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The structure of a universally employed enzyme: V8 protease from *Staphylococcus aureus*

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V8 protease, an extracellular protease of *Staphylococcus aureus*, is related to the pancreatic serine proteases. The enzyme cleaves peptide bonds exclusively on the carbonyl side of aspartate and glutamate residues. Unlike the pancreatic serine proteases, V8 protease possesses no disulfide bridges. This is a major evolutionary difference, as all pancreatic proteases have at least two disulfide bridges. The structure of V8 protease shows structural similarity with several other serine proteases, specifically the epidermolytic toxins A and B from *S. aureus* and trypsin, in which the conformation of the active site is almost identical. V8 protease is also unique in that the positively charged N-terminus is involved in determining the substrate-specificity of the enzyme.

1. Introduction

Drapeau *et al.* (1972) first isolated V8 protease and since then it has been widely used for determining the primary structure of proteins. This extracellular proteolytic enzyme of *Staphylococcus aureus* possesses unique substrate specificity. V8 protease cleaves peptide bonds on the carbonyl side of aspartate and glutamate residues and the exact specificity is dependent on the buffer (Sørensen *et al.*, 1991). V8 protease does not have significant sequence identity to other serine proteases (29 and 32% with epidermolytic toxins A and B, respectively, and 13% with bovine trypsin), except in regions involving catalytically important residues. There are no disulfide bonds in V8 protease. This is a major evolutionary difference, as the pancreatic serine proteases have at least two disulfide bridges (James *et al.*, 1978). However, the catalytic triad (His, Asp, Ser) is in the same linear order along the polypeptide chain as that of the pancreatic serine proteases. V8 protease is inhibited by diisopropyl fluorophosphates (Drapeau, 1978). The diphenyl phosphonate analogues of aspartate and glutamate have also been shown to inhibit V8 protease (Hamilton *et al.*, 1998).

We report here for the first time the X-ray crystallographic structure of V8 protease.

2. Experimental

V8 protease crystals suitable for X-ray diffraction studies were first obtained by Gehrig *et al.* (1985). The crystals used in the present study were grown in a hanging drop containing 7 mg ml^{-1} protein in 50 m*M* *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) pH 8.6, 50 m*M* KCl and 15% polyethylene glycol (PEG) 5000 monomethyl ether

Table 1
Data-collection and refinement statistics for V8 protease.

Values in parentheses are for the outer resolution shell.

Data collection	
Resolution range (Å)	40.0–1.90 (1.93–1.90)
No. reflections measured	97623
No. unique reflections	20095 (790)
R_{sym}	0.09 (0.41)
Completeness (%)	92.0 (76.2)
Redundancy	6.3 (4.7)
$I/\sigma(I)$	8.1 (1.1)
Refinement statistics	
Resolution range (Å)	10.0–1.90 (1.97–1.90)
Total No. reflections	19878
No. non-H protein atoms	1645
No. water molecules	69
R value (for all 19 878 reflections)	0.20 (0.30)
R_{free} value (962 reflections)	0.23 (0.36)
Average B factor (Å^2)	18.82
R.m.s. deviations from ideal geometry	
Bond distances (Å)	0.01
Bond angles (°)	1.38
Dihedral angles (°)	25.73
Improper angles (°)	0.76

Table 2
MAD data (osmium) and phasing.

(*a*) Data collection.

	Absorption edge	Inflection point	High-energy remote
Wavelength (Å)	1.1391	1.1532	1.0898
Resolution (Å)	100.0–2.0	100.0–2.0	100.0–2.0
No. unique reflections	13664	14430	16539
R_{sym}	0.10	0.07	0.08
Completeness (%)	93.1	98.8	94.3

(*b*) MAD phasing.

No. of primary heavy-atom sites per AU	1
No. of secondary sites per AU	2
Phasing power	1.6
Figure of merit (%)	66.9

(MME) over a reservoir containing 100 m*M* KCl, 100 m*M* HEPES and 20% PEG 5000 MME at 293 K. V8 protease crystallized in the hexagonal space group $P6_522$, with unit-cell parameters $a = b = 62.7$, $c = 225.9$ Å. 1.9 Å resolution X-ray diffraction data for the native protein were collected on beamline BL18B at 290 K at the Photon Factory, Tsukuba, Japan. The data-collection statistics are given in Table 1.

