Tobacco Etch Virus Protease: Crystal Structure of the Active Enzyme and Its Inactive Mutant


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Abstract—Tobacco Etch Virus Protease (TEV protease) is widely used as a tool for separation of recombinant target proteins from their fusion partners. The crystal structures of two mutants of TEV protease, the active autolysis-resistant mutant TEV-S219D in complex with the proteolysis product, and the inactive mutant TEV-C151A in complex with a substrate, have been determined at 1.8 and 2.2 Å resolution, respectively. The active sites of both mutants, including their oxyanion holes, have identical structures. The C-terminal residues 217–221 of the enzyme are involved in formation of the binding pockets S3–S6. This indicates that the autolysis of the peptide bond Met218–Ser219 exerts a strong effect on the fine-tuning of the substrate in the enzyme active site, which results in a considerable decrease in the enzymatic activity.

Key words: autolysis, crystal structure, cysteine proteases, TEV protease, tobacco etch virus

INTRODUCTION

Tobacco etch virus (TEV) belongs to the family of potyviruses, which, in turn, is a subfamily of a large group of positive-strand RNA viruses that are responsible for a number of plant and animal diseases [1]. A single open reading frame of the TEV RNA encodes a polyprotein with a molecular mass of about 346 kDa [2]. TEV was found to contain three proteolytic enzymes [3]: P1, HC-Pro, and NI (nuclear inclusion). At the initial stage, all of them are autocatalytically released from the polyprotein N-terminus, but only NI participates in all the subsequent stages of proteolysis. NI has a molecular mass of 49 kDa and consists of two subunits with M 21 and 27 kDa. The larger subunit is a cysteine protease; at late stages of infection, it has been shown to exist as an independent protein, which was named TEV protease. It was established that TEV protease is structurally similar to serine proteases, such as trypsin and chymotrypsin; however, it utilizes the cysteine thiol group instead of a serine hydroxyl as the active nucleophile [4, 5].

Due to its stringent sequence specificity, TEV protease is widely used in biotechnology for the endoproteolytic removal of both affinity tags and fusion proteins from recombinant target proteins [6]. According to literature data, in contrast to factor Xa, enterokinase, and thrombin, TEV protease has so far not been reported to cleave genetically engineered fusion proteins at unintended locations. A consensus cleavage sequence for TEV protease starting from the position P6 is Glu-Xaa-Xaa-Tyr-Xaa-Gln*Ser/Gly (symbol * indicates the scissile bond) [7], whereas the best sequence for cleavage is Glu-Asn-Leu-Tyr-Phe-Gln*Gly/Ser [8]. Although the specificity of TEV protease toward these amino acid sequences is very high, it is not absolute. It was shown that the enzyme can cleave itself at the bond Met218–Ser219 producing a truncated protein with a significantly lower activity [3, 9]. Such autolysis was observed only in vitro and the cleavage site turned out to be unexpected, since the sequence that includes this site, -Gly-His-Lys-Val-Phe-Met*Ser-, has little in common either with the consensus or the best-processed sequences: only the Phe in position P3 and Ser in position P1 coincides with the canonical sequences. For these reasons, the autolysis phenomenon has been a subject of recent studies [9], and a number of stable mutants of this enzyme have been proposed.

We present here the crystal structures of two variants of TEV protease. One of them is TEV-S219D mutant, which is an active enzyme stable to autolysis. Another mutant, TEV-C151A, is inactive, since the catalytically active Cys151 residue is replaced by Ala. Both proteins have been crystallized as complexes with either the product of proteolytic reaction (in the case of TEV-S219D) or the intact substrate (TEV-C151A) [10].
RESULTS AND DISCUSSION

Overall Structure

There is a dimer consisting of molecules A and B in the asymmetric unit of both TEV-S219D (Fig. 1) and TEV-C151A crystals, however, in each case it has quite different structure. Under physiological conditions and in solution, TEV protease exists as a monomer (unpublished data). Since the mode of enzyme dimerization is not the subject of this paper, we are not going to discuss this problem here, the detailed description of the aggregation can be found in [10]. Despite the fact that the dimers of the mutants of TEV protease are quite different, the structures of monomers are very close (the root-mean-square deviation between the positions of Ca-atoms is only 0.24 Å).

