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Structure of a New Alkaline Serine Protease (M-Protease) from *Bacillus* sp. KSM-K16

BY TAKASHI YAMANE, TAKUMA KANI, TAICHI HATANAKA, ATSUO SUZUKI AND TAMAICHI ASHIDA

Department of Biotechnology, School of Engineering, Nagoya University, Chikusa-Ku, Nagoya 464-01, *Japan*

TOHRU KOBAYASHI AND SUSUMU ITO

Tochigi Research Laboratories of Kao Corporation, 2606 *Akabane, Ichikai, Haga, Tochigi* 321-34, *Japan*

AND OSAMU YAMASHITA

Wakayama Research Laboratories, Kao Corporation, 1334 *Minato, Wakayama* 640, *Japan*

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Abstract

An alkaline serine protease, M-protease, from *Bacillus* sp. KSM-K16 has been crystallized. Two morphologically different crystal forms were obtained. Crystal data of form 1: space group $P2_12_12_1$, a = 47.3, b = 62.5, c = 75.6 Å, $V = 2.23 \times 10^5$ Å^3, Z = 4 and V_m = 2.09 Å^3 Da^{-1}. Crystal data of form 2: space group $P2_12_12_1$, a = 75.82 (2), b = 57.79 (2), c = 54.19 (1) Å, $V = 2.29\,(2) \times 10^5$ Å^3, Z = 4 and V_m = 2.15 Å^3 Da^{-1}. The crystal structure of M-protease in form 2 has been solved by molecular replacement using the atomic model of subtilisin Carlsberg (SBC) which is 60% homologous with M-protease, and refined to the crystallographic R factor of 0.189 for 7004 reflections with $F_o/\sigma(F) > 3$ between 7 and 2.4 Å resolution. The final model of M-protease contains 1882 protein atoms, two calcium ions and 44 water molecules. The three-dimensional structure of M-protease is essentially similar to other subtilisins of known structure. The 269 C^α positions of M-protease have an r.m.s. difference of 1.06 Å with the corresponding positions of SBC. The crystal data of form 2 are close to those of SBC, though the structure determination of form 2 made it clear that it is not isomorphous to the crystal structure of SBC. The deletions of amino acids occur at the residues 36′ and 160′–163′ compared with SBC (numerals with primes show the numbering for SBC). The deletion of the four residues (160′–163′) may significantly affect the lack of isomorphism between M-protease and SBC.

Introduction

Bacillus strains produce extracellular serine proteases (Tsuru & Yoshimoto, 1987). Several strains of alkalophilic *Bacillus* produce alkaline serine proteases which show their optimal activity with alkaline pH (Horikoshi & Akiba, 1982). These alkaline serine proteases are classified as belonging to the subtilisin family. Many X-ray structural studies on the subtilisin family have been carried out in order to understand the structure–function relationship of subtilisin on the basis of the three-dimensional structure; for example, subtilisin BPN′ (Wright, Alden & Kraut, 1969; Alden, Birktoft, Kraut, Robertus & Wright, 1971), subtilisin Novo (Drenth, Hol, Jansonius & Koeboek, 1972), subtilisin Carlsberg (SBC) (Neidhart & Petsko, 1988), the complex of subtilisin BPN′ with *Streptomyces* subtilisin inhibitor (Hirano, Akagawa, Mitsui & Iitaka, 1984), the complex of subtilisin Novo with chymotrypsin inhibitor 2 (McPhalen, Svendsen, Jonassen & James, 1985), the complex of subtilisin Carlsberg with eglin (McPhalen, Schnebli & James, 1985) or with eglin c (Bode, Papamokos & Musil, 1987), the complex of subtilisin Novo with eglin (Heinz, Priestle, Rahuel, Wilson & Grütter, 1991) and many modified or mutant enzymes such as peroxide-inactivated subtilisin BPN′ (Bott *et al.*, 1988). Recently the three-dimensional structure of an alkaline serine protease, PB92, from *Bacillus alcalophilus* has also been determined (van der Laan *et al.*, 1992), though the coordinates do not appear to have been published yet.

A new alkaline serine protease, M-protease, has been purified from *Bacillus* sp. KSM-K16 and some properties of M-protease have been studied (Kobayashi *et al.*, 1994). The amino-acid sequence of M-protease has already been determined by the analysis of its nucleotide sequence (Hakamada, Kobayashi, Hitomi, Kawai & Ito, 1994). It consists of 269 amino-acid residues and has a molecular weight of 26 723. Comparison of the amino-acid sequence of M-protease with subtilisin Carlsberg shows that the sequence identity between them is 60%. M-protease differs from PB92 only at four residues. The amino-acid sequence of M-protease is compared with that of SBC and PB92 in Fig. 1. M-protease is stable in the pH range between 5 and 12, and it optimal pH range is between 11 and 12.3. This shows that the activity and the stability of M-protease at the high-alkaline pH range are suitable for the additive use in detergent. In order to make clear the three-dimensional structure of M-protease and to obtain the information to design a

series of more useful enzymes, we have thus initiated the structural study of M-protease. Upon crystallization two morphologically different crystal forms of M-protease, 1 and 2, were obtained, of which form 1 seems to be isomorphous to the crystal structure of PB92. Therefore, the structure analysis of form 2 have at first been carried out. Here the X-ray structure determination of form 2 and comparison of the structure of M-protease with that of SBC are presented.

