Crystal Structure of the tRNA 3’ Processing Endoribonuclease tRNase Z from Thermotoga maritima*

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The maturation of the tRNA 3’ end is catalyzed by a tRNA 3’ processing endoribonuclease named tRNase Z (tRNase Z or 3’-tRNase) in eukaryotes, Archaea, and some bacteria. The tRNase Z generally cuts the 3’ extra sequence from the precursor tRNA after the discriminator nucleotide. In contrast, Thermotoga maritima tRNase Z cleaves the precursor tRNA precisely after the CCA sequence. In this study, we determined the crystal structure of T. maritima tRNase Z at 2.6-Å resolution. The tRNase Z has a four-layer αβ/βα sandwich fold, which is classified as a metallo-β-lactamase fold, and forms a dimer. The active site is located at one edge of the β-sandwich and is composed of conserved motifs.

Based on the structure, we constructed a docking model with the tRNAs that suggests how tRNase Z may recognize the substrate tRNAs.

All tRNA molecules are transcribed as long precursors with extra sequences at their 5’ and 3’ termini. Hence the precursor tRNAs (pre-tRNAs) undergo several processing steps, including the removal of the 5’ and 3’ extra sequences, to generate mature tRNAs (1–4).

Several ribonucleases are involved in the 5’ and 3’ processing. The removal of the 5’ extra sequence is accomplished by RNase P, a ribonuclease protein, in all three kingdoms.

On the other hand, the mechanisms of the 3’ extra sequence removal differ among the three kingdoms. In eukaryotes, the tRNA genes do not encode the 3’-terminal CCA sequence, which is essential for aminocacylation. The eukaryotic 3’ processing is done mainly by a tRNA 3’ processing endoribonuclease named tRNase Z (EC 3.1.26.11; RNase Z or 3’-tRNase), which cuts the pre-tRNAs with the 3’ extra sequence after the discriminator base (5–11). Then the CCA sequence is added by tRNA nucleotidyltransferase (12). Some as yet unidentified exoribonucleases may remove the 3’ extra sequence in some circumstances (7, 13). In Archaea, the 3’ terminus is processed by tRNase Z (9, 14, 15).

In bacteria, the 3’ processing pathways are more diverse. In Escherichia coli, all tRNA genes encode the CCA sequence, and the removal of the 3’ extra sequence is catalyzed by a combination of exo- and endoribonucleases (2, 16). The endonucleolytic cleavage downstream of the 3’-CCA sequence is catalyzed mainly by RNase E. Then the exonucleolytic processing of the 3’ extra sequence is catalyzed by a combination of RNases T, PH, D, BN, and II and polynucleotide phosphorylase (16) following the removal of the 5’ extra sequence. On the other hand, in Bacillus subtilis, some tRNA genes encode the CCA sequence, and others do not. tRNase Z processes only the pre-tRNAs without the CCA sequence, while an unidentified tRNase Z-independent tRNA processing pathway may exist for tRNAs with the CCA sequence (17). In Thermotoga maritima, the 3’-CCA sequence exists in almost all of the tRNA genes with only one exception (18). The T. maritima tRNase Z can correctly process the pre-tRNAs with the CCA sequence; it cleaves the pre-tRNAs precisely after the CCA sequence, not after the discriminator base (15), unlike the common tRNase Zs.

tRNase Zs belong to the ELAC1/ELAC2 family within the metallo-β-lactamase superfamily (9, 19–21). The ELAC1 proteins are the short forms of tRNase Z (tRNase ZS) consisting of 300–400 amino acids and are widely distributed among the three kingdoms (9, 22). On the other hand, the ELAC2 proteins are the long forms of tRNase Z (tRNase ZL) consisting of 800–900 amino acids and are found only in eukaryotes (9, 19, 22). tRNase ZS and the C-terminal half of tRNase ZL are homologous to each other. Both of them have the metal binding motifs conserved in the metallo-β-lactamase superfamily, although their overall sequence similarities to metallo-β-lactamases are very low. In addition, the N-terminal half of tRNase ZL shares a weak sequence homology to the C-terminal half of tRNase ZL and tRNase ZS, although it lacks the metal binding motifs (19–21). Hence it has been suggested that the tRNase ZL gene was produced by the duplication of the tRNase ZS gene.

