcript (and also those of $tRNA^{Phe}$ and $tRNA^{Gly}$) was constructed by using the T7 RNA polymerase technique (*3*). Aminoacylation of these tRNAs with a ^{14}C-labeled amino acid was conducted with each aminoacyl tRNA synthetase contained in the *E. coli* S100 fraction. As mentioned above, I used dipeptides as possible catalysts, and 1 μl of a 1 M solution of each of them being mixed with 1 μl of an aminoacyl tRNA solution containing 0.05 A_{260} unit, 0.6 μl of 100 mM $MgCl_2$, and 1 μl of homopolynucleotides (100 mg/ml), at pH 7.3. The mixture was incubated at 37°C for 1 h. Then 0.6 μl of a 0.2 N potassium hydroxide solution was added, followed by further incubation at 37°C for 2 h to hydrolyze the aminoacyl tRNA. Then 0.3 μl aliquots were spotted onto a Merck HPTLC silica plate (10 × 10 cm) with a concentration zone, and the plate was developed with a mixture of butanol, water, and acetic acid (volume ratio, 4 : 1 : 1) (*2*). The radioactive spots on the TLC plate were detected with a FUJIX 2000 imaging analyzer to identify the oligopeptides formed.

The catalytic non-radiogenic peptides used in this experiment were purchased from Sigma and Bio-Synthesis. Many of them contained histidine (HA, HK, HD, AH, VH, KH, DH; I also tried H itself), but other kinds of peptides (AG, AV, VD, KD, KL, VK, LR, KK, MA, AM, and AC) were also employed to check their possible catalytic roles. Histidine had no catalytic activity, but alanylhistidine

alone of the above dipeptides was found to have significant peptidyl transferase activity, as described below. In this context, the catalytic activity is quite specific. The R_f values of Phe, Lys, Pro, and Gly, and their oligopeptides were measured separately, using non-labeled authentic molecules purchased from Ajinomoto, Sigma, and Biosynthesis, after staining with ninhydrin. Oligomers of phenylalanine moved faster than its monomer on TLC, but oligomers of lysine, proline, and glycine moved slower than their monomers.

Figure 1a illustrates the dimerization of phenylalanine during the peptidyl transfer reaction. Lanes F1 and F2 show authentic phenylalanine and its dipeptide, which were visualized on the imaging plate by adding ^{14}C-labeled phenylalanine to the spots of the developed phenylalanine and its dipeptide stained with ninhydrin. Lanes F3 and F4 show the results of experiments without poly U and AH, respectively, while Lane F5 shows that with both poly U and AH. The spot due to the formation of phenylalanylphenylalanine was only observed in the presence of both the poly U template and the catalyst, AH. Lane F6 for phenylalanine demonstrates the importance of the sequence of the dipeptide. The exchange of alanine and histidine in the sequence severely affected the peptidyl transfer efficiency.

Figure 1b illustrates the oligomerization patterns of the three other amino acids. The left lane for each amino acid (P1, K1, and G1) shows the spot formed by each radioactive amino acid derived from aminoacyl tRNAs through hydrolysis after incubation with templates but without AH. The spots in the lanes for P2 and G2 show dipeptides resulting from oligomerization of the amino acids due to the peptidyl transfer reaction on the templates with AH. In Lane K2, the tripeptide spot can be seen overlapping the spot of the dipeptide. Again the polymerization of amino acids occurred only in the presence of both the template and catalytic dipeptide, AH. Lane G3 clearly shows that the reaction is template-dependent, because in this case the template was replaced by poly C.

In the case of phenylalanine, the dimer spot appears above the monomer spot, but in the other cases the dimer spots come under the monomer spots, as already mentioned. It should be remarked that the monomer spots disappear in the cases of $tRNA^{Lys}$ and $tRNA^{Gly}$ (100% conversion), and it almost disappears in the case of $tRNA^{Pro}$ (97% conversion). These differences may be interpreted in terms of the template effect: Stacking among the nucleic acid bases (*4–6*) is the strongest in poly A and the weakest in poly U. Consequently, $tRNA^{Lys}$ is geometrically well aligned on poly A and peptidyl transfer among $tRNA^{Lys}$ occurs easily to form oligolysines, while the direction of $tRNA^{Phe}$ on poly U is random and some of the $tRNA^{Phe}$ molecules will miss the chance to form dipeptides. The $tRNA^{Gly}$ and $tRNA^{Pro}$ cases are intermediate between those of the above tRNAs. Transcripts of $tRNA^{Phe}$ and $tRNA^{Gly}$ gave the same results as the intact tRNAs (data not shown). This indicates that a base modification in a tRNA does not play a role in the peptidyl transfer reaction.

It was reported that peptidyl transfer from a small RNA fragment carrying methionine, which had been derived from the 3′-terminal region of methionyl-tRNA, to puromycin was observed in the presence of protein-depleted ribosomes (*7*). However, the peptidyl transfer reaction between phenylalanyl tRNAs on the model template, poly U, on protein-depleted ribosomes was not detected in the thin layer chromatography experiment of Nitta, Ueda, and Watanabe (*2*). The presence of pyridine, a tertiary amine, was necessary to observe phenylalanine oligomers.