Experimental

Crystallization

M-protease was purified in the inactivated form by treatment with phenylmethylsulfonyl fluoride (PMSF) and freeze dried. The crystallization was carried out by the hanging-drop vapor-diffusion method. The freeze-dried enzyme was dissolved in an 50 m*M* acetate buffer solution (pH 5.5) to a concentration of 10 mg ml^{-1}. A 5 µl droplet of the protein solution was mixed in an equal amount of a reservoir solution (50 m*M* acetate buffer at pH 5.5 containing 1.4 *M* ammonium sulfate). This droplet was equilibrated against a 1 ml reservoir solution at 291 K. Two morphologically different crystal forms, 1 and 2, were obtained 2 to 3 months later.

Generally needle-shaped crystals of form 1 with maximum dimensions of 0.6 × 0.2 × 0.2 mm were obtained. The crystals diffract X-rays beyond 2.3 Å resolution using a normal X-ray source. They belong to space group $P2_12_12_1$ with a = 47.3, b = 62.5, c = 75.6 Å, $V = 2.23 \times 10^5$ Å^3, $Z = 4$, $V_m = 2.09$ Å^3 Da^{-1}, which seem to be isomorphous to the crystals of PB92, however (van der Laan *et al.*, 1992). Occasionally in a droplet one or two pillar crystals of form 2 with maximum dimensions of 1.0 × 0.2 × 0.1 mm were grown. The diffraction power of crystal form 2 is rather weak and the resolution limit is almost 2.8 Å using a Rigaku RU300 X-ray generator (focus size 0.3 × 3 mm, 50 kV, 100 mA). The crystal data of form 2, space group $P2_12_12_1$, a = 75.82 (2), b = 57.79 (2), c = 54.19 (1) Å, $V = 2.29\,(2) \times 10^5$ Å^3, $Z = 4$ and $V_m = 2.15$ Å^3 Da^{-1} are, on the whole, close to those of SBC (Neidhart & Petsko, 1988), *i.e.* $P2_12_12_1$, a = 76.67, b = 55.65, c = 53.08 Å, $V = 2.26 \times 10^5$Å^3, $Z = 4$, though the structure analysis of form 2 made it clear that the crystal structures of form 2 and SBC are not isomorphous to each other.

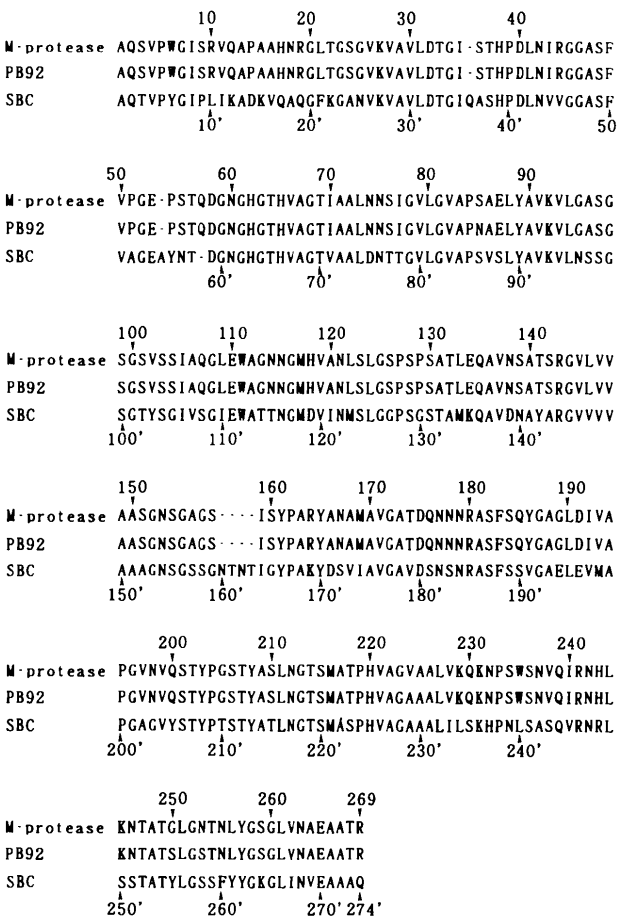


Fig. 1. Comparison of the amino-acid sequence of M-protease, PB92 and SBC. The amino-acid numbering scheme for PB92 is based on the alignment of M-protease. That for SBC is given as the lower numerals with primes.

