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Bioorganic & Medicinal Chemistry Letters 13 (2003) 21–24

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

True Interaction Mode of Porcine Pancreatic Elastase with FR136706, a Potent Peptidyl Inhibitor

Takayoshi Kinoshita,[a,*] Isao Nakanishi,[a] Akihiro Sato[b] and Toshiji Tada[c]

[a]*Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan*
[b]*Analytical Research Laboratories, Fujisawa Pharmaceutical Co. Ltd., 5-2-3, Tokodai, Tsukuba, Ibaraki 300-2698, Japan*
[c]*Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 593-8570, Japan*

Received 19 September 2002; accepted 5 October 2002

Abstract—The crystal structure of porcine pancreatic elastase (PPE) complexed with a potent peptidyl inhibitor, FR136706, was solved at 2.2 Å resolution. FR136706 fits snugly into the extended active site pocket. The benzene moiety of FR136706 induced dramatic movement of the side chain moiety of Arg217 and both moieties formed a π–π interaction, which has never been found previously in structures of PPE complexed with inhibitors. This novel interaction mode may lead to design of new types of inhibitors.
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Introduction

Elastase is a serine protease classified in the chymotrypsin family, and is possibly the most destructive enzymes having the ability to degrade virtually all of the connective components in the body. Uncontrolled proteolytic degradation by elastase has been implicated in a number of pathological conditions. Pancreatic elastase (EC 3.4.21.36) causes the fatal disease pancreatitis, and leukocyte elastase (EC 3.4.21.37) has been implicated in a number of inflammatory disorders. Highly selective drugs for each elastase can suppress the side effects due to other elastases.[1]

Structural studies have been described using two elastases: human leukocyte elastase (HLE) consisting of 218 amino acids and porcine pancreatic elastase (PPE) consisting of 240 amino acids. Although the amino acid sequence homology of the two elastases is only 37%, the backbone architecture of both elastases is conserved, and the structures of the active sites near the cleavage site are very similar. The S1 pocket recognizes small hydrophobic groups such as alanine, valine or leucine. The S2 and S3 pockets are wide and broad hydrophobic areas. On the other hand, the S4 and S5 pockets, which are remote from the cleavage site, are quite different. Careful observation of this difference may lead to compatible structure based drug design (SBDD) and may allow design of selective inhibitors for the respective elastases. Nevertheless, a large number of non-selective inhibitors have been researched and developed by SBDD.

FR136706, 2-[4-[(*S*)-1-[(*S*)-2-[(*RS*)-3,3,3-trifluoro-1-isopropyl-2-oxopropyl]aminocarbonyl]pyrrolidin-1-yl-] carbonyl]-2-methylpropyl]aminocarbonyl] benzoylamino]-acetic acid (Fig. 1a), is an example of a potent peptidyl inhibitor with no selectivity for the two elastases.

FR136706 is structurally similar to MDL101146 (Fig. 1b), and structures of HLE and PPE complexed with MDL101146 have been already reported.[2] Based upon those structures, the binding modes of FR136706 can be easily predicted because of the similarity in their chemical structures. Thus, the terminal valine residue is deduced to bind at the S1 pocket. The P1–P4 residues of FR136706 can then be assigned corresponding to the S1–S4 pockets of the enzyme. However, the terminal glycine moiety can not be confirmed as a P5 residue from the assumed binding model.

Selectivity for the two elastases may be easily achieved if the real interaction modes of an inhibitor are available for both enzymes. The precise and complete structure of

*Corresponding author. Fax: +81-298-478313; e-mail: takayoshi_kinoshita@po.fujisawa.co.jp

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PII: S0960-894X(02)00852-1

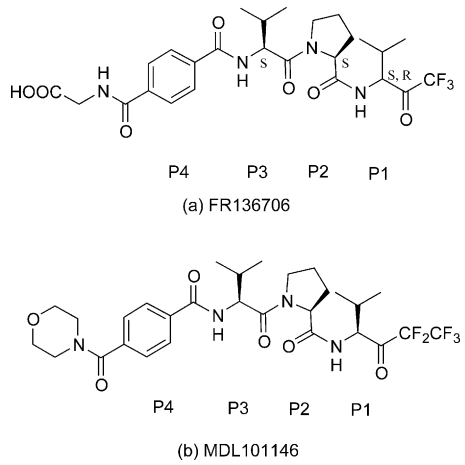

Figure 1. Chemical structures of elastase inhibitors. (a) Structure of FR136706. This compound is suggested to have P1–P4 residues from a model of the complex. The terminal glycine moiety can not be assigned. (b) Structure of MDL101146. P1–P4 residues assigned based upon the structure of the complex.