In the present study, we determined the crystal structure of T. maritima tRNase Z at 2.6-Å resolution. Based on the structure, we constructed a local docking model that provides new insight into how tRNase Z recognizes the pre-tRNA structure.
**Crystal Structure of tRNase Z**

**Table I**

Summary of data collection and refinement statistics

|                      | SeMet | Edge | Remote | Native |
|----------------------|-------|------|--------|--------|
| **Data Collection**  |       |      |        |        |
| Wavelength (Å)       | 0.979 | 0.974| 0.9841 | 1.0000 |
| Resolution (Å)       | 50–3.0| 50–3.0| 50–3.0 | 50–2.6 |
| Unique reflections   | 15,769| 15,733| 15,823 | 23,807 |
| Redundancy           | 8.7   | 6.0  | 6.0    | 5.7    |
| Completeness, all data (last shell) (%) | 99.8 (99.6) | 99.5 (99.0) | 99.4 (99.2) | 98.8 (97.8) |
| I0(I), all data (last shell) (%) | 27.4 (4.0) | 23.2 (3.3) | 22.5 (3.1) | 28.3 (2.9) |
| R<sub>sym</sub>, all data (last shell) (%) | 8.4 (29.7) | 6.8 (29.3) | 6.8 (30.1) | 5.3 (31.2) |
| Refinement           |       |      |        |        |
| R<sub>work</sub> (%) |       |      |        | 22.6   |
| R<sub>free</sub> (%) |       |      | 27.9   |        |
| r.m.s.d. bond length (Å) |       |      | 0.008  |        |
| r.m.s.d. bond angles (°) | 1.40  |      |        |        |

<sup>a</sup> R<sub>sym</sub> = Σ|I<sub>hkl</sub> - (I<sub>hkl</sub>)|/ΣI<sub>hkl</sub>, where (I<sub>hkl</sub>) is the mean value of I<sub>hkl</sub>.  
<sup>b</sup> The free R factor was calculated using 5% of reflections omitted from the refinement.

**FIG. 1.** Ribbon diagram displaying the overall structure of *T. maritima* tRNase Z (stereoview). A, the *T. maritima* tRNase Z subunit structure. The α-helices, β-strands, and ζ helices are colored yellow, cyan, and orange, respectively. The conserved motifs of the metallo-β-lactamase superfamily are represented by ball-and-stick models and are colored red. The disordered regions are represented by dashed lines. B, the *T. maritima* tRNase Z dimer structure. The two subunits are colored pink and green. The non-crystallographic symmetry 2-fold axis is perpendicular to the paper. All of the graphic figures in the present study were drawn with CueMol (cuemol.sourceforge.jp/en/).