Data collection

X-ray intensity data of form 2 were collected on Fuji imaging plate using a Weissenberg camera (Sakabe, 1983) and synchrotron radiation light source at the BL6A2 station at the Photon Factory, National Laboratory for High Energy Physics. The diffraction intensities on the imaging plates were digitized by a Fuji BAS-2000 analyzer, and processed by the *WEIS* program system (Higashi, 1989). The X-ray wavelength was 1.00 Å. The cassette radius used was 483 mm, and the size of collimator was 0.2 mm. A total of 24 185 reflections were obtained by the rotation around the needle (a) axis. There were 7897 independent reflections with $I/\sigma(I) > 1$ between 100 and 2.4 Å resolution, giving an $R_{\rm merge}$ of 0.119, which is defined as,

$$R_{\rm merge} = (\Sigma_h \Sigma_i |I_h - I_{hi}|)/(\Sigma_h I_{hi}),$$

where I_{hi} is the intensity of the ith observation of a reflection h and I_h is its mean value. The completeness of the data set is 80.6% and that in the highest resolution shell (2.49 to 2.40 Å) is 63.4%.

Structure determination

The molecular-replacement search model adopted was the 2.5 Å crystal structure of SBC (PDB entry number 1SBC). All the atoms of SBC except for a Ca^{2+} ion and water molecules were used as the search model. The search model was placed in a $P1$ orthogonal cell with $a = b = c$ = 120 Å, and the structure factors were calculated using the program *FFTSF* (Ten Eyck,

Table 1. *Standard deviations after the last cycle of refinement*

	R.m.s. deviations	Target deviations	No. of parameters
Bond lengths (1–2) (Å)	0.016	0.020	1911
Bond angles (1–3) (Å)	0.038	0.030	2607
Dihedral angles (1–4) (Å)	0.047	0.040	661
Planar groups (Å)	0.014	0.020	1681
Chiral volume (Å^3)	0.175	0.200	306
Planar (0,180) (°)	2.6	3.0	273
Single torsion contact (Å)	0.181	0.500	612
Multiple torsion contact (Å)	0.269	0.500	554
Possible hydrogen bond (Å)	0.232	0.500	117

1973). Cross-rotation functions were calculated using the fast-rotation function (Crowther, 1972), in which the resolution ranges and the outer Patterson radii for the sphere of integration were varied; the optimum results were obtained with the data between 8 and 3.5 Å resolution and the radius of 20.4 Å. The cross-rotation searches were carried out in 5° steps in all three spherical polar angles (ψ, φ, χ). The highest peak located at ψ = 35, φ = 170, χ, = 220°, which clearly showed that the crystal structure of M-protease was not isomorphous to that of SBC. The rotational parameters were refined using *PROTEIN* (Steigemann, 1974). Translation functions were calculated using the *T* function (Crowther & Blow, 1967), as implemented in the program *ELTFCALC* (Lattman, 1985). The highest peak in each Harker section calculated using 6–4 Å data gave a unique consistent solution. The molecular-replacement model (1920 non-H atoms included but Ca^{2+} ions excluded) gave an initial *R* factor of 0.472 for 1671 reflections between 7 and 4 Å resolution.

Refinement

The molecular-replacement model was refined using a combination of simulated-annealing refinement with the *X-PLOR* system (Brünger, 1990) and computer-graphics model building with the *Turbo-FRODO* software (Cambillau, 1992). The rigid-body refinement gave an *R* factor of 0.456 and after the first cycle of the slowcool protocol the *R* factor dropped to 0.262, at 7–4 Å resolution. Then the resolution range was extended to 2.4 Å resolution [7004 reflections with a $3\sigma(F)$ cut-off] and individual temperature factors for the non-H atoms were applied. The second cycle of the simulated-annealing refinement gave an *R* factor of 0.251. At this stage most amino-acid residues different to those in M-protease were changed and the side chains were fitted into a $2F_o - F_c$ map. The third of the seven cycles of refinement reduced the *R* factor to 0.209. In total, 1882 non-H protein atoms, two Ca ions and 44 water molecules were added after the following eight cycles of the *X-PLOR* refinement, lowering the *R* factor to 0.175. Final positional refinement was carried out with *PROLSQ* (Hendrickson & Konnert, 1980). After five cycles of refinement the r.m.s. deviations from ideal stereochemistry are converged, as shown in Table 1. The final *R* factor is 0.189 for 7004 reflections between 7 and 2.4 Å resolution. The coordinates of M-protease have been deposited with the Protein Data Bank.*

Results and discussion

The Luzzati plot (Luzzati, 1952) in Fig. 2 indicates that an upper limit for the estimated errors in the atomic coordinates were 0.25 Å for the refined structure.

