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MOLECULAR STRUCTURE OF FR901277, A NOVEL INHIBITOR OF HUMAN LEUKOCYTE ELASTASE, AND ITS BINDING MODE SIMULATION

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Abstract: X-ray crystal structure analysis of FR901277, a novel inhibitor of human leukocyte elastase, was performed and revealed that the lipophilic side chains are located towards the outside of the molecule. Binding simulation using computational methods showed that these lipophilic moieties could bind to the hydrophobic binding pockets of HLE.

Human Leukocyte Elastase(HLE) is an enzyme that hydrolyzes several connective tissue components such as elastin, proteoglycan and certain types of collagen, and may play a role in the destructive processes associated with chronic inflammatory diseases such as emphysema.[1] FR901277 is a natural product which was isolated from the culture filtrate of *streptomyces resistomicificus* and is a potent inhibitor of HLE; $IC_{50}=1.8x10^{-7}$ M.[2] The gross chemical structure of FR901277, shown in Fig. 1, was determined by amino acid analysis and various spectroscopic methods such as MS and NMR, and consists of four normal amino acids (L-ORN(1), L-THR(2), L-PHE(5) and L-VAL(7)) and three unusual amino acids (dehydroxyTHR(3), AA(4) and AA(6)) and is a unique bicyclic peptide compound. Utilizing the above mentioned spectroscopic methods, the absolute configuration (L or D) of the Cα atoms of AA(4) and AA(6), and the absolute configuration (R or S) of the hydroxy group of the side chain in AA(4) could not be explicitly determined. It is of importance for medicinal chemists to obtain structural information regarding ligand molecules. Also it is helpful if complex structures with their receptors are available in order to rapidly and efficiently create new drug candidates. Generally, elastases possess a narrow binding pocket called S1,[3] which specifically recognizes small hydrophobic moieties of ligands.[4] It is also known that an oxyanion hole next to the S1 binding pocket stabilizes the ligand-elastase complex by electrostatic effects.[4] FR901277, a selective inhibitor of elastase, is considered to be using these binding sites. However, it was deemed quite difficult to predict the binding mode of FR901277 to HLE without full information on the three-dimensional structure, since this compound has several hydrophobic moieties which may bind to the S1 pocket. Therefore, we carried out a crystal structure determination of FR901277 and its binding mode simulation applying the above mentioned binding mode assumption, by means of computational methods. In this letter, we describe the structural features of FR901277 and discuss the predicted binding mode to HLE.

Experimental

Crystal structure analysis

Colorless prismatic crystals of FR901277 hexahydrate ($C_{47}H_{63}N_9O_{13}\cdot 6H_2O$) were obtained from ethylene glycol/water solution and the intensity data was measured with graphite monochromatized Mo-Kα radiation using

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a Rigaku AFC-5R four-circle diffractometer. Crystal data : space group $P2_12_12_1$, a = 21.352(9), b = 21.529(9), c = 13.262(9) Å and Z = 4. A total of 6554 independent reflections were collected using a crystal sealed into a glass capillary tube, up to 55.0° in 2θ. The initial phase was solved by SHELX86[5] and one FR901277 molecule was completely located after several Fourier syntheses. Also, water molecules could be found at eight sites in an asymmetric unit from the difference Fourier map. Based on peak height analysis, four of the eight peaks were assigned as water molecules with full occupancy whereas the other four were those with half occupancy. The refinement of the atomic coordinates was done using 2760 reflections with $I > 1.5\sigma(I)$ by full-matrix least-squares methods in the teXsan program package.[6] The final R-value was 0.10, and the positive and negative residual electron densities were 0.77 and -0.31 e/Å^3, respectively.

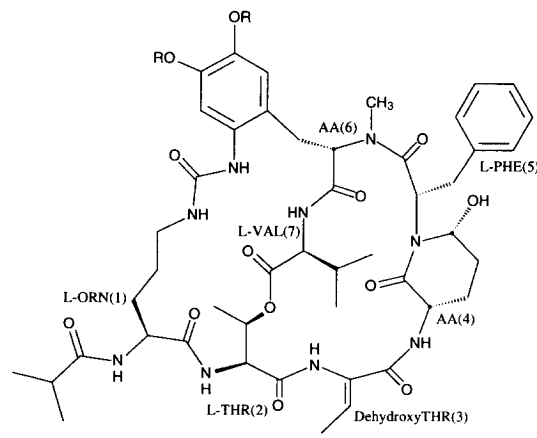

Figure 1 Chemical structure of FR901277 (R=H) and FR134043 (R=SO_3Na). Amino acid name is followed by number in parenthesis. The chiralities of the three dotted atoms in AA(4) and AA(6) were unknown, while those of the other amino acids are L configuration.