the FR136706–HLE complex can be easily modeled based upon the MDL101146–HLE complex. On the other hand, the binding mode of the FR136706–PPE complex is difficult to predict due to the following reasons. First, the morpholino moiety of MDL101146 in the PPE complex is exposed to the solvent region. Second, the Arg217 side chain is unnaturally fastened by an adjacent elastase molecule in the MDL101146–PPE complex crystal. Arg217 is essential for the recognizing P4 residue[3] and for biological activity. The chemical modification of Arg217 leads to an 85% loss of activity toward the specific substrate *N*-succinyltrialanine *p*-nitroanilide.[4] The crystal packing of the MDL101146 complex is mainly responsible for the structural unreliability around Arg217. The crystal of the complex belongs to the trigonal form with space group *R*3, which is different from the orthorhombic form of the native and many other complexes. This packing ensures that MDL101146 complex molecules can not make the same interaction network as in the orthorhombic crystals.

In order to succeed in the selective SBDD, it is necessary to investigate the true and precise interaction mode including the side chain of Arg217. Here we determine the structure of FR136706–PPE complex with natural interactions. Subsequently, careful observation and comparison of these regions in both elastases may lead to successful SBDD (Table 1).

Materials and Methods

Commercially available PPE was purchased from Worthington Biochemicals and used without further purification. FR136706 was produced at our company. The complex solution was prepared by directly dissolving the inhibitor at a final concentration of 1 mM into the protein solution. Crystals of the complex were prepared under the similar crystallization conditions of native PPE.[5]

Table 1. Data collection and refinement statistics of FR136706 complexed with porcine pancreatic elastase

Data collection and processing	
Space group	$P2_12_12_1$
Unit cell parameters (Å)	$a=51.29$, $b=58.31$, $c=75.52$
Resolution (Å)	2.20
R_{sym} (%)	8.2 (15.1)
Completeness (%)	94.6 (90.9)
Redundancy	3.1 (1.5)
$I/\sigma(I)$	9.6 (4.4)
Refinement	
R_{cryst} (%)	21.5 (22.7)
R_{free} (%)	24.1 (26.2)

Values in parentheses are for the highest shell (2.35–2.20 Å).

X-ray data for the complex were collected at room temperature on a Rigaku R-AXIS-IIc. Graphite monochromatized MoK_α radiation ($\lambda=0.7107$ Å) was produced by a Rigaku RU-200 X-ray generator operated at 50 kV and 90 mA. The oscillation images were indexed and integrated by the program PROCESS.[6]

The difference Fourier map was calculated using the phases and amplitude obtained from the native structure[7] (PDB code: 3EST) without ions and solvent molecules. Active serine Oγ was in an envelope of the density continuous with the inhibitor and likewise covalently attached. A model with the *S* configuration at Cα of the P1 valine residue could easily fit onto the difference map.

After fitting the inhibitor onto the difference Fourier map, the areas of ions existing in the native structure were checked. A sulphate ion and a calcium ion were conserved in the native structure, and these ions were assigned at the envelope center with the highest density of the map. The model of the complex was used as the starting point of refinement, which included a rigid-body refinement step, slow cooling step, Powell minimization step and a temperature factor refinement. Water molecules were then picked up in a difference map at the 3 σ level and minimization was performed. The programs X-PLOR,[8] Quanta (Accelrys Inc.) and X-solvate (Accelrys Inc.) were used for refinements, model building and picking water molecules, respectively. Coordinate data have been deposited in the Protein Data Bank under the code 1MMJ.

Results and Discussion

Crystals of FR136706 complexed with PPE grew in 1–2 months and had typical dimensions of 0.4 × 0.15 × 0.15 mm. These crystals belonged to the orthorhombic form, which is the same as those of the other many complexes. Within a 5 Å distance around the side chain of Arg217, no other PPE molecules except for water molecules were present. The side chain of Arg217 therefore has no conformational change due to alignment in the crystal. Therefore, the conformation in this crystal should reflect the in vivo situation.