Structural comparison with subtilisin Carlsberg (SBC)

The three-dimensional structure of M-protease is essentially similar to other subtilisins of known structure where eight helices form a basic skeleton. The overall folding of M-protease is shown in Fig. 3, superimposed on that of SBC (Neidhart & Petsko, 1988). The prominent structural differences between M-protease and SBC were observed around the deletion regions. Superposition of SBC on to M-protease using all the 269 C^{α} positions gave an overall r.m.s. difference of 1.06 Å, where five residues in SBC, 36′ and 160′–163′, were

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory (Reference: 1MPT, R1MPTSF). Free copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Reference: AM0016). A list of deposited data is given at the end of this issue.

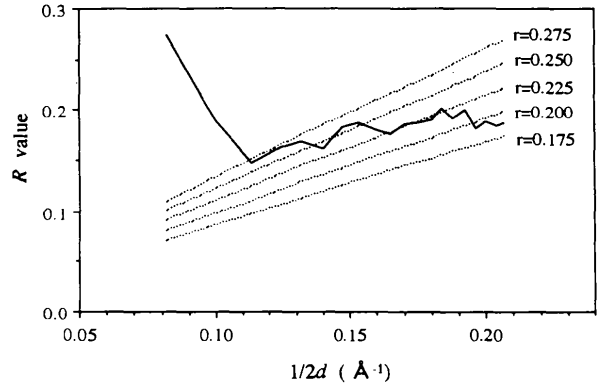


Fig. 2. Luzzati plots of *R* factor *versus* resolution (1/2*d*). All reflections in the resolution range 7.0–2.4 Å with $F_o > 3\sigma(F)$ are used.

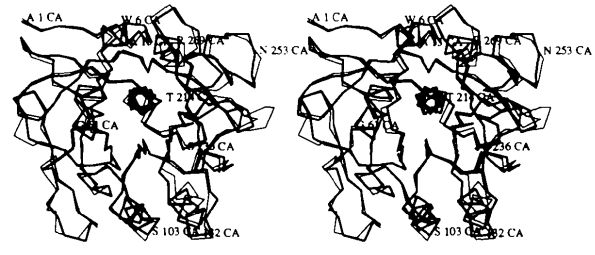


Fig. 3. Stereoview of the superposition of the C^{α} atoms in M-protease (thick lines) and SBC (thin lines). The N-terminus, initial residues in the eight helices and the C-terminus are labeled.

Table 2. *Comparison of helices in M-protease and SBC*

Helix	M-protease residues	SBC residues	R.m.s. (Å)
H1	6–11	6′–11′	0.97
H2	13–19	13′–19′	0.71
H3	62–70	63′–71′	0.29
H4	102–114	103′–115′	0.39
H5	131–143	132′–144′	1.26
H6	214–231	219′–236′	0.40
H7	237–246	242′–250′	0.58
H8	254–257	259′–262′	1.10

omitted because of the deletion in M-protease (Fig. 1). The largest differences in C^{α} positions are near the deletion region, that is, 157′ (difference 2.48 Å), 158′ (4.80 Å) and 159′ (8.40 Å). The overall r.m.s. value dropped to 0.87 Å when these three residues were excluded and to 0.82 Å when two more residues 37′–38′ (differences 3.48 and 2.48 Å, respectively) were also excluded. Results of the superposition between M-protease and SBC are shown in Fig. 4, as a function of residue number. Table 2 shows the results from the superposition of individual helices. A common Ca^{2+} ion between M-protease and SBC, Ca276, shows a positional difference of 0.38 Å.

There are three fragments of the main chain where the C^{α} atoms deviate by more than 2 Å from each other. Those of the C-terminal region (residues 262–269) have an average difference of 1.45 Å (the highest difference is 2.59 Å at Arg269) between M-protease and SBC. A large difference between PB92 and SBC is also observed at the C-terminal region (van der Laan *et al.*, 1992). Other large differences are observed on the surface loops. Larger differences occur at Asp175 (2.04 Å) and Gln176 (2.40 Å) in the surface loop (residues 175–189). The main chain for residues 175–176 is in contact with the side chain of Trp6. The corresponding contacts in SBC are those between residues 180′–181′ and Tyr6′. The indole moiety of Trp6 and the phenol moiety of Tyr6′ are in the same orientation between M-protease and SBC. The side-chain dihedral angle χ_1 is 76.5° for Trp6 and 86.7° for Tyr6′. The movement of this part, therefore, may be attributable to the larger size of Trp compared with Tyr. The other large difference of 2.01 Å between M-protease and SBC occurs at residue 252. The shift in the surface loop (residues 247–253) connecting the H7 helix with the H8 helix may be induced by a packing effect rather than by the deletion of an aromatic side chain due to the substitution of Tyr255′ (SBC) to Gly250 (M-protease). The other high deviations occur at the region from Pro129 to Arg143 a large part of which is contained in the H5 helix. This shows that the overlap of the exposed H5 helices on each molecular surface is poor. The shift of H5 may be due to the amino-acid substitution from Gly (130′ in SBC) to Pro (129 in M-protease).

