Note

Kinetics of Hyperprocessing Reaction of Human Tyrosine tRNA by Ribonuclease P Ribozyme from *Escherichia coli*

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Human tyrosine tRNA and fly alanine, histidine, and initiator methionine tRNAs are generally cleavable internally by bacterial ribonuclease P ribozyme. The unusual internal cleavage reaction of tRNA, called hyperprocessing, occurs when the cloverleaf structure of the tRNA molecule is denatured to form a double-hairpin-like structure. The hyperprocessing reaction of these tRNAs requires magnesium ions. We analyzed details of this reaction using human tyrosine tRNA and *Escherichia coli* RNase P ribozyme. The usual processing reaction occurred efficiently with magnesium at 5 mM, but for the hyperprocessing reaction, higher concentrations were needed. With such high concentrations, hyperprocessing cleaved both mature tRNA and tRNA precursor as substrates. When mature tRNA was the substrate, the apparent *K*₅₀ was almost the same as in the usual reaction, but *kₐ₉* was smaller. These results indicated that the occurrence of hyperprocessing depends on the magnesium ion concentration, and suggested that magnesium ions contribute to the recognition of the shape of the substrate by bacterial RNase P enzymes.

Key words: *Escherichia coli*; tRNA; RNase P; hyperprocessing; tyrosine

The tRNA is found in all cellular organisms and is important in protein synthesis. Most tRNA molecules have a cloverleaf structure with four major arms (acceptor-stem, D-stem/loop, anticodon-stem/loop, and T-stem/loop) and an extra variable loop (Fig. 1A, top left).¹,² For a long time, the cloverleaf structure was thought to be stable and rigid. Recently, however, certain tRNAs have been found to be disrupted, some without base modification and some under in vitro conditions.³⁻⁴ In other words, not all tRNAs are rigid, and their cloverleaf shape is not always stable. The study of such destabilized or unstable tRNAs should give clues about the ways in which tRNA molecules are stabilized and also about the origin of tRNA molecules.

Figure 1 (B and C) shows human tyrosine tRNA as an example of unstable tRNAs. This molecule has an alternative double-hairpin structure: the acceptor- and anticodon-stems in the cloverleaf disappear and the D- and T-stems are extended, retaining the D- and T-stems/loops as core hairpins. The same shape change has been found with *Drosophila* alanine, histidine, and initiator methionine tRNAs.³⁻¹⁰ The change from a cloverleaf to a double-hairpin can be monitored by the reaction of ribonuclease P from *Escherichia coli*.³⁻¹⁰

Ribonuclease P also has been found in all species examined. The enzyme catalyzes the 5'-processing reaction of tRNA precursors to produce a mature 5'-end in tRNA molecules.¹,² Bacterial RNase P RNA is a ribozyme that can catalyze the RNase P reaction without the protein component in vitro.¹,² The enzymes from *E. coli* and *Bacillus subtilis* in particular have been studied.³⁻²⁵ In the bacterial RNase P reaction, hairpin RNA with a CCA-3' tag sequence can be a substrate as well as tRNA precursors, and is cleaved by the enzyme in the same way. If the 3'-half of a tRNA molecule forms hairpin, it can be a substrate yielding internally cleaved fragments of tRNA.⁶⁻¹¹ Using the RNase P reaction, we can detect the changes in shape (usually to double-hairpin folds) of destabilized tRNA molecules.

Recently, we found the hyperprocessing reaction with human tyrosine tRNA as well as fly tRNAs,⁵ but the kinetic mechanism of the is not yet known. In this study, we analyzed the magnesium ion dependence of the reaction and the response of the reactions.

Figure 1C shows the products of the RNase P reaction of human tyrosine pre-tRNA by the *E. coli* ribozyme in the presence of 5 and 60 mM magnesium ions.⁹ The *E. coli* ribozyme requires magnesium ion for the cleavage reactions. With 5 mM magnesium, only the usual processing reaction was observed. With 60 mM magnesium, two hyperprocessed products were observed: product1 was from the cleavage at the A³⁸-U³⁹ bond, and product2 was from the cleavage at the U³⁹-C⁶⁰ bond.

Figure 2A shows the magnesium ion dependence of the RNase P reaction. The tRNA precursor was the
substance. Mature tRNA and two hyperprocessed products were observed when the magnesium ion concentration was 10 mM or more, when the concentration was less than 10 mM, no hyperprocessed products were detected. These results indicated that for the hyperprocessing reaction, magnesium ions at a concentration of more than 10 mM were needed. The magnesium ions are said to raise the affinity be-

![Fig. 1](image)

**Fig. 1. Hyperprocessing Reaction of Human Tyrosine tRNA by E. coli RNase P.**

(A) Schematic representation of substrate recognition by bacterial RNase P. The enzyme recognizes the tRNA precursor in the cloverleaf structure (left) and the hairpin RNA with the CCA-3' tag sequence (right) as its substrate. The scissors represents the cleavage site in the RNA. (B,C) Hyperprocessing of human tyrosine tRNA in vitro. The regions U18G6 and U18,A1' contribute to the change in the formation of the tRNA. The hyperprocessing of this tRNA by E. coli RNase P occurred at the A18,U19 bond (corresponding to product1) and at the U18-C36 bond (product2). Photograph is from earlier reference. Numbering system for bases, Sprinzl et al.

