Crystal structure analyses of thermolysin in complex with its inhibitors

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Introduction

Thermolysin (TLN) is a metalloprotease with 316 amino acid residues. This enzyme contains a Zn^{2+} ion in the active site, which is required for its enzyme activity. We have analyzed interaction between TLN and its inhibitors by thermodynamic methods. We have revealed that a series of stereo-isomeric inhibitors, benzyloxycabonyl-amino acids (hereafter Z-L(D)-amino acid), are ideal compounds to analyze thermodynamics of TLN-inhibitor interaction. To date, thermodynamic parameters for the interaction between TLN and Z-L(D)-amino acid have been experimentally determined. In order to improve and establish the method to predict the binding thermodynamics, computer simulation work has also been carried out. To validate these results, we have tried to determine the crystal structures of TLN in complex with various inhibitors, Z-L(or D)-amino acid.

Method

TLN was crystallized using a sitting drop vapor diffusion method as described earlier [1]. Crystals of TLN were soaked in the standard buffer containing an inhibitor for a few days to prepare the crystal of the TLN-inhibitor complex. Obtained crystals were mounted a glass capillary. Data collection was carried out at room temperature using the ADSC CCD detector of BL6A at PF. The data were processed using the program MOSFLM and scaled using the CCP4 suite. Crystal structures were determined using the molecular replacement method and refined with the program CNS.

Results

The crystal structures of TLN-inhibitor complexes have been refined (Table 1). The comparison of these structures will give detailed insights into the interaction between TLN and the inhibitors.

Reference

[1] Colman PM et al. J.Mol.Biol. (1972) **70**, 701

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Table 1. Current status of crystallographic refinement

Inhibior	Z-L-Asp	Z-D-Asp	Z-L-Glu	Z-D-Glu	Z-L-Thr	Z-D-Thr
Resolution (Å)	20 – 1.6	20 – 1.7	20 – 1.6	20 – 1.8	20 – 1.6	20 – 1.7
Completeness (%)	98.9	99.1	98.8	99.9	95.8	99.8
R-merge (%)	7.1	8.3	7.2	8.0	7.3	9.6
R-factor (%)	17.1	16.7	17.3	16.5	17.3	16.8
Free R-factor (%)	18.6	18.9	18.3	18.5	18.3	18.4
Rmsd(bond) (Å)	0.0045	0.0045	0.0045	0.0049	0.0053	0.0045
Rmsd (angle) (°)	1.17	1.18	1.17	1.18	1.19	1.18