The average temperature (*B*) factor of main-chain atoms is 8.6 $Å^2$, of side-chain atoms 9.1 $Å^2$, and of all protein atoms 8.8 $Å^2$. The variation of the *B* factors for both the main-chain and the side-chain atoms as a function of residue number is shown in Fig. 5. The several surface-loop regions exhibit higher *B* factors. The average *B* value for residues 53–57, 21.1 $Å^2$, is highest in M-protease as shown in Fig. 5. This shows the great flexibility of the surface loop region (residues 52–58) where the insertion at 55′ and the deletion at 57 occur among M-protease, PB92 and SBC. The same flexibility is observed for the corresponding surface loops of SBC and PB92. The average *B* value for the region 124–128

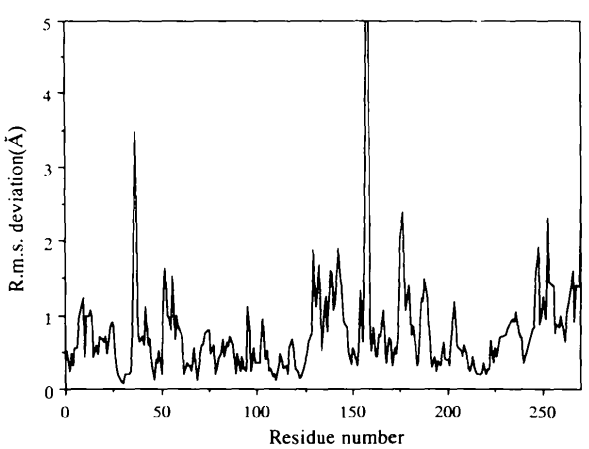


Fig. 4. Plot of the positional difference of equivalent C^{α} atoms between M-protease and SBC as a function of residue number. A total of 266 equivalent C^{α} positions are superposed.

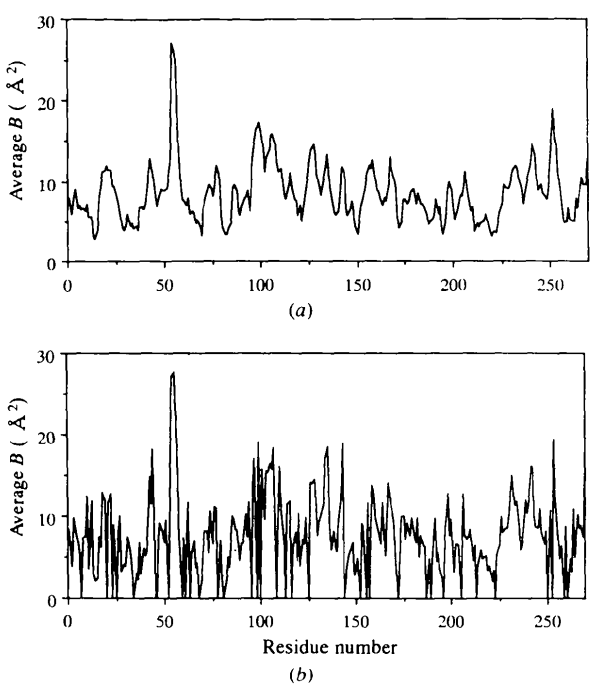


Fig. 5. The average temperature factors for (*a*) main-chain and (*b*) side-chain atoms as a function of residue number.

is highest in PB92, though that for the corresponding regions in M-protease and SBC is not so high. The average B value for residues 153′–155′ is second highest in SBC. On the other hand that for the corresponding regions in M-protease and PB92 is moderate. These results show that the flexibility of surface loops among M-protease, SBC and PB92 differs. The B factors of two Ca^{2+} ions are 10.2 and 26.6 Å^2, respectively. In the final refinement a total of 44 solvent water molecules were contained in the model. The B factors of the water molecules range from 2.0 to 48.3 Å^2 with an average of 16.1 Å^2. The rather small number of the water molecules found in the crystal structure may be partly attributed to the resolution limit of 2.4 Å and partly to the criteria for acceptance being the formation of at least two hydrogen bonds and the B value less than 50.0 Å^2.