TEV protease consists of two domains in the form of antiparallel β-barrels, with the catalytically important amino acid residues (His46, Asp81, and Cys151) located at the interdomain interface (Fig. 2). The C-terminal fragment of TEV protease (amino acid residues 222–236) is disordered; however, we localized the last seven residues 230–236 belonging to the molecule B of the active mutant TEV-S219D, i.e., to the molecule B that is symmetry mate of the independent molecule B in the crystal. These residues form β-strand parallel to the strand 62–67, extending the existing four-stranded β-sheet (Fig. 1). The interaction of this kind could arise only in the process of crystallization and must therefore be considered to be a crystallization artefact.

Active Site

The active sites of both mutants of TEV protease have exactly the same structures, including both the oxyanion hole and the binding pockets. Moreover, the conformations of polypeptides bound to the active sites are also almost the same, with both adopting extended conformations (Fig. 3). As expected, in the case of the mutant TEV-S219D, the heptapeptide substrate Ac-Glu-Asn-Leu-Tyr-Phe-Gln-Gly was hydrolyzed at the bond between the Gln and Gly residues, and the larger Glu-Asn-Leu-Tyr-Phe-Gln-Gly was hydrolyzed at the Met218–S219 bond producing a truncated enzyme with greatly diminished activity [9]. The structures of the S219D and C151A mutants of TEV protease show that the cleavable bond Met218–S219 is located at the distance of only 5 Å from the active site and that the residues that immediately follow S219 are involved in the formation of binding pockets S1–S6 (Fig. 3). It seems therefore likely that the autolysis is an intramolecular event. We also found that TEV protease would not cleave the polypeptides having the sequence of the internal cleavage site Gly-Gly-His-Lys-Val-Phe-Met*Ser-Lys-Pro-Arg-Arg corresponding to amino acid residues 212–223. Therefore, we suggest that the concentration-inducible autolysis must be initiated by a conformational change of the flexible C-terminal tail of the enzyme, so that the sequence mentioned above could be accommodated in a proper way to be cleaved. The crystal structures of the active and inactive mutants also provide an explanation why the truncated enzyme is much less active. The reason is that the residues that immediately follow the peptide group cleaved during the autolysis are involved in the formation of the S1–S6-binding pockets. Therefore, their removal will certainly affect the enzyme–substrate interaction.

CONCLUSIONS

As already mentioned above, wild type TEV protease undergoes autolysis in vitro at the Met218–Ser219 bond producing a truncated enzyme with greatly diminished activity [3, 9]. There is no evidence that this process occurs in vivo and has any physiological significance. However, autolysis is certainly a problem during the enzyme production and purification, since it may be induced by increase in the enzyme concentration. The mutant TEV-S219D is tenfold more stable to autolysis than the native protein without any decrease in the enzymatic activity [9]. The structures of the S219D and C151A mutants of TEV protease show that the cleavable bond Met218–S219 is located at the distance of only 5 Å from the active site and that the residues that immediately follow S219 are involved in the formation of binding pockets S1–S6 (Fig. 3).

EXPERIMENTAL

Preparation, isolation and crystallization of the proteins. Both mutants of TEV protease were expressed as maltose-binding fusion proteins (MBP); the N-terminal tags Gly-His6 (for TEV-S219D) and His6 (for TEV-C151A) were used as affinity tags for the corresponding mutants of TEV protease [12]. Either TEV protease or tobacco vein mottling virus (TVMV) cleavage sites were constructed between MBP and the His tags. The carrier proteins were cleaved by TEV protease or TVMV protease. Both proteins were produced in E. coli BL21(DE3) cells that contained an accessory plasmid encoding either TVMV or TEV protease for intacellular cleavage of MBP.

The cells were grown at 37° C Erlenmeyer shaken flasks containing Luria broth supplemented with the appropriate antibiotics. The culture was induced for 4 h by IPTG to a final concentration of 1 mM with the