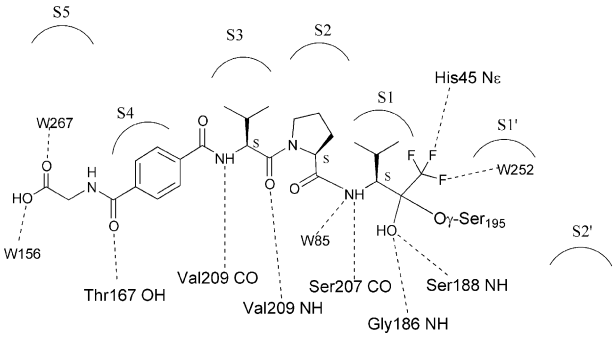

Figure 2. Interaction of FR136706 complex. FR136706 binds to PPE by seven hydrogen bonds, a covalent bond and many hydrophobic interactions. Dotted lines show hydrogen bonds in the complex.

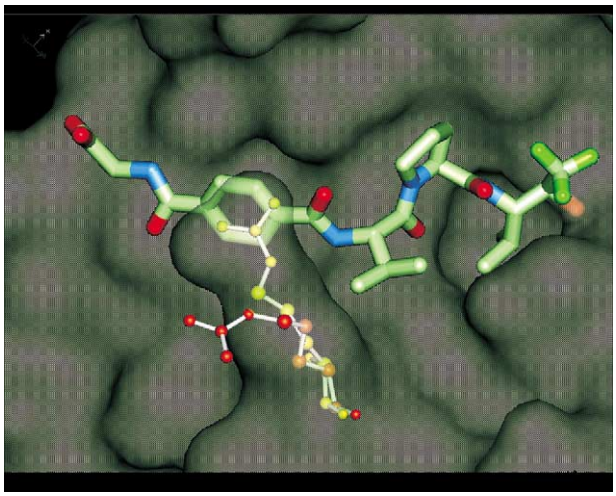

Figure 3. FR136706 (stick model) on the surface of PPE. The two side chains of Arg217 in the FR136706 and MDL101146 complexes are shown as yellow and red ball-and-stick models, respectively. The side chain moiety of Arg217 greatly moves and caps the benzene moiety of FR136706. Both moieties form a π–π interaction.

The Cα backbone structures of holoenzyme in the native and FR136706 complex form are very similar to each other, with a r.m.s.d value of 0.31 Å. The Cα backbone structures of holoenzyme in the native and MDL101146 complex are also similar with a r.m.s.d value of 0.48 Å. Concerning backbone atoms, the structure of PPE itself is very rigid and unchanged when inhibitors bind to PPE, although some enzymes, such as aldose reductase, are known to have big movement in backbone structure through inhibitor-inducing.[9] Therefore, careful and precise observation of the slight but critical movement of side chains can directly lead to successful SBDD.

Near the cleavage site, FR136706 fits snugly in the extended active site pocket with a similar interaction mode to the other inhibitors.[10,11] FR136706 makes a total of seven hydrogen bonds, a covalent bond and many hydrophobic interactions with the PPE molecule (Fig. 2). The oxy-anion hole, which plays a key role in the recognition and stabilization of the carbonyl group of a substrate, is occupied by a hydroxyl group, which forms hydrogen bonds with Gly186 NH and active

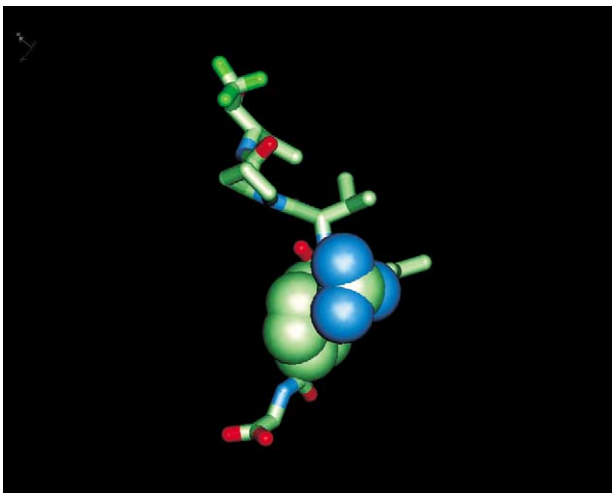

Figure 4. An interaction between the benzene moiety of FR136706 and the guanidyl moiety of Arg217. Both moieties forming a π–π interaction are shown by space-filled sphere model. The other part of FR136706 is shown as a stick model.