Since V8 protease is a serine protease, attempts were made to solve the structure by molecular replacement using the structure of bovine trypsin as a model. However, the attempts failed, probably owing to the very low sequence identity between V8 protease and bovine trypsin (13%). The structure was solved using MAD data to 2.0 Å resolution collected at beamline BM14-D, BioCARS, APS, Argonne, Illinois, USA on an osmium derivative. Both native and MAD data sets were processed using *DENZO* (Otwinowski & Minor, 1997) and the *CCP*4 (Collaborative Computational Project, Number 4, 1994) suite of programs. Three heavy-atom (Os) sites were located and refined (Table 2) using the *CNS* suite of programs (Brünger *et al.*, 1998). The figure of merit (FOM) after solvent flattening and density modification was 0.93, with an FOM-weighted R value of 0.14. An electron-density map was calculated using phases obtained from the heavy-atom sites. The first 216 of the 274 residues of the protein were modeled using this map. The final 58 C-terminal residues did

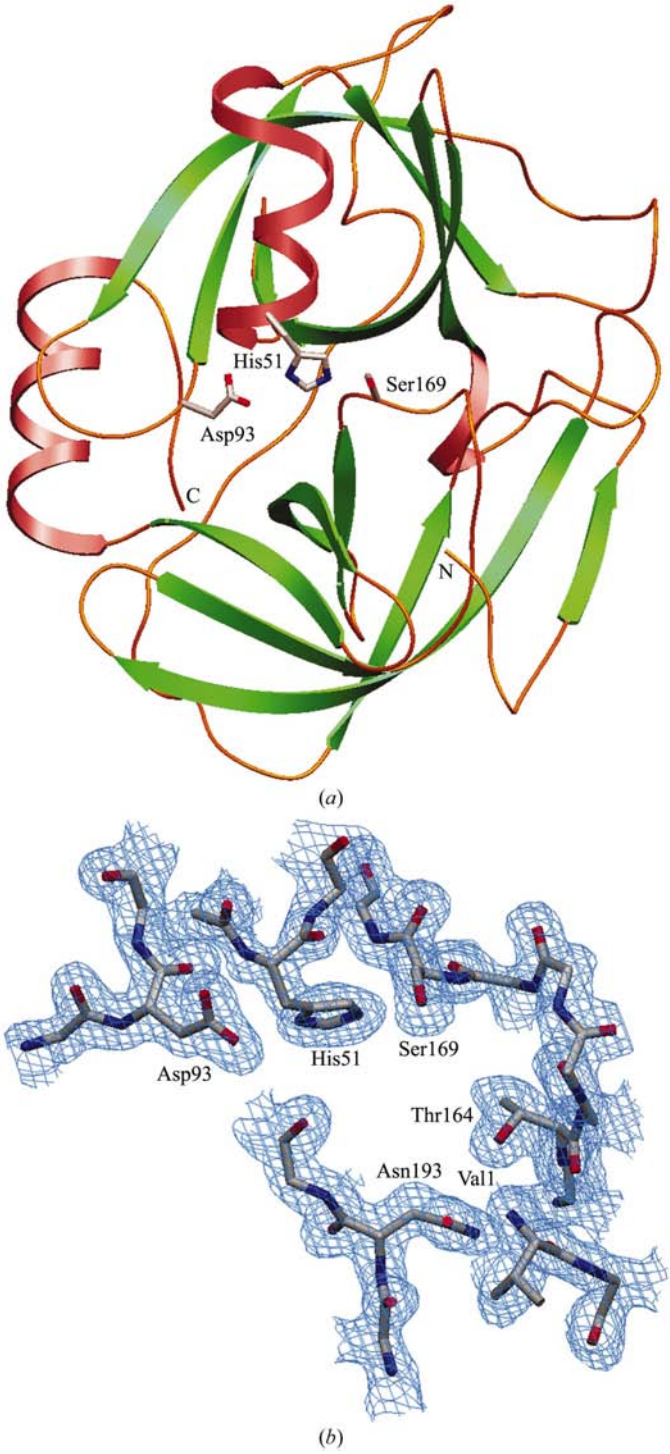

Figure 1
(*a*) C$^\alpha$ trace of V8 protease showing the catalytic triad. All figures were drawn using the program *SETOR* (Evans, 1993). (*b*) Active-site residues and the electron-density map. Contours are at the 1σ level.

not have sufficient corresponding electron density to be modeled in all the subsequent maps that were calculated. The absence of electron density for the 58 C-terminal residues is probably owing to the autocatalytic activity of the enzyme (Drapeau, 1977).

The structure was refined with the native data in the 10–1.9 Å resolution range using the *X-PLOR* (Brünger, 1992) and *CNS* (Brünger *et al.*, 1998) program packages. The final R value is 0.20, with an $R_{\rm free}$ value (5% of 19878 reflections) of 0.23; the refinement statistics are shown in Table 1. The model contains 69 ordered water molecules.