A Ramachandran (Ramakrishnan & Ramachandran, 1965) plot is shown in Fig. 6. Most of the (φ, ψ) angles are within the acceptable regions, except for Ser55, Asn178 and Ala266. The dihedral angles (φ, ψ) of these residues are (–99.0, –104.4°), (100.0, 21.2°) and (84.5, –61.1°), respectively. All the three residues are located on flexible regions. Ser55 is contained in the most flexible loop region, and the average B value of the side chain of Ser55 is highest in M-protease (Fig. 5). Asn178 and Ala266 are also contained in a surface loop (residues 175–189) and the C-terminal region, respectively, where large differences of the C^{α} positions between M-protease and SBC were observed (Fig. 4). There remain some ambiguities in these residues because the corresponding electron densities are fairly broad. A break of the H1 helix occurs at residue 12 and helix H2 begins at residue 13. The (φ, ψ) angles of Gln12 and Lys12′(SBC) are (52.7, 39.9°) and (55.6, 38.8°), respectively. The substitution of Pro for Asp at residue 14 does not cause a break in this helix. The (φ, ψ) pair of Pro14 is (–50.8, –39.6°).

M-protease differs in sequence by four residues from PB92. Val224 is substituted by an alanyl residue in both PB92 and SBC. The side chain of Val224 is in contact with that of Ala264. Side-chain exchanges of a compensatory nature, Val224 and Ala264 in M-protease in place of Ala229′ and Val269′ in SBC, are observed. It is interesting that such compensatory changes do not occur in PB92 because Ala264 is conserved in PB92. Asn253 is substituted by a seryl residue also in both PB92 and SBC. M-protease forms intramolecular hydrogen bonds from Asn253 $O^{\delta 1}$ to Asn255 N and to Leu256 N, as shown in Fig. 7(*a*), though Ser258′ does not form any intramolecular hydrogen bonds in SBC (Fig. 7*b*). The side chain of Ser85 in M-protease and possibly that of Asn85 in PB92 have no intramolecular contacts. On the other hand, the side chain of Ser86′ forms a weak hydrogen bond to that of Lys22′ in SBC. Residue 250 is completely different among these subtilisins, that is, Gly in M-protease, Ser in PB92 and Tyr in SBC. The side chain of Tyr255′ in SBC is located on the molecular surface and has no intramolecular

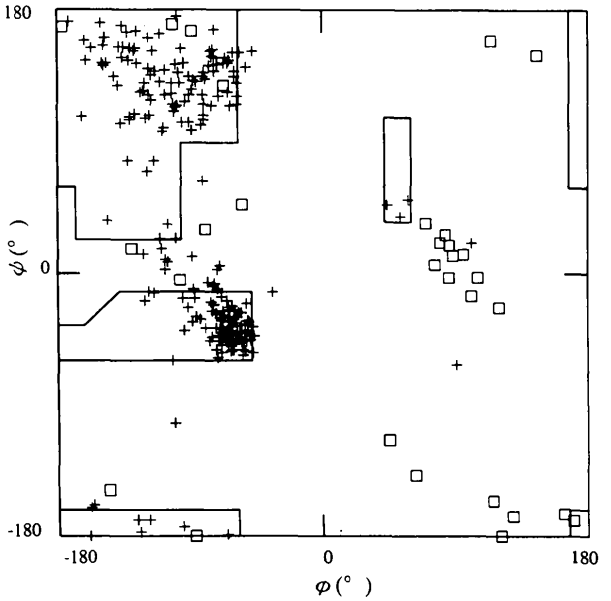


Fig. 6. A Ramachandran plot for M-protease: (□) glycyl residues, (+) the other residues.

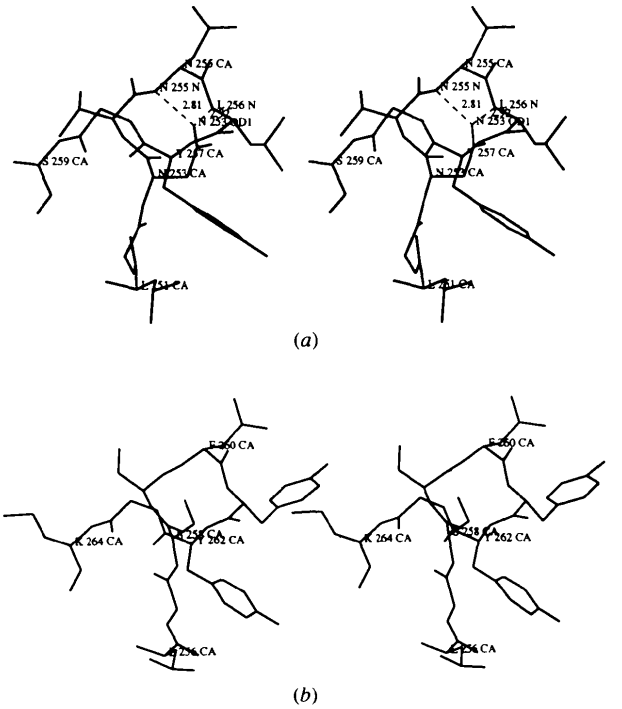


Fig. 7. Stereoviews of the portion of the H8 helix in (*a*) M-protease and (*b*) SBC. The portion in SBC is not superposed onto that in M-protease because of the large r.m.s. differences between them (Table 3). In (*a*) dashed lines show the intramolecular hydrogen bonds concerned with the side chain of Asn253.

hydrogen bond, though it is rather hydrophobic. The details of these local structures of PB92 are not clear. However these structural changes may partly explain the pH stability of M-protease.