Ser188 NH (2.72 Å and 2.65 Å). FR136706 also forms an antiparallel sheet structure with Ser207 and Val209 by three hydrogen bonds: P1 NH to Ser207 CO: 3.08 Å; P3 CO to Val209 NH: 2.86 Å; P3 NH to Val209 CO: 2.71 Å. A fluorine atom and His45 Nε make a hydrogen bond with a distance of 2.66 Å. Hydrophobically, the P1, P2 and P3 side chains interact with the corresponding S1–S3 pockets. The terminal glycine turns to the solvent region, and the carbonyl group forms hydrogen bonds with two water molecules as well as with the side chains of Arg21 and Ser52 in the symmetry-related PPE molecule. The carbonyl group of the P4 residue also makes a hydrogen bond with Thr167 Oγ (2.86 Å).

The side-chain moiety of the noteworthy Arg217 residue locates on the benzene moiety of FR136706 and both moieties form a π–π interaction (Fig. 3). The average distance between the benzene atoms of the inhibitor and the guanidyl atoms of Arg217 is 3.5 Å. In the MDL101146 complex, Arg217 does not cap the benzene moiety at all. Arg217 in the native structure is apart from the protein surface and exposed to the solvent region. Therefore, the conformation of Arg217 is suggested to be induced by FR136706 binding and to be important for inhibitor recognition. As a result of Arg217 movement, the S3 pocket becomes clearer, and is extended along with the ethylene chain of Arg217. In the native structure, the S3 pocket is broad and seems to be unimportant for inhibitor recognition. Furthermore, Arg217 movement results in emergence of a clear S5 pocket, although the side chain of Arg217 partially occupies the pocket in the MDL101146 complex (Fig. 4).

Conclusion

A novel interaction mode in an FR136706–PPE complex involving unique Arg217 movement has been discovered. Based upon this structure, we believe the activity of FR136706 can be improved effectively. Initi-

ally, PPE selective design is considered, since HLE selective design can be applied more effectively if PPE structures are available and are understood precisely.

The P1 valine residue exactly fits the S1 pocket, suggesting that the P1 residue is critical. The S2 pocket has an extra hydrophobic and broad area extended in the directions to the Cβ and Cγ atom of the P2 proline residue. Practically, several modifications toward this space to obtain effective inhibitors have already been reported.[12]

The P3 valine residue can be replaced with bigger groups such as leucine or isoleucine, since Arg217 movement causes extension of the S3 pocket. In the native or other complexes, the S3 pocket is broad or ambiguous because Arg217 is in the solvent region. Therefore, it has been difficult to come up with effective design for the S3 pocket.

The P4 benzene moiety sits down on the hydrophobic surface consisting of the side chains of Phe208, Val88 and Ala89, and is capped by the Arg217 side chain. The benzene moiety sandwiched by the side chains of PPE is essential for inhibitor binding. A water molecule, forming a hydrogen bond with Asp86, is also present 4.8 Å from the same plane of the benzene moiety. As an application of a method for mimicking the structural water molecule,[13] the water molecule can be introduced to the benzene moiety with two or three spacer atoms.

The terminal glycine residue is exposed to the solvent region and interacts with an adjacent PPE molecule. Therefore, the glycine can not be assigned as a P5 residue at all, and does not participate in the interaction, although it does play an important role to increase the solubility of FR136706 itself. In fact, the inhibitor with the glycine removed from FR136706 has the same activity for elastases. On the other hand, the S5 pocket, newly emerged by Arg217 movement, is empty and a hydrophobic group can be introduced to this pocket, consisting of Trp164 and ethylene chain of Lys219.

Based upon the structural information from this experiment, FR136706 derivatives selective for PPE may be produced. Furthermore, compatible SBDD using the structures of both complexes with PPE and HLE may lead to selective and effective inhibitors. Careful observation of the structural differences of the S3, S4 and S5 pockets in both elastases should lead to success.

Acknowledgements

We would like to thank Dr. D. Barrett, Medicinal Chemistry Research Laboratories, for helpful discussion and critical evaluation of the manuscript.

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