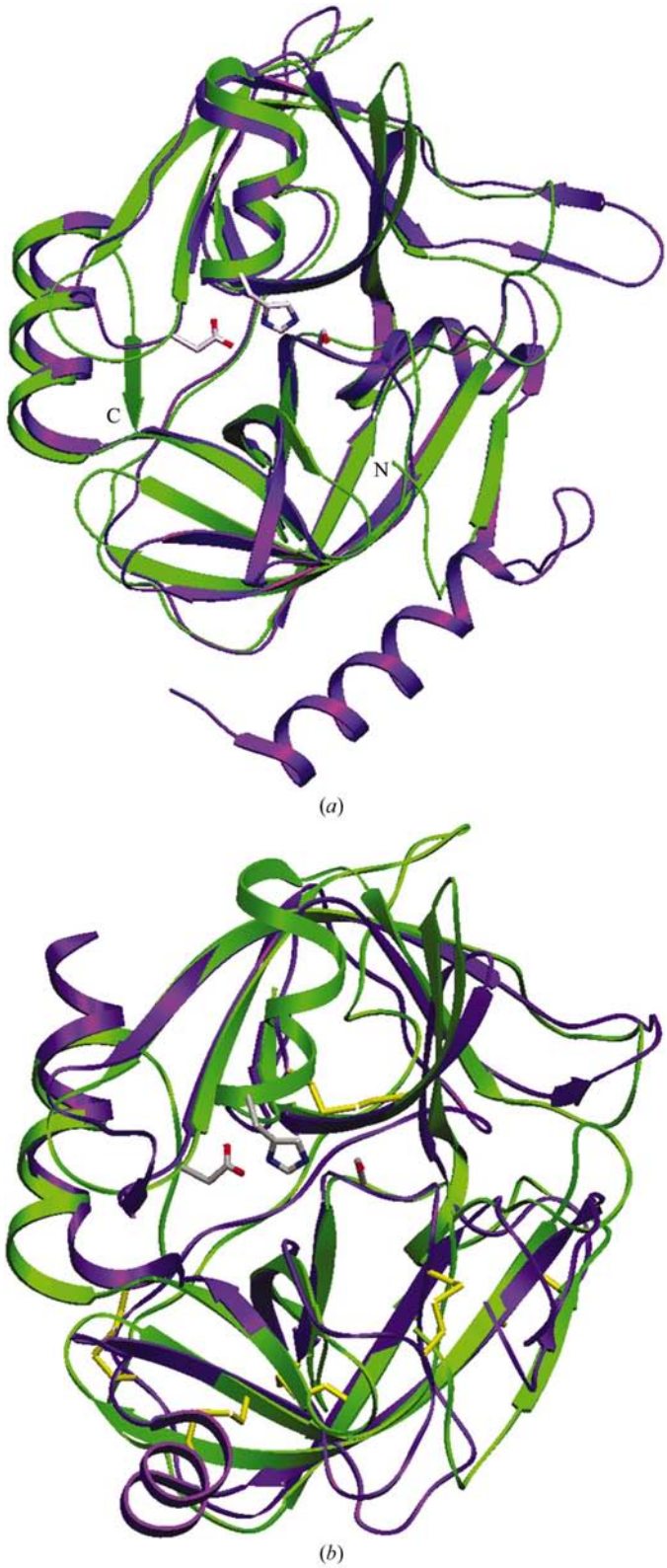

Figure 2
(*a*) Superposition of the C^{α} traces of V8 protease (green) and epidermolytic toxin A (purple). (*b*). Superposition of the C^{α} traces of V8 protease (green) and porcine pancreatic trypsin (purple).

3. Results and discussion

The structure of V8 protease from *S. aureus* is shown in Fig. 1(*a*) and the active site of the enzyme consisting of the residues His51, Asp93 and Ser169 is shown in Fig. 1(*b*). The linear amino-acid sequence numbering of V8 protease is used in this work rather than the amino-acid sequence alignment with bovine trypsin. Owing to the lack of significant sequence identity between V8 protease and other serine proteases, a search was made for structural similarity using the DALI server (Holm & Sander, 1994). Three structures, the epidermolytic toxins A and B (Vath *et al.*, 1997; Cavarelli *et al.*, 1997) from *S. aureus* and bovine beta trypsin (PDB code 5ptp; Finer-Moore *et al.*, 1992), had significant structural homology with the V8 protease (r.m.s.d.s of 1.6, 1.7 and 2.2 Å, respectively). V8 protease was superposed on the structures of epidermolytic toxin A (PDB code 1agj) and porcine pancreatic trypsin (PDB code 1avw; Song & Suh, 1998) and the results are shown in Figs. 2(*a*) and 2(*b*). Using the structural similarity of trypsin to V8 protease, the binding of inhibitors to V8 protease was modeled in order to determine the residues responsible for the specificity of the enzyme. The structure of V8 protease was superposed on the trypsin portion of the structure of the complex between porcine pancreatic trypsin and soybean trypsin inhibitor (Song & Suh, 1998). A section of the soybean trypsin inhibitor, residues 550–570, was visually docked to the active site of V8 protease, after replacing Arg563 of the inhibitor by Glu and subsequently by Asp. The energy of the modeled complex of V8 protease and inhibitor was then minimized using *MacroModel* (Mohamadi *et al.*, 1990). The results are shown in Figs. 3(*a*) and 3(*b*).