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The double-stranded DNA encoding the *T. maritima* tRNase Z gene was amplified by PCR from the plasmid pQE7/TnW(T), which produces a histidine-tagged tRNase Z protein (15), to remove the tag-encoding sequence and was cloned between the Ndel and SalI sites of the expression vector pET26b(+) (Novagen). The *T. maritima* tRNase Z protein was overexpressed in *E. coli* strain BL21(DE3). Cells were grown in LB medium containing 50 μg/ml kanamycin at 37 °C until the A<sub>600</sub> was ~0.5, and then isopropyl β-D-thiogalactopyranoside was added to a 1 mM final concentration. Cells were grown at 37 °C for an additional 3 h and were harvested by centrifugation. The pellet was resuspended in 50 mM sodium phosphate buffer (pH 7.0) containing 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 10 mM β-mercaptoethanol, DNase I, and a protease inhibitor mixture (Complete EDTA-free, Roche Applied Science) and was homogenized by sonication. The extract was heated for 30 min at 80 °C to denature most of the *E. coli* proteins. The heat-treated fraction was centrifuged, and the supernatant fraction was loaded onto an SP-Sepharose Fast Flow column (Amersham Biosciences) equilibrated with 50 mM sodium phosphate buffer (pH 7.0) containing 50 mM NaCl and 10 mM β-mercaptoethanol. The protein was eluted with a linear gradient of 0.05–1.0 M NaCl. The protein eluate was diluted with an equal volume of 20 mM HEPES buffer (pH 7.0) containing 10 mM β-mercaptoethanol, and the resultant solution was then loaded onto a HiTrap heparin column (Amersham Biosciences) equilibrated with 20 mM HEPES buffer (pH 7.0) containing 500 mM NaCl and 10 mM β-mercaptoethanol. The protein was eluted with a linear gradient of 0.5–1.5 M NaCl and was purified to more than 95% homogeneity with a yield of about 1.5 mg of purified protein from 1 liter of LB medium cell culture as judged by SDS-PAGE.

**Crystallization of tRNase Z**—Prior to crystallization, the protein fraction was dialyzed against 20 mM HEPES buffer (pH 7.0) containing 400 mM NaCl and 10 mM β-mercaptoethanol and was concentrated to ~3.5 mg/ml using a Vivaspin2 filter (Vivasience). The atomic absorption experiment revealed the presence of zinc ions in the *T. maritima* tRNase Z solution, although no zinc had been added. An initial screen for crystallization was accomplished by the hanging drop vapor diffusion method at 20 °C with Crystal Screen I, Crystal Screen II, and Natrix (Hampton Research). The volume of the reservoir solutions was 500 μl (400 μl of the screening kit reagent mixed with 40 μl of 5 M NaCl), and the drop was prepared by mixing equal volumes (1 μl each) of the protein solution and the screening kit reagent. The crystals were obtained within 3 days in drops containing 0.1 M Tris-Cl buffer (pH 8.5)
FIG. 2. Sequence alignment of tRNase Zs and the C-terminal halves of tRNase ZLs. The amino acid sequence of *T. maritima* tRNase Z is aligned with those of *B. subtilis* tRNase Z, *E. coli* tRNase Z, *Methanococcus jannaschii* tRNase Z, *Pyrobaculum aerophilum* tRNase Z, *Thermoplasma acidophilum* tRNase Z, *Arabidopsis thaliana* tRNase ZS (A.tha_tRNaseZS), the C-terminal domain of *A. thaliana* tRNase ZL (A.tha_tRNaseZL_C), human tRNase ZS (H.sap_tRNaseZS), and human tRNase ZL (H.sap_tRNaseZL_C). The secondary structures for α-helices, β-strands, and 3_10-helices are denoted by the Greek characters, α, β, and η, respectively, and are colored as in Fig. 1. The red box shows the fully conserved residues, and the blue rectangle shows the moderately conserved residues throughout the tRNase Zs. The green triangles indicate the characteristic motifs of the metallo-β-lactamase superfamily. The blue triangles indicate the amino acid residues involved in the dimer formation. These amino acid sequences were aligned using the program ClustalW (40), and the figure was prepared by ESPript (41).
with 1.25 M ammonium phosphate, and thin, large crystals grew to dimensions of 0.7 × 0.5 × 0.05 mm² within a week. The selenomethionine (SeMet) derivative was prepared by overexpression of tRNase Z in the E. coli strain BS44(DE3) and was purified and crystallized by the same procedures used for the native protein.

