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Crystal structure of tRNA-specific ribonuclease, colicin D

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Introduction

Colicin D (697 residues) has recently been characterized as a tRNase that kills sensitive *Escherichia coli* cells via a specific tRNA cleavage. It targets exclusively the four isoaccepting tRNAs for Arg, whose anticodon sequences are ICG, CCG, U*CU, (U*; 5-methylaminomethyluridine), and CCU, and cleaves the phosphodiester bond between positions 38 and 39, at the 3' junction of the anticodon stem and the loop. The residue is A at position 38, and G or C at 39 in the four isoacceptors; these sequences at positions 38 and 39 are also shared by some other tRNAs, however. If colicin D recognizes only the cleavage site, as with another tRNase colicin E5, its tartget would not exclusively be $tRNA^{Arg}$. It could, therefore, recognize some higher order structural features of the targets with or without base recognition.

The molecular mechanisms for recognition of tRNAs have been closely studied and characterized on aminoacy-tRNA synthetases (aaRS). There are, however, no homologies between colicin D and aaRSs. Thus, we started to analyze the crystal structure of colicin D to identify its specific recognition mechanism.

Materials and Methods

The $tRNA^{Arg}$-specific ribonuclease activity of colicin D exclusively locates its C-terminal domain (CRD; the C-terminal Ribonuclease Domain). The expression plasmids used here have operons consisting of ORFs coding for either CRD604 (604-697 residues of colicin D) or CRD595 (595-697 residues), and Imm D (inhibitor protein; 94 residues) tagged with six histidines at the C-terminus, expressed under control of the colicin promoter. For a MAD experiment, selenomethionine (SeMet) substituted CRD604-ImmD was prepared.

CRD604-ImmD crystal was obtained in mother liquor condition with 0.09 M Mes at pH .5, 10% MPD, 5 mM DTT, 18% PEG8000, and 0.18 M magnesium acetate at 4 °C. The space group is $P4_12_12$ and the unit cell dimensions are a = b = 58.5 Å, c = 149 Å for both native and SeMet crystals.

Results and Discussion

In the complex structure, CRD consists of a four-stranded antiparallel β-sheet, with two helices on one side and a short helix on the other side. ImmD proved to be a four-helix bundle protein, in which the first helix parallels the second and the third parallels the fourth, and each parallel pair is in a twisted position to each other (Fig. 1).

The complex was formed mainly by electrostatic interaction between positive charges on CRD and negative charges on ImmD.

All ribonucleases reported to date have at least one histidine as a catalytic residue. There are two histidines in CRD, and crystal structure supports that His611 is responsible for the catalysis since it located on the surface of the protein whereas the other one was buried inside of the molecule.

Interestingly, the basic residues, Arg602, Lys603, Lys607, Lys608, Lys610 and Arg624 line the surface having a slight curve. The trace of these residues is reminiscent of the backbone curve of tRNA. The distance between Nζ, Nη1 or Nη2 atoms of adjacent resi1ues are 4.8-9.7 Å. The distance between the atoms of the basic residues can change according to the conformations of the side chains. These basic residues may therefore play an important role in tRNA specific binding of CRD and recognition of the higher order structure of tRNA.

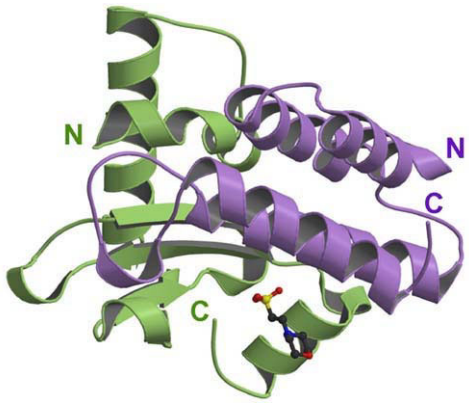

Figure 1. The complex structure of CRD-ImmD.

References

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