Binding mode simulation

As a base model of the FR901277 molecule, the molecular structure determined here was used. The structure of HLE registered in the PDB with the code name, 1hne,[7] was used as a receptor model, after removing all water and ligand molecules. Based on the binding mode hypothesis mentioned above, the docking simulation was performed using the program GREEN(Ver.5.01),[8] followed by FlexiDock equipped with a SYBYL6.4 modeling system;[9] At first, following six parts were chosen as P1 small hydrophobic moieties next to negatively charged carbonyl groups, which locate in the oxyanion hole. Thus, the isopropyl group at the N terminus, the side chain atoms of L-THR(2), dehydroxyTHR(3), AA(4) and L-VAL(7), and the N-methyl group of AA(6). Next, in all cases, the FR901277 molecule was manually placed into the binding site so as to locate a hydrophobic moiety and a negatively charged group in the S1 binding pocket and the oxyanion hole of HLE, respectively. Docking simulation was then done using the Monte Carlo simulation mode of GREEN, using a precalculated energy grid around the HLE active site. During each simulation, the bicyclic framework of FR901277 was regarded as rigid whereas the N-terminal and side chain groups of L-PHE(5) and L-VAL(7) were treated as flexible. Further investigation by FlexiDock, considering the side chain flexibility around the active site of HLE, was applied to the most significant orientations obtained from the GREEN simulation as the last step.

Results and Discussion

Structural features of FR901277

All water molecules hydrogen-bonded with FR901277 and formed complicated hydrogen bond networks. The molecular structure of FR901277 in a crystalline state is shown in Fig. 2. All the normal Cα atoms were L configuration while the hydroxy group in the AA(4) side chain was R configuration. The orientation of the methyl group in the dehydroxyTHR(3) side chain was Z form. Except for the N-methyl amide bond between L-PHE(5) and AA(6), all amide bonds were trans configuration. These amide bonds, including cis N-methyl amide bond were not largely distorted. Also, the φ-ψ angles of the normal amino acids were in the stable region.[10] These features were also observed in the solution structure of FR134043 (see Fig. 1),[11] a disulfonated derivative of FR901277. The RMSD for the main chain atoms of both structures was 0.48 Å and they were conformationally quite similar. One large difference is the number of intramolecular hydrogen bonds which stabilize the bicyclic framework of these molecules; that is, the number of hydrogen bonds in crystalline FR901277 was two while that in the solution structure of FR134043 was one. The hydrogen bond between the NH of L-VAL(7) and OH of AA(4) was common in both structures. The newly found hydrogen bond was formed between the NH of AA(4) and the carbonyl oxygen atom of L-VAL(7). With this new hydrogen bond, the cyclic framework composed of the main chain atoms from L-THR(2) through L-VAL(7) became more rigid.

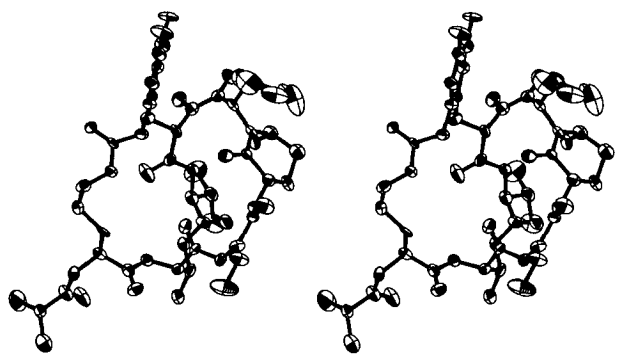

Figure 2 Stereoview of molecular structure of FR901277 showing 40% probability displacement ellipsoids. Hydrogen atoms are omitted for clarity.

The two phenyl rings in the molecule were close to each other and they are almost perpendicular. Since the shortest distance between the two phenyl rings was 2.8 Å, this perpendicular orientation is stabilized by a CH/π interaction. From these observations, including the solution structure of FR134043, the overall molecular conformation of FR901277 is considered to be rigid and the active conformation for binding to HLE would not be far from this conformation. The most characteristic feature of this molecule is that many hydrophobic moieties, such as the isopropyl groups at both the N-terminus and L-VAL(7), side chain atoms in dehydroxyTHR(3), AA(4), L-PHE(5) and so on, are located on the outside of the whole molecule. This feature allows interesting speculation regarding the binding mode between HLE and FR901277, since it is well known that there are several hydrophobic binding pockets in the active site of HLE.[12] The details about this are described in the next section.