The peptide bond between Tyr161 and Pro162 takes a *cis* conformation (ω = 6.5°). The *cis* peptide bond is also found in the same position in SBC and PB92.

Crystal packing

The crystal data of form 2 are close to those of SBC, though the structure determination of form 2 using the molecular-replacement method made it clear that crystals of M-protease are not isomorphous with those of SBC. Fig. 8 shows the packing scheme of M-protease and that of SBC in each crystallographic unit cell. One of the differences of the intermolecular contacts between M-protease and SBC is shown in Fig. 9. The residues 158′–161′ which form a β-turn in SBC protrude on its molecular surface. By the deletion of the residues 160′–163′, a short-cut in the main chain occurs in M-protease; that is, Ser158 moves to Ile159, occupying the nearby position of residue 163′ in SBC. The difference of the C^{α} positions between Gly157 and Ser158′ is 0.63 Å, and that between Ile159 and Ile164′ is 0.80 Å. Both values lie below the overall r.m.s. difference. The surface loop of residues 247–253 of the neighboring molecule related by crystallographic symmetry occupies the space which is made available by the deletion of the β-turn. As a result of the deletion in M-protease, two intermolecular hydrogen bonds are formed (Fig. 9*a*). If the β-turn is conserved in M-protease, Asn246 and Thr247 of the neighboring molecule almost coincide with residues 158′ and 159′, respectively. This leads to breakage of the packing scheme of M-protease. Therefore, we conclude that the deletion of the four residues (160′–163′) is responsible for the stable packing of M-protease, resulting in the lack of isomorphism with SBC.

Structures of the catalytic triad

The catalytic triad of M-protease is composed of Asp32, His62 and Ser215. The electron density for the catalytic triad in the final $2F_o - F_c$ map is shown in Fig. 10(*a*), and the structure of the catalytic triad is compared with that of SBC in Fig. 10(*b*). There is a significant peak (2.3σ level on a $2F_o - F_c$ map and 6σ level on an $F_o - F_c$ map) adjacent to His62 $C^{\delta 2}$. The distance between the peak center and His62 $C^{\delta 2}$ was 2.40 Å. The peak is highest in an $F_o - F_c$ map, and may be retainable as a water molecule. However it was not assigned to a water molecule because it did not fit the aforementioned criteria. The arrangement of the side chains in the catalytic triad is well conserved between M-protease and SBC, except for the disposition of Ser215 O^{γ}. The torsion angles χ_1 of Ser215 are 18.3° for M-protease and −135.7° for SBC. The difference of the O^{γ} positions between M-protease and SBC is 1.93 Å. On the contrary the differences of His62 $N^{\varepsilon 2}$ and $N^{\delta 1}$ between M-protease and SBC are 0.43 and 0.33 Å, respectively. The interatomic distances concerning the catalytic triad

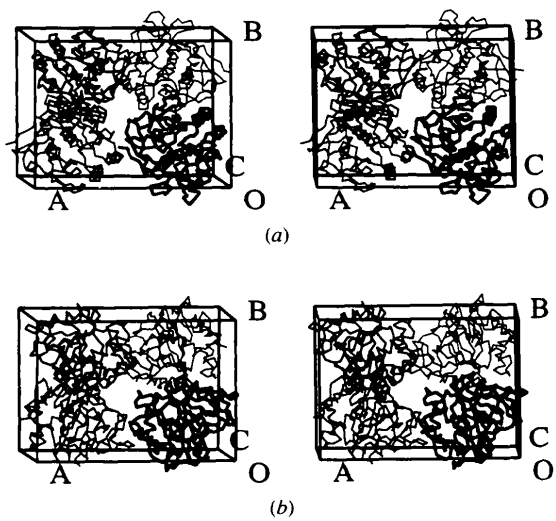


Fig. 8. Stereoviews of the crystal packing of (*a*) M-protease and (*b*) SBC.