Data Collection and Structure Determination—The data set of the native protein and the multilengthwidth anomalous dispersion data set of the SeMet derivative protein were collected from BL41XU and BL26B1 at SPring-8 (Harima, Japan), respectively. Data processing and scaling were performed with HKL2000 (23). The crystals belong to the orthorhombic space group P2₁2₁2₁ with unit cell parameters a = 111.9 Å, b = 73.6 Å, and c = 92.0 Å for the native protein and a = 115.0 Å, b = 72.5 Å, and c = 90.1 Å for the SeMet derivative protein. Ten selenium sites were picked of the 12 selenium sites expected in the asymmetric unit using SnB (24). The selenium parameters were refined, and the initial phases were calculated using SOLVE at 3-Å resolution (25). The resulting initial phases were extended to 2.7 Å by density modification with the program RESOLVE (25). The atomic model was manually built using O (26) and was refined using CNS (27). Phasing of the native crystal data set was carried out by molecular replacement using MOLREP (28) with the 2.7-Å SeMet derivative models as a search model. The model of the native crystal was built and refined in a similar manner as that of the SeMet derivative structure. There are two molecules (A and B) in the asymmetric unit. One flexible region and two short loop regions (amino acid residues 152–174 of molecule A, and amino acid residues 133–138 and 211–222 of molecule B) were not visible due to disorder and were excluded from the model. The crystal structure was refined to the R and R_free factors of 22.6 and 27.9%, respectively, within the resolution range of 50–2.6 Å. The data collection, phasing, and refinement statistics are shown in Table I.

RESULTS AND DISCUSSION

Overall Structure—The crystal structure of T. maritima tRNase Z (32.6 kDa) was solved by the multilengthwidth anomalous dispersion method using the SeMet derivative at 2.6-Å resolution and was refined to R and R_free factors of 22.6 and 27.9%, respectively (Fig. 1A). In the crystal, the asymmetric unit contains two molecules, A and B. The root mean square deviation (r.m.s.d.) value between these two molecules was 0.5 Å for 236 Ca atoms, indicating that they share almost identical structures. They form a dimer composed of the molecules related by the non-crystallographic symmetry 2-fold axis. Actually the gel filtration and analytical ultracentrifugation analyses revealed that T. maritima tRNase Z is a dimer in solution (data not shown), which is consistent with the results that other tRNase Zs are also dimers in solution (9, 29).

The overall architecture of T. maritima tRNase Z is a four-layer aββα sandwich fold (Fig. 1A). The structure consists of two ß-sheets, each composed of seven ß-strands and three ß-helices on both sides (Fig. 1A). The structure is divided into two nearly equivalent domains: an N-terminal domain (ß1–6, ß14, and ß1–3) and a C-terminal domain (ß7–13 and ß4–6), consisting of one ß-sheet and three ß-helices. In the crystal structure, there is a long disordered region between ß9 and ß10 (amino acid residues 152–174 of molecule A, and amino acid residues 152–175 of molecule B) that may protrude from the core structure into the solvent. Additionally two short loop regions (amino acid residues 134–136 and 218–220 of molecule A, and amino acid residues 133–138 and 211–222 of molecule B), located at the edge of the C-terminal ß-sheet, are also disordered (Fig. 1A).

The dimer interface is mainly composed of the N-terminal ß-helices of both subunits (Fig. 1B). The non-crystallographic symmetry 2-fold axis runs approximately perpendicular to helices ß2 and ß3. Therefore, the direction of the ß2 and ß3 helices of each subunit is antiparallel. The buried surface area is about 1400 Å²/monomer, which is 13% of the total surface area. The directly interacting residues in the dimer are Phe-11 in ß2, Glu-28 in the loop connecting ß3 and ß1, Ser-31 and Thr-32 in ß1, Val-51 in the loop connecting ß4 and ß2, Trp-58 and Ile-63 in ß2, and Glu-88 and Arg-95 in ß3 within the cut-off distance of 3.5 Å. These amino acid residues are not conserved in the tRNase Z family (Fig. 2). Another dimer composed of crystallographic symmetry-related molecules is also formed in the crystal. When tRNase Z forms this type of dimer, the N and C termini of both subunits are located close to each other and are involved in the dimer interface. However, tRNase Z molecules with N- or C-terminal histidine tags are active, indicating that the crystal packing makes this type of dimer.

