Crystal structures of ribonuclease LE complexed with substrate fragments

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Introduction

Ribonuclease LE (RNase LE) from cultured tomato (*Lycopersicon esculentum*) cell is a member of the RNase T_2 family showing broad base specificity. RNase LE consists of 205 amino acid residues and has a molecular mass of 22,666 Da.

The objects of crystal structure analysis of RNase LE are to gain a basis for further studies on the base-recognition and catalytic mechanisms of the RNase T_2 family enzymes and to know the basic framework of plant ribonucleases.

Methods

Crystals of RNase LE were obtained by the hanging-drop vapor diffusion method [1]. The crystals belong to an orthorhombic space group $P2_12_12_1$, with cell dimensions of a = 74.10 Å, b = 78.72 Å, and c = 33.00 Å. Assuming one molecule per asymmetric unit, a V_M value of 2.1 $Å^3$/Da, corresponding to a solvent content of 41 %, is obtained.

Initially, we tried to solve the structure of RNase LE by molecular replacement techniques. The structure of RNase Rh [2], having a ca. 30 % sequence identity with RNase LE, was used as a search model. Since this attempt failed, the structure was determined by the multiple isomorphous replacement (MIR) method.

X-ray data collections for native and heavy-atom derivative crystals were performed using a R-AXIS IIc area detector with CuKα radiation. High-resolution data collections were performed by an oscillation method using two native crystals at the beam line BL18B.

The structure has been solved by the MIR method using five heavy-atom derivatives and refined to an R value of 0.219 at 1.65 Å resolution [3].

To obtain a basis for base-recognition and catalytic mechanisms of RNase LE, substrate-fragment complex crystals were obtained by transferring the native crystals into a series of substrate-fragment solutions containing guanosine 2'-monophosphate (2'-GMP: inhibitor), guanosine 3'-monophosphate (3'-GMP: substrate fragment), guanosine 5'-monophosphate (5'-GMP: substrate fragment), or deoxyguanylyl-3,5-deoxyguanosine (d(GpG): substrate analogue). The structures of these four complexes have been solved by the difference Fourier method and refined to R values below 0.20 at 1.8 Å resolution.

Results and Discussion

The structure consists of seven α-helices and seven β-strands, belonging to an α+β type structure. Comparison of the structure of RNase LE with that of RNase Rh reveals that while the overall folding topologies are similar to each other, major insertions and deletions are found at the N-terminal regions. The structural comparison and an amino acid sequence alignment of the RNase T_2 enzymes show that the structure of RNase LE is the basic framework of the animal/plant subfamily of RNase T_2 enzymes, and the structure of RNase Rh is that of the fungal subfamily of RNase T_2 enzymes. Subsequently, we superposed the active site of the RNase LE with that of RNase Rh and found that (1) His39, Trp42, His92, Glu93, Lys96, and His97 of RNase LE coincided exactly with His46, Trp49, His104, Glu105, Lys108, and His109, respectively, of RNase Rh, and (2) two conserved water molecules were found at the putative P_1 sites of both enzymes. These facts suggest that plant RNase LE has a very similar hydrolysis mechanism to that of fungal RNase Rh, and almost all the RNase T_2 enzymes widely distributed in various species would share a common catalytic mechanism.

The substrate-fragment complexes reveal interesting features: the substrate fragments (3'-GMP and 5'-GMP) and substrate analogue (d(GpG)) bound in the productive binding mode while inhibitor (2'-GMP) bounds non-productive binding mode; and the binding mode of 3'-GMP at B_1 subsite and that of 5'-GMP at B_2 subsite are similar to that of d(GpG) bound at both B_1 and B_2 subsite. These observations show that the binding of substrate fragments may conform to the principles of additivity (Tanaka, N. et al., in preparation).

Acknowledgements

We thank Drs. M. Suzuki and N. Igarashi for help with data collection at the Photon Factory.

References

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