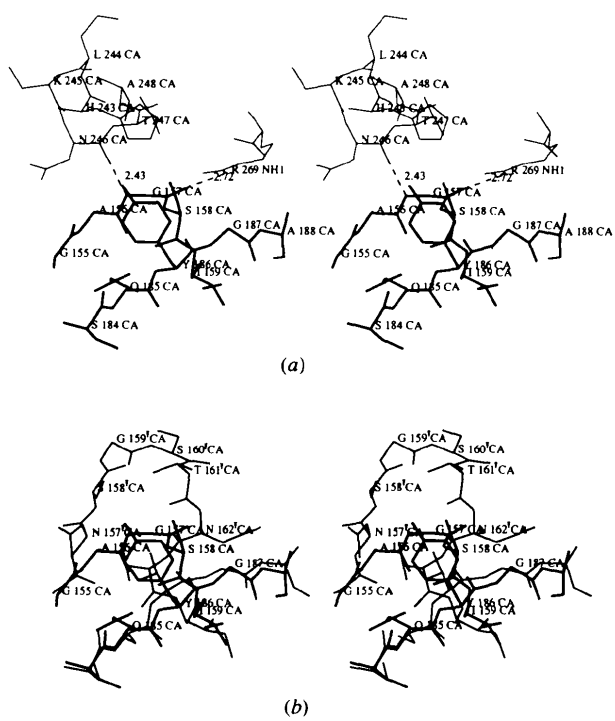


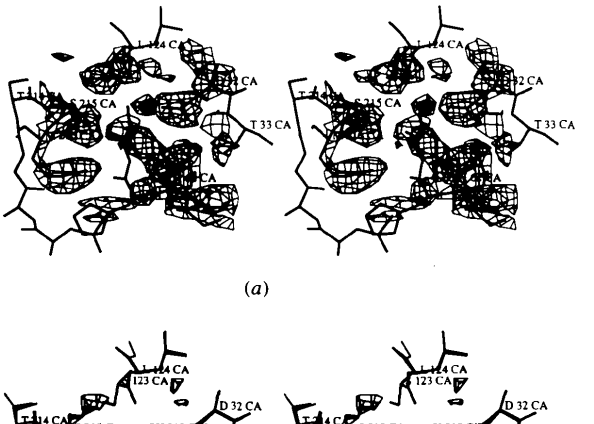
Fig. 9. Stereoviews of residues 155–159 and 184–188 of M-protease (thick lines). (*a*) A contact region of the neighboring molecule of M-protease is drawn with thin lines. Dashed lines show the intermolecular hydrogen bonds. (*b*) The corresponding portion of SBC is superposed with medium lines. By the deletion of the residues 160′–163′, it is made possible for residues 246–247 of the neighboring molecule to be in contact with Gly157 as shown above.

Table 3. *Summary of interatomic distances among the active sites* (Å)

	M-protease	SBC	PB92
Ser215 $O^{\gamma}\cdots$His62 $N^{\epsilon 2}$	3.37	2.65	2.98
His62 $N^{\delta 1}\cdots$Asp32 $O^{\delta 1}$	2.83	3.29	2.50
His62 $N^{\delta 1}\cdots$Asp32 $O^{\delta 2}$	2.80	2.65	3.10

are given in Table 3. Table 3 shows that one or two hydrogen bonds are formed between His62 $N^{\delta 1}$ and the carboxyl group of Asp32. The rather long distance of 3.37 Å between Ser215 O^{γ} and His62 $N^{\epsilon 2}$ and the angles of Ser215 C^{β}—$O^{\gamma}\cdots$His62 $N^{\epsilon 2}$ (82.2°) in M-protease suggest that no hydrogen bond is formed between Ser215 O^{γ} and His62 $N^{\epsilon 2}$. On the other hand one is formed in both SBC (Neidhart & Petsko, 1988) and PB92 (van der Laan *et al.*, 1992).

The different orientation of Ser215 O^{γ} may be induced by the modification with PMSF. An $F_o - F_c$ map calculated using the final model showed some perturbation around Ser215 O^{γ} (Fig. 10*b*). There remained a positive peak about 2 Å from the O^{γ} position. This was then treated as an SO_2 moiety in PMSF and included in the refinement, and the R value went up to 0.202. The refined B values for the tentative SO_2 group were 42.2 Å^2 for S, 41.2 Å^2 for O_1 and 41.7 Å^2 for O_2. In addition appropriate electron density for phenylmethyl moiety in PMSF did not appear in an $F_o - F_c$ map. Therefore, the SO_2 moiety was removed from the model. To construct the PMSF group and check the effect on the orientation of the catalytic triad, the structure refined at higher resolution will be required. Unfortunately, the quality of form 2 is not suitable for high-resolution analysis. We could, however, collect the X-ray data of form 1 at 1.6 Å resolution, and structure analysis is in progress in order to make a detailed comparison of the side-chain orientation of Ser215 in the catalytic triad of M-protease, SBC and PB92, and to clarify the pH stability of M-protease.



(*a*)

(*b*)

Fig. 10. Stereoviews of the active site in M-protease. (*a*) The $2F_o - F_c$ electron density contoured at 1.4σ level is superposed. (*b*) The structure in SBC (thin lines) and the $F_o - F_c$ electron density contoured at 2σ level are superposed.

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