Structure of V8 Protease from *Staphylococcal aureus*

L. Prasad, Y. Leduc, L. T. J. Delbaere
Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada

Introduction

The staphylococcal V8 protease is an extracellular protease of *staphylococcal aureus* that is probably related to the mammalian serine proteases. The enzyme exhibits a unique substrate specificity as it cleaves exclusively on the carbonyl sides of peptide bonds of either glutamic or aspartic acid residues [1]. Although it exhibits significant sequence homology with other serine proteases in the regions involving catalytically important residues, no other regions of sequence homology are apparent. In addition, V8 protease possesses no disulphide bridges, which is a major evolutionary difference from all other pancreatic and related serine proteases, which have at least two of these groups.

Methods and Materials

V8 protease crystals were grown in a drop containing 7 mg/mL protein in 50 mM KCL, 50 mM Hepes (pH 8.6), and 15% PEG 5000 MME over a reservoir containing 100 mM KCL, 100 mM Hepes (pH 8.6), and 20% PEG 5000 MME at 20°C. V8 crystallizes in the hexagonal space group $P6_522$, with unit cell dimensions of a = b = 62.72 Å and c = 225.88 Å.

Multiwavelength anomalous diffraction (MAD) data were collected by using an Osmium derivative at beamline 14-BM-D of the Bio Consortium for Advanced Radiation Sources (BioCARS) at the APS. The data were processed by using DENZO and SCALEPACK.

Three heavy atom sites were located and refined by using CNS [Crystallography & NMR (nuclear magnetic resonance) System] software. After solvent flattening and density modification, the figure of merit was 0.93 and an electron density map was calculated. The entire protein was traced by using this map. Refinement of the structure to 1.9-Å resolution was carried out by using native data collected at the Photon Factory, Tsukuba, Japan. The final R-value is 0.198, and the R_{free}-value is 0.257.

Results

Electron density maps calculated by using phases obtained from the MAD experiment were of sufficient quality to trace the entire protein. The ribbon representation of the alpha-carbon trace of V8 protease is shown in Fig. 1.

Discussion

The active site of V8 protease (Fig. 2) is very similar to those of other serine proteases, even though the overall folding is different. Attempts are underway to prepare and solve structures of V8 protease-inhibitor complexes.

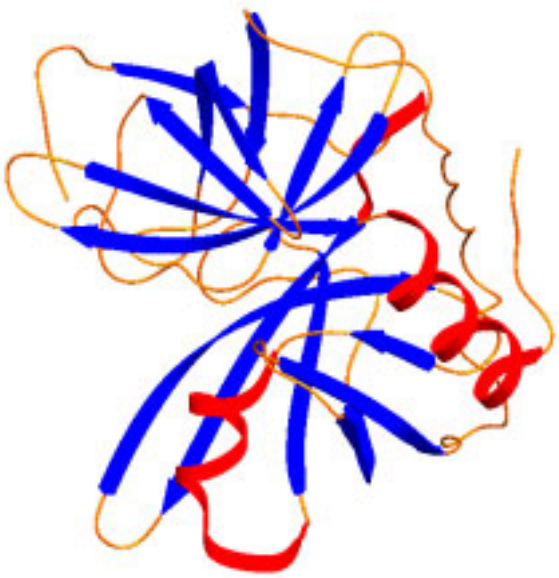

FIG. 1. Ribbon diagram of the C-alpha trace of V8 protease.

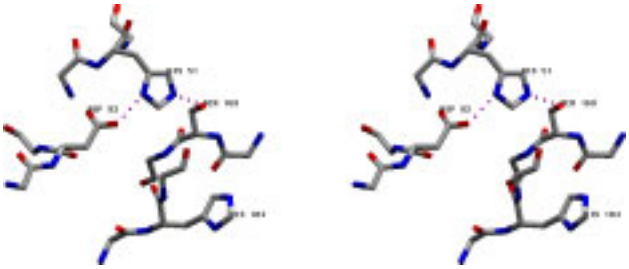

FIG. 2. Active site of V8 protease.

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References

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