Simulated Binding Mode

Docking simulation by GREEN gave only one significant orientation of FR901277 in the HLE binding site when the side chain atoms of dehydroxyTHR(3) were initially placed at the S1 binding pocket. That is, after simulation there were no large intermolecular contacts between FR901277 and HLE, and the S1 site was shallowly occupied by the methyl group of the dehydroxyTHR(3) side chain. In the other five cases, stable orientations could not be found and the S1 pocket was no longer occupied after simulation. Further investigation of the complex structure for the energetically most stable orientation obtained by the GREEN simulation was performed by FlexiDock, rotating the side chains of HLE around the FR901277 binding site, as well as the side chains and the N-terminus of FR901277. After this simulation, the S1 pocket was more deeply occupied by the methyl group, and improved recognition by the S1 pocket was noted. The binding state resulting from this simulation is shown in Figs 3 and 4. A most remarkable feature in this binding mode is that all the hydrophobic moieties, except the N-terminal isopropyl group of FR901277, occupied the hydrophobic binding sites or area in the HLE active site. That is, the hydrophobic side chain of dehydroxyTHR(3), L-THR(2), AA(4) and L-PHE(5) were located at S1, S2, S1' and S2' binding sites, respectively. Also, the isopropyl side chain of L-VAL(7) was towards the hydrophobic wall constructed by the His-57, Ala-60, Asn-61 and Val-62 residues of HLE. The catechol part of the AA(6) side chain was located towards the outside of the binding site, facing the two OH groups to the solvent region, and was deemed not to be involved in the direct interaction with the HLE since there were no HLE amino acid residues within 4.6 Å of this catechol ring. However, a positively charged region, shown as red surfaces in Fig. 3, derived from the Arg-147 side chain presented near the catechol ring. This result explains the fact that FR134043, a disulfonated derivative of the catechol group in FR901277, has a higher affinity to HLE, since a coulombic interaction between a positively charged arginine side chain and negatively charged sulfonate groups could be anticipated by this model. The L-ORN(1) and the N-terminal moiety did not have a strong interaction with HLE. In the complex structures of serine proteases with single chain peptide or peptide-mimic inhibitors, this L-ORN(1) corresponds with the P3 subsite[12] of inhibitors which form a short antiparallel β-sheet with the peptide backbone of Val-216 in HLE.[4,12] Though such an interaction, which is typical for the binding of peptides to serine proteases, was not observed in this model, it is expected that this kind of interaction will be possible if induced fit occurs or FR901277 slightly changes its bicyclic conformation. From the above mentioned observations, therefore, this simulated binding model can reasonably explain the high affinity and specificity of FR901277 against HLE. If this model reproduces well the true complex structure, the factor most contributing to stabilization of the complex is considered to be hydrophobic interactions.

Conclusion

We have succeeded in crystal structure analysis of FR901277 and shown its unique structural features.[14] The bound state to HLE was predicted using computational methods. We believe this structural information will be helpful for the improvement of several properties of FR901277, such as water solubility, molecular weight and inhibitory activity.