A ribosomal protein, L2, was reported to be important

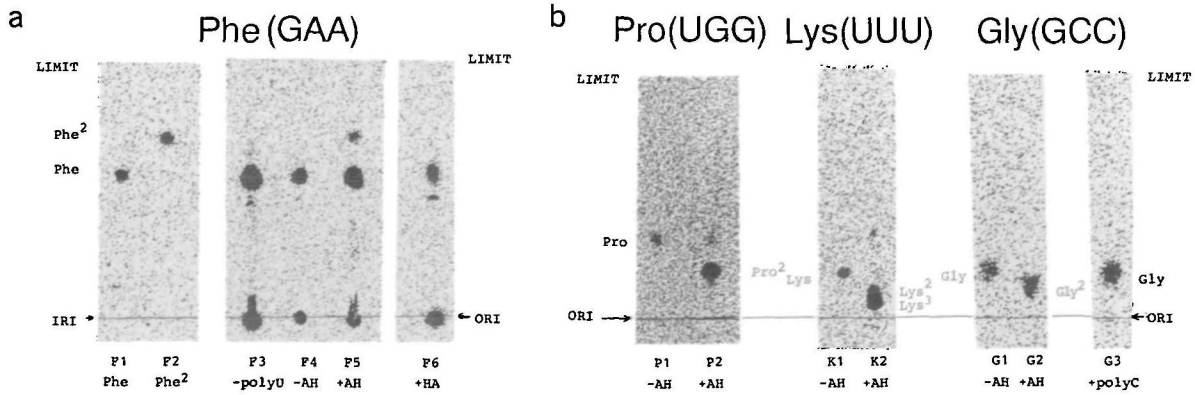

Fig. 1. **HPTLC diagrams obtained in the template-directed peptidyl transfer experiment.** Lanes F1-F6 are for phenylalanine, P1-P2 for proline, K1-K2 for lysine, and G1-G3 for glycine. The arrows with ORI indicate the locations of the concentration zones for HPTLC, namely the origins of elution. The uppermost end of each lane marked LIMIT corresponds to the limit of elution. The R_f values for F, F^2/P, P^2/K, K^2, K^3/G, and G^2 are 0.52, 0.69/0.34, 0.19/0.16, 0.10, 0.05/0.18, and 0.11, respectively. (a) Lanes F1 and F2 show the positions of the spots for phenylalanine and its dimer (see text), while Lanes F3, F4, and F5 illustrate the results of the peptidyl transfer experiments without the template, poly U, without the catalyst, AH, and with both poly U and AH, respectively. In Lane F6, in which histidinylalanine was used instead of alanylhistidine, no formation of phenylalanylphenylalanine was observed. At the origin of elution for F3-F6, spots due to the ^{14}C-labeled phenylalanyl-tRNA can be seen. Stacking of the side chain of phenylalanine on the RNA moiety appears to interfere with alkali hydrolysis leaving undissociated phenylalanyl-tRNA. The percentages of the radioactivity in the spots of the origin, monomer and dimer in Lane F5 are 25, 64, and 11, respectively. (b) In the cases of the three other amino acids, the spot in each number 1 lane is due to the ^{14}C-labeled amino acid monomer separated from its aminoacyl-tRNAs due to alkali hydrolysis after the peptidyl transfer experiment without AH. The spot in each number 2 lane is due to the amino acid dimer or trimer formed during the peptidyl transfer reaction joining the amino acid moieties of each aminoacyl-tRNA. No formation of glycylglycine was seen in Lane G3, for which the template was changed from poly G to poly C.

for the peptidyl transfer reaction (*8*). Four histidines, which are tertiary amines, are found in L2, and the amino acid was suggested by a chemical modification experiment to play an important role in the peptidyl transfer activity (*9*). Consequently, my finding above may imply that the peptidyl transfer between the peptidyl tRNA at the P site and the aminoacyl tRNA at the A site on a ribosome would be related to the presence of a histidine residue in L2, and that the ribosome may simply be providing a space suitable for the peptidyl transfer processes. However, a real understanding of the complex ribosomal process remains for the future.

The author expresses his sincere thanks to Prof. K. Watanabe and Dr. T. Ueda of the University of Tokyo for their valuable comments regarding this work. He also thanks to Prof. N. Okada of the Tokyo Institute of Technology for providing the opportunity to use their facilities for isotope experiments.

REFERENCES

1. Shimizu, M. (1995) Specific aminoacylation of C4N hairpin RNAs with cognate aminoacyl-adenylates in the presence of a dipeptide: Origin of the genetic code. *J. Biochem.* **117**, 23-26
2. Nitta, Z., Ueda, T., and Watanabe, K. (1994) Template-dependent peptide formation on ribosomes catalyzed by pyridine. *J. Biochem.* **115**, 803-807
3. Sampson, J.R. and Uhlenkeck, O.C. (1988) Biochemical and physical characterization of an unmodified yeast phenylalanine transfer RNA transcribed in vitro. *Proc. Natl. Acad. Sci. USA* **85**, 1033-1037
4. Simpkins, H. and Richards, E.G. (1967) Preparation and properties of oligouridylic acids. *J. Mol. Biol.* **29**, 349-356
5. Brahms, J., Michelson, A.M., and Van Holde, F. (1966) Adenylate oligomers in single and double strand conformations. *J. Mol. Biol.* **15**, 467-488
6. Brahms, J., Maurizot, J.C., and Michelson, A.M. (1967) Conformation and thermodynamic properties of oligocytidylic acids. *J. Mol. Biol.* **25**, 465-480
7. Noller, H.F., Hoffarth, V., and Zimniak, L. (1992) Unusual resistance of peptidyl transferase to the protein extraction procedure. *Science* **256**, 1416-1419
8. Nierhaus, K.H. (1979) Analysis of the assembly and function of the 50S subunit from *E. coli* ribosomes by reconstruction in *Ribosomes: Structure, Function, and Genetics* (Chambliss, G., Craven, G.R., Davies, J., Davies, K., Kahan, L., and Nomura, M., eds.) pp. 267-294, Univ. Park Press, Baltimore
9) Kimura, M., Kimura, J., and Watanabe, K. (1985) The primary structure of ribosomal protein L2 from *B. stearothermophilus*. *Eur. J. Biochem.* **153**, 289-297