Structural Similarity with Metallo-ß-lactamases—The tRNase Z family belongs to the metallo-ß-lactamase superfamily (19, 21). A structural similarity search using DALI (30) with known three-dimensional protein structures also revealed that the overall architecture of T. maritima tRNase Z shares a common fold with those of the metallo-ß-lactamases (31), such as the T. maritima putative zinc-dependent hydrolase of the metallo-ß-lactamase superfamily (TM0207) (Z = 12.0), Protein Data Bank code 1VJN), Bacteroides fragilis metallo-ß-lactamase (Z = 9.0, Protein Data Bank code 1A7T) (32), and Stenotrophomonas maltophilia L1 metallo-ß-lactamase (Z = 9.0, Protein Data Bank code 1SML) (33). Despite the modest Z score and the low level of structure-based sequence identity (less than 10%), T. maritima tRNase Z was structurally aligned with the three top scoring proteins (Fig. 3, A–D), TM0207 with an r.m.s.d. of 2.5 Å for 155 Ca atoms, B. fragilis metallo-ß-lactamase with an r.m.s.d. of 2.8 Å for 121 Ca atoms, and S. maltophilia L1 metallo-ß-lactamase with an r.m.s.d. of 2.5 Å for 121 Ca atoms, by the program Secondary Structure Matching (34).

Active Site—The metallo-ß-lactamase superfamily has five conserved motifs (20, 21, 35, 36). Motif I consists of the conserved aspartic acid, and motif II, which is typical of the superfamily, consists of the conserved signature motif HXXHDH and is located at the edge of the N-terminal ß-sheet. Motifs III, IV, and V are located at the edge of the C-terminal ß-sheet. Motifs III and V each have a conserved histidine residue, and motif IV has an acidic residue or a cysteine (20, 21, 35, 36). The active site of the metallo-ß-lactamase superfamily comprises motifs II-V located at one edge of the ß-sandwich (19).

These five conserved motifs are also conserved in the tRNase

FIG. 3. Comparison of the structure of T. maritima tRNase Z with those of other metallo-ß-lactamase proteins. A, T. maritima tRNase Z; B, T. maritima putative zinc-dependent hydrolase of metallo-ß-lactamase superfamily (TM0207); C, B. fragilis metallo-ß-lactamase; D, S. maltophilia L1 metallo-ß-lactamase. The ß- and 3₁₀ helices are colored yellow, and the ß-strands are colored cyan. The structure comparison was made using Secondary Structure Matching (34).
Z family (Fig. 2); motif I (Asp-25) is located at the end of β3, motif II (His-48, His-50, Asp-52, and His-53) is located at the region connecting β4 and α2, motif III (His-134) is located at the loop connecting β8 and β9 but is disordered in this structure, motif IV (Asp-190) is located at the end of β10, and motif V (His-244) is located at the end of β12 (Figs. 1A and 2). A previous study revealed that Asp-25 (motif I), His-48, His-50, Asp-52, and His-53 (motif II) of T. maritima tRNase Z are essential for the activity, suggesting that these residues form a part of the active site (15). In fact, four of the residues, His-48, His-50, Asp-52, and His-53, are located close to each other and form half of the active site pocket in the tRNase Z structure (Fig. 4A). Asp-25 is located in the disallowed region of the Ramachandran plot and interacts with the side chain of Thr-47, which is either Thr or Ser in the tRNase Z family (Fig. 2), and may fix the active site pocket (Fig. 4A). Notably the motif I aspartate residue adopts a sterically restrained conformation in other metallo-β-lactamases (31–33). Motifs IV and V create the other half of the active site pocket (Fig. 4B), and motif III may also contribute, but it is disordered in the crystal structure. Indeed, as shown in Fig. 4B, the active site residues of T. maritima tRNase Z superimpose well on those of B. fragilis metallo-β-lactamase: His-48, His-50, Asp-52, His-53 (motif III), Asp-190 (motif IV), and His-244 (motif V) correspond to His-82, His-84, Asp-86, Ser-87 (motif II), Cys-164 (motif IV), and His-206 (motif V), respectively (Fig. 4B).