Despite the lack of sequence identity between V8 protease and other serine proteases, the tertiary structure is very similar to that of the pancreatic type enzymes, especially in the active-site region, as shown in Figs. 2(*a*) and 2(*b*). In most serine proteases, the conformation of the sequence of residues 166–169 (192–195 in trypsin) containing the active-site Ser169 (195) is such that the oxyanion hole is preformed, but not in the structures of the epidermolytic toxins A and B (Vath *et al.*, 1997; Cavarelli *et al.*, 1997), which are specific for glutamates only and hence are classified as Glu endopeptidases (Dancer *et al.*, 1990). His184 and Tyr160 (His210 and Tyr186 in trypsin) are likely to stabilize the S1 pocket as occurs in other serine proteases (Cavarelli *et al.*, 1997).

The modeling of V8 protease complexed to an oligopeptide shows that the Glu or Asp residue is hydrogen bonded/ion paired to the positively charged N-terminus, which subse-

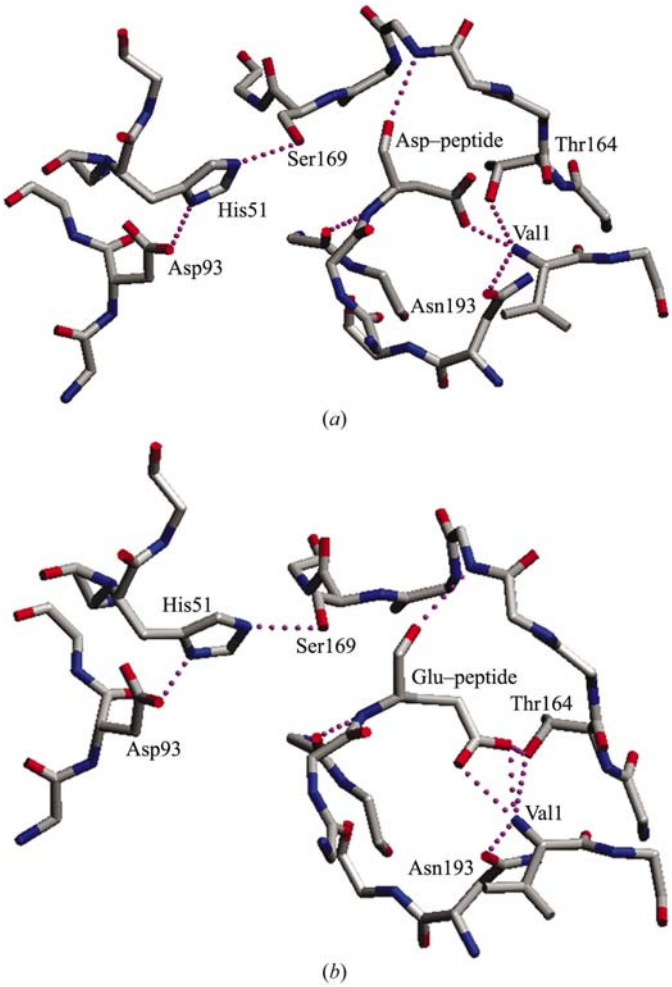

Figure 3
(*a*) Model of a complex between V8 protease and an oligopeptide with an aspatrate residue bound to the active site. (*b*) Model of a complex between V8 protease and an oligopeptide with a glutamate residue bound to the active site.

quently correctly positions the corresponding peptide bond to be attacked by the active-site Ser169 of the enzyme. To our knowledge, this is the first example of a protease where the specificity is determined by the N-terminus.

4. Conclusion

The tertiary structure of V8 protease is similar to that of the pancreatic serine proteases and modeling studies strongly indicate that the enzyme specificity of V8 protease for acidic amino-acid residues is determined by the positively charged amino-terminus.

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