![Fig. 2](image)

**Fig. 2. RNase P Reaction of Human Tyrosine tRNA.**

Open circles, pre-tRNA remaining; closed circles, mature tRNA; open triangles, the product1 RNA; and closed triangles, the product2 RNA. (A) Dependence on the magnesium ion concentration. The tRNA precursor the substrate. The reaction was at pH 7.6, 37°C, for 10 min (58 μM pre-tRNA, 0.39 μM E. coli RNase P RNA, 100 mM NH₄Cl, 5%(w/v) polyethylene glycol 6000, and 50 mM Tris-HCl; in 10-μl scale). (B) Dependence on time. The tRNA precursor was used as a substrate. Reactions were done at pH 7.6, 37°C (58 μM pre-tRNA, 0.78 μM E. coli RNase P RNA, 60 mM MgCl₂, 100 mM NH₄Cl, 5%(w/v) polyethylene glycol 6000, and 50 mM Tris-HCl; in 10-μl scale). (C) Reaction dependence of the hyperprocessed products. Purified mature tRNA was the substrate. Reactions were done at pH 7.6, 37°C, for 10 min (3.13 μM mature tRNA, 4.85 μM E. coli RNase P RNA, 60 mM MgCl₂, 100 mM NH₄Cl, 5%(w/v) polyethylene glycol 6000, and 50 mM Tris-HCl; in 12.5-μl scale). The reaction was stopped by the addition of 4 μl of 0.5 M EDTA (pH 8.0), and development was on 10% PAGE. Amounts of products were measured from the radioactivity as described elsewhere.

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![Diagram of RNase P Reaction](image)

**Fig. 3.** Schematic Representation of RNase P Reaction. See text.

between the substrate and the enzyme, but our results that a high concentration of magnesium ions affected the magnitude of the reaction.

Figure 2 shows the changes in the reaction with time with about 60 μM pre-tRNA and about 3 nM mature tRNA as the substrate (B and C), respectively. The reaction that produced mature tRNA and product2 was more rapid than that that produced product1. Product2 alone can be produced from mature tRNA under the conditions used in Fig. 2C if the RNase P reaction is stopped within 20 min. The reaction to produce product2 from mature tRNA is catalyzed by standard Michaelis-Menten equations (with 4.85 mM *E. coli* RNase P RNA, 1.5-100 nM mature tRNA, 60 mM MgCl2, 100 mM NH4Cl, 5% polyethylene glycol 6000, and 50 mM Tris-HCl, pH 7.8 at 37°C; the reaction was for 10 min and then the reaction mixture was incubated for 90 min at 37°C), and the kinetic parameters were a *kcat* of 0.037 min⁻¹ and *Km* of 63 nM. The kinetic values of the RNase P reactions reported by others are a *kcat* of 0.2 to 20 min⁻¹ and a *Km* from 30 to 340 nM. Comparison of the values suggests that the hyperprocessing of mature tRNA occurs as efficiently as the usual processing reaction of tRNA precursors.

Figure 3 is a schematic representation of the RNase P reaction of human tyrosine pre-tRNA. The pre-tRNA is cleaved by RNase P to mature tRNA. Some of the mature tRNA fits into the hairpin fold and is hyperprocessed. Some of the pre-tRNA may also fit into the hairpin fold and will be hyperprocessed as we found earlier by guide DNA technique. In hyperprocessing, pre-tRNA produces more efficiently than mature tRNA. Either mature tRNA or pre-tRNA in the hairpin fold is hyperprocessed to produce both product1 and 2 RNAs. The RNA products in the RNase P reaction depend on the magnesium ion concentration. The reaction that produces product2 from mature tRNA is as efficient as the reaction that produces mature tRNA from pre-tRNA. The reaction that produces the product1 from mature tRNA is slower than the other reactions, perhaps partly because the affinity to the RNA substrate may be lower, and partly because the long pre-steady-state may cause slow release of the product. Several conformers of RNAs may coexist in the reaction mixture, possibly giving various products such as product1 and product2.

Why does hyperprocessing of tRNA occur? The catalytic center of RNase P requires magnesium, and the enzyme has several magnesium binding sites. Magnesium affects the affinity of the tRNA and the RNase P, and also affects the folding of RNase P. According to Fang *et al.*, the dissociation constant of the *B. subtilis* ribozyme for magnesium ions at 37°C is 6.5 mM, which suggests that the substrate...
selectivity of the E. coli ribozyme may also switch at about the same magnesium concentration as for the B. subtilis enzyme. The results suggest that the effects of magnesium on tRNA and RNase P interactions are important. A high magnesium ion concentration may give the bacterial RNase P the unusual ability to accept the hairpin-folded tRNA-derived substrate that leads to the hyperprocessing reaction. Excess magnesium ions with RNase P would harm the tRNA molecules, which might explain the role of the protein component of the enzyme.

The occurrence of the hyperprocessing reaction of tRNA is, of course, the unusual and unexpected event. Exploration of the hyperprocessing reaction may give clues about how tRNA is stabilized and how its the origin shape originated.

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