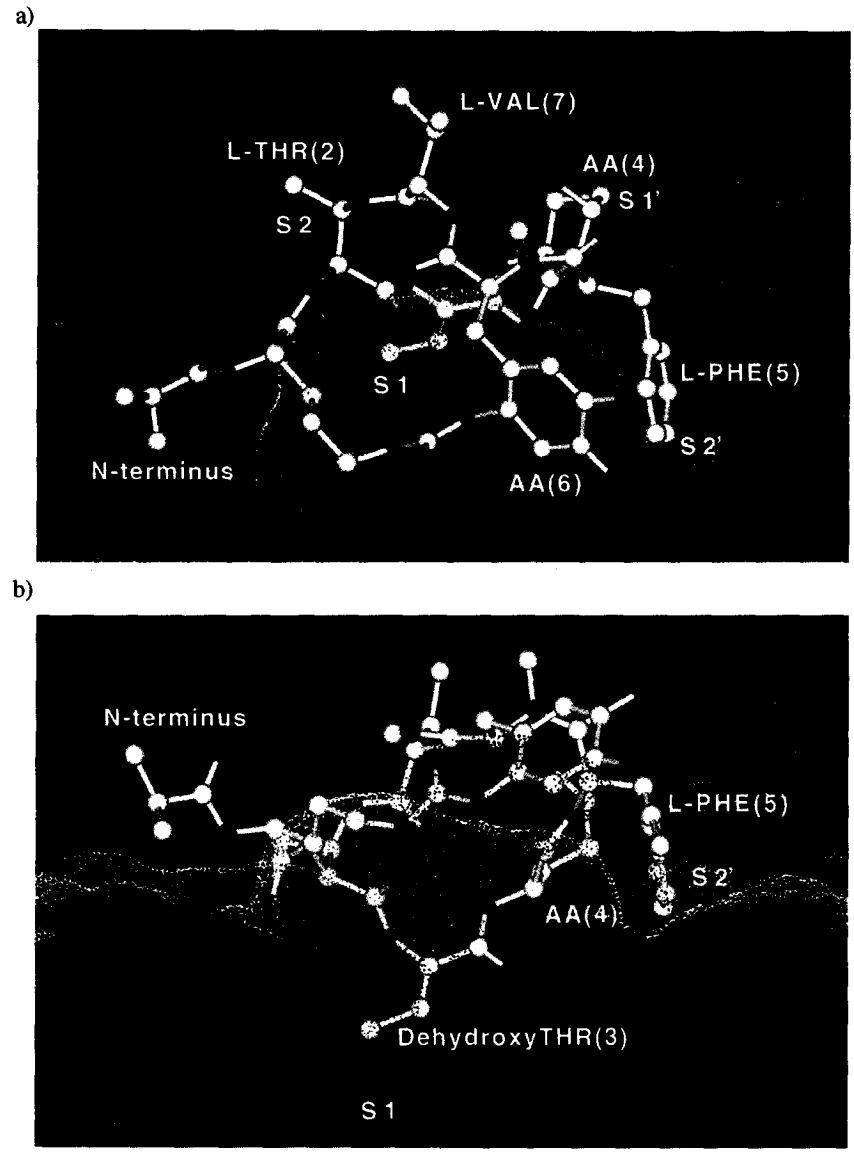

Figure 3 The simulated binding mode between FR901277 and HLE. The active site of HLE is depicted by the dot surface. Yellow to green regions are electrostatically neutral while the red area is positively charged. FR901277 is shown by a ball and stick representation. Atom colors are as follows:carbon in yellow, nitrogen in blue and oxygen in red. All hydrogen atoms are omitted for clarity. Several residue names and binding pockets were denoted with white letters. b) is viewed from lower side of a). Both figures are drawn by the program GREEN.

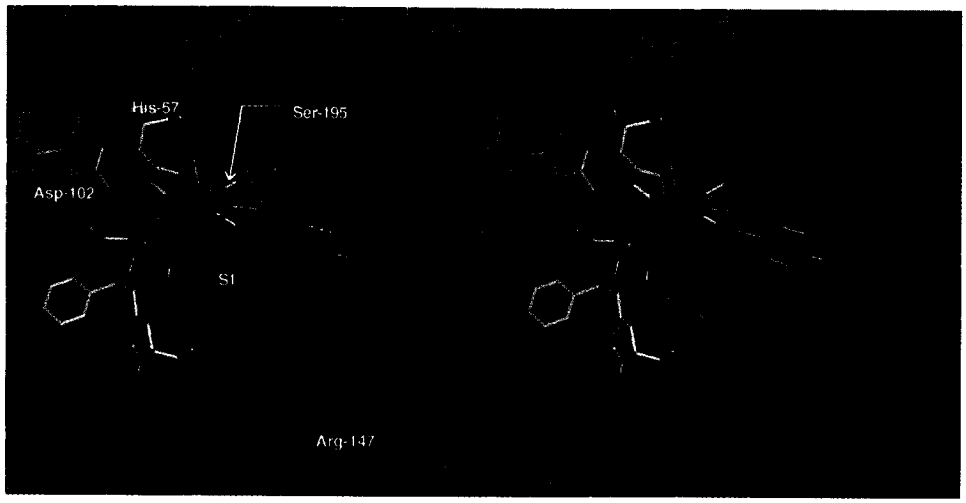

Figure 4 Stereoview of the simulated active site structure of the complex. All hydrogen atoms are omitted for clarity. The FR901277 molecule is coloured by atoms: carbon in green, nitrogen in blue and oxygen in red. The catalytic triad (Ser-195, His-57 and Asp-102) is coloured yellow. Three amino acid residues which form an antiparallel β-sheet with peptidyl inhibitors, are coloured white. The Arg-147 residue near the catechol part of FR901277 is coloured light blue. Other amino acid residues of HLE are depicted by magenta thin lines. This figure is drawn by InsightII.[13]

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14. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 124113. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).