In the present electron density map for the T. maritima tRNase Z crystal, a strong electron density is tightly bound to the side chain of Asp-52, Asp-190, and His-244. By analogy to the structures of metallo-β-lactamases, we assigned this electron density as a zinc ion. However, the distance between the putative zinc ion and the side chains of Asp-52, Asp-190, and His-244 are longer (3.5, 3.3, and 3.1 Å, respectively, in molecule A) than the usual distance between a zinc ion and the side chains of protein residues (2.0–2.3 Å) (37). This may be because the binding of the zinc ion to T. maritima tRNase Z is weak. In fact, the zinc binding positions slightly differ between the two molecules in the asymmetric unit. In molecule B, the position of the zinc ion is slightly shifted toward Asp-190 as compared with that in molecule A, and as a result, the distances between the zinc ion and Asp-52, Asp-190, and His-244 change (3.2, 2.7, and 3.2 Å, respectively). We could not exclude the possibility that this density is due to a different metal, such as Mg$^{2+}$ or Mn$^{2+}$, which is required for the catalysis, although neither Mg$^{2+}$ nor Mn$^{2+}$ was added exogenously.

**Relationship with the Phosphodiesterase (PDE) Motif**—A previous report suggested that tRNase Z has a PDE motif, one of the zinc binding motifs (HX$_3$HX$_{20–129}$E), in the region overlapping with that of motif II (9). The PDE motif was originally described in cAMP phosphodiesterase (38), which catalyzes the hydrolysis of cAMP into AMP to regulate the cAMP concentration. The PDE4D2 catalytic domain has a compact structure composed of 16 α-helices and contains two metal ions (38), which is completely different from that of T. maritima tRNase Z. In the structure of PDE4D2, the two PDE motifs jointly create a single pocket for binding the two metal ions (38). One is the zinc ion and the other may be Mg$^{2+}$ or another divalent cation. When we compared the putative active site of the T. maritima tRNase Z structure with the AMP binding site of the human PDE4D2 catalytic domain-AMP complex structure, only four residues, Asp-52, His-53, Asp-190, and His-244 in T. maritima tRNase Z and Asp-201, His-200, Asp-318, and
Z requires Mg\(^{2+}\) and no bridging water in the crystal structure of tRNase Z as in PDE4D2. Although there is no second metal ion to serve as a proton donor in the catalysis of RNA in PDE4D2, Asp-318 also forms hydrogen bonds with a water molecule bridging two metal ions, and His-160 is located near the O-3' of AMP (38). Therefore, it was suggested that Asp-318 works as a general base to activate the bridging water for a nucleophilic attack on the phosphodiester bond of cAMP, and His-160 may protonate the O-3' of AMP (38). Since there are several histidine residues at the active site, Asp-190 may serve as a general base, and a histidine residue at the active site may serve as a proton donor in the catalysis of RNA in T. maritima tRNase Z as in PDE4D2. Although there is no second metal ion and no bridging water in the crystal structure of T. maritima tRNase Z, the previous study showed that T. maritima tRNase Z requires Mg\(^{2+}\) or Mn\(^{2+}\) for its activity, suggesting that it is bound to the tRNase Z together with the substrate RNA.

**RNA Binding Model**—It is interesting to examine how tRNase Z may recognize the substrate tRNAs. Fig. 6a shows the residue identity mapped on the molecular surface of the dimer of T. maritima tRNase Z, based on the alignment shown in Fig. 2, ranging from pale green (70% similarity) to deep green (100% identity). The conserved residues are mainly concentrated at the active site (Fig. 6a). The electrostatic potential shows that one side of T. maritima tRNase Z is mostly negatively charged, and the other side is mostly positively charged (Fig. 6b). However, there is no clear correlation between the distribution of conserved amino acids and that of the electrostatic potential except around the active site (Fig. 6, A and B). It was reported that T. maritima tRNase Z and other tRNase Zs require the whole tRNA structure for optimal activity, and tRNase Zs require the acceptor stem and the T arm of tRNA (14, 22, 39). Although the anticondor arm is not essential for the activity, the T arm and D arm are very important for the catalysis by tRNase ZS (14). This implies that tRNase ZS recognizes the top half of the tRNA molecules. Taking these aspects into account, we constructed a docking model of the T. maritima tRNase Z dimer and the two whole tRNAs (yeast tRNA\(^{Phe}\)) (Fig. 7). In this model, the tRNAs bind the positively charged surface of the T. maritima tRNase Z dimer, and one protomer of the dimer recognizes the 3'-CCA terminus of the tRNA, while the other protomer recognizes the remaining part of the tRNA (Fig. 7). The 2-fold axis of the T. maritima tRNase Z dimer is perpendicular to the tRNA binding surface, and the two tRNAs could bind to the enzyme at the same time without steric hindrance. The tRNase Z interacts with the acceptor stem, the D stem, and the T stem (Fig. 7).

On the other hand, tRNase Zs from organisms other than T. maritima cleave the pre-tRNAs after the discriminator at position 73 (3, 5–11). These pre-tRNAs enter the active site more deeply, suggesting that the structures of the common tRNase Zs are different from that of T. maritima tRNase Z. For T. maritima tRNase Z, the S31Q mutation shifts the cleavage site from position 76 to position 74 or 73, while the T33Q mutation shifts it to position 75 or 74 (15). These residues are located on or next to the dimer interface, which is formed by Phe-11, Glu-28, Ser-31, Thr-32, Val-51, Trp-58, Ile-63, Glu-88, and Arg-95 (Fig. 2). Surprisingly all of these interface amino acid residues, except for Glu-28, of T. maritima tRNase Z are different from the well conserved residues in the corresponding positions of the common tRNase Zs. For instance, the residues corresponding to Phe-11, Ser-31, and Trp-58 in T. maritima tRNase Z are commonly Pro, Gln, and Pro, respectively, in other tRNase Zs (Fig. 2). Hence the difference in the residues involved in the dimer formation appears to be correlated with the difference in the pre-tRNA cleavage site. As described above, one pre-tRNA molecule is likely to bind with the two subunits of the T. maritima tRNase Z dimer (Fig. 7). Therefore, the relative orientation of the two subunits in the dimer probably defines the cleavage site distinctly between the common tRNase Zs and T. maritima tRNase Z.

**Implication for the structure of tRNase ZL**—Both the N-terminal and C-terminal domains of tRNase ZL share sequence similarity with tRNase Z (19–21). This implies that both domains also have the same metallo-\(\beta\)-lactamase fold, and the structure of tRNase ZL may resemble that of the tRNase Z dimer. In the dimer structure of T. maritima tRNase Z, the C terminus of one protomer is located adjacent to the N terminus of the other protomer, although the directions of both ends are opposite (Fig. 1B). Hence only a local structural change, such as the movement of \(\beta\)-strands, is needed to connect the N- and
C-terminal domains in the structure of tRNase ZL, and it retains the same relative orientation as that of the tRNase ZS dimer. The N- and C-terminal β-strands are located on the tRNA binding face in our model, and thus the local movement of the N and C termini may cause the different substrate specificities between tRNase ZS and tRNase ZL (22). To elucidate the precise mechanism of the substrate recognition of the tRNase Zs, further studies, such as the structure determination of a complex of tRNase Z and an RNA substrate or the structure determination of tRNase ZL are necessary.

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