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Molecular insight into pseudolysin inhibition using the MM-PBSA and LIE methods

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Abstract

Pseudolysin, the extracellullar elastase of *Pseudomonas aeruginosa* (EC: 3.4.24.26) plays an important role in the pathogenesis of *P. aeruginosa* infections. In the present study, molecular dynamics simulations and theoretical affinity predictions were used to gain molecular insight into pseudolysin inhibition. Four low molecular weight inhibitors were docked at their putative binding sites and molecular dynamics (MD) simulations were performed for 5.0 ns, and the free energy of binding was calculated by the linear interaction energy method. The number and the contact surface area of stabilizing hydrophobic, aromatic, and hydrogen bonding interactions appears to reflect the affinity differences between the inhibitors. The proteinaceous inhibitor, Streptomyces metalloproteinase inhibitor (SMPI) was docked in three different binding positions and MD simulations were performed for 3.0 ns. The MD trajectories were used for molecular mechanics-Poisson–Boltzmann surface area analysis of the three binding positions. Computational alanine scanning of the average pseudolysin–SMPI complexes after MD revealed residues at the pseudolysin–SMPI interface giving the main contribution to the free energy of binding. The calculations indicated that SMPI interacts with pseudolysin via the rigid active site loop, but that also contact sites outside this loop contribute significantly to the free energy of association.

Keywords: Pseudolysin; Molecular dynamics simulations; Free energy of binding; MM-PBSA calculations; SMPI; Protein–protein interactions; LIE calculations; Pseudolysin inhibition; Computational alanine scanning

1. Introduction

Theoretical prediction of protein–protein and protein–ligand binding affinities complements experimental analysis by adding molecular insight into the macroscopic properties measured by the experimental analysis. The experimental binding free energy does not refer to a single conformation of the associated complex and the separated molecular partners in solution, but to ensembles of structures representative of the associated and dissociated states. During the last years, free energy models that consider only the initial and final states of the association process have been developed. Compared with the more rigorous methods like free energy perturbation (FEP) these methods are less computational expensive making them suitable for a variety of molecular systems. In the present study, two such methods, the molecular mechanics-Poisson–Boltzmann surface area (MM-PBSA) and the linear interaction energy (LIE) methods were used to predict the association free energy and the interaction modes of the proteinaceous metalloproteinase inhibitor SMPI and four low molecular weight inhibitors of pseudolysin.

Pseudolysin, the extracellullar elastase of *Pseudomonas aeruginosa* (EC: 3.4.24.26), is a zinc metalloendopeptidase of the thermolysin family, involved in the pathogenesis of *P. aeruginosa* infections. The three dimensional (3D) structure of pseudolysin is very similar to that of thermolysin (Thayer et al., 1991), the prototype enzyme of the family. The amino terminal domain consists mainly of antiparallel β strands, while the carboxyl terminal domain is mainly

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α-helical. The structure contains a catalytic zinc ion, a calcium ion and two disulfide bonds, (Cys30–Cys58 and Cys270–Cys297). His140, His144, Glu164 and a water molecule ligate the catalytic zinc ion. Site directed mutagenesis studies have identified Glu141 and His223 as essential for the elastase activity.

Experimental and clinical studies showed that pseudolysin cleaves casein, elastin, and synthetic peptides (Morihara, 1995), human IgG (Doring et al., 1981; Holder and Wheeler, 1984), collagen types III and IV (Heck et al., 1986), serum α1-proteinase inhibitor (Morihara et al., 1979) and human bronchial mucosal proteinase inhibitor (Johnson et al., 1982). Studies on mice indicated that pseudolysin also has severe hemorrhagic and muscle damaging activities (Komori et al., 2001), and is involved in various lung infections (Mariencheck et al., 2003). Strong evidences suggest that pseudolysin is implicated in chronic ulcers by degradation of human wound fluids and human skin proteins (Schmidtchen et al., 2003) and corneal infection causing corneal liquefaction which could be sight threatening (Hobden, 2002). Experimental attempts were made to treat rabbit cornea infections using antibiotics and *P. aeruginosa* elastase specific inhibitors as adjuncts to antibiotics (Kessler and Spierer, 1984; Burns et al., 1990), and some inhibitors showed promising effects (Kessler and Blumberg, 1987). A detailed knowledge about the active site geometry and the interaction modes of known inhibitors is the key for designing new inhibitors of clinical use.

Streptomyces metalloproteinase inhibitor (SMPI), from *Streptomyces nigrescens* TK-23, was the first known proteinaceous inhibitor of metalloproteinases. It inhibits the gluzincin metalloproteinases that also include the thermolysin family. The X-ray crystal structure of SMPI is not known, but the NMR structure (Ohno et al., 1998) shows that the protein (102 amino acids) contains two disulphide bridges. The inhibitor function has been connected to the Cys64–Val65 segment of the Arg60–Ala73 loop. This loop also contains a disulphide bridge between Cys64 and Cys69, resulting in a rigid loop structure (Seeram et al., 1997a; Seeram et al., 1997b). The stronger (several thousands fold) binding affinity of SMPI to pseudolysin compared to other available inhibitors of pseudolysin indicates a huge potential in designing inhibitors mimicking the pseudolysin binding region of SMPI. Such inhibitors may have an immersed therapeutic value. Binding studies indicate that SMPI binds stronger to pseudolysin that to thermolysin (a 100-fold higher binding affinity) (Morihara et al., 1979). Thermolysin is the prototype enzyme of the thermolysin family and at least 26 complexes of thermolysin with low molecular weight inhibitors are deposited in the PDB database (Matthews, 1988). However, very little is known about the binding mode of inhibitors to pseudolysin. The X-ray structure of pseudolysin was published in 1991 (Thayer et al., 1991), and in July 2004 the first X-ray structure of pseudolysin (PDB-acquisition: 1u4g) in complex with a small molecule inhibitor was deposited in the PDB database. However, neither the interaction mode of SMPI with pseudolysin nor thermolysin is known in detail. Ideally, the X-ray structure of the pseudolysin–SMPI complex would be used to guide a comprehensive functional survey of residues present at the interaction interface. Protein–protein interfaces usually have some residues ('hot spot' residues) that give the main contribution to the free energy of binding (Clackson et al., 1998). Identifying these residues is an important starting point for a rational design of small molecule mimics.

The aim of the present study was to gain molecular insight into pseudolysin inhibition using molecular dynamics (MD) simulations and theoretical affinity predictions. Three different binding modes of SMPI were explored by 3 ns of MD simulations and MM-PBSA calculations of the free energy of protein–protein association. The three binding modes were also studied by computational alanine scanning of residues at the pseudolysin–SMPI interaction interfaces to determine the hot spot residues responsible for high affinity binding. Further, four small molecule inhibitors were docked at the active site and their interactions were studied by MD simulations for 5 ns and calculations of the free energy of binding using the LIE method.

2. Materials and methods

The calculations were performed using four processors on a HP Superdome with 550 MHz CPUs. The X-ray crystal structure of pseudolysin (PDB-acquisition: 1ezm) was used as starting structure. The coordination of the zinc and calcium ions within the pseudolysin structure was described by non-bonded energy terms in the calculations (Terp et al., 2000). Zinc was assigned a formal charge of +2.0, van der Waals radius 0.69 Å and well depth $\varepsilon = 0.014$ kcal/mol. Calcium was assigned a formal charge of +2.0, van der Waals radius 1.6 Å and well depth $\varepsilon = 0.1$ kcal/mol (Terp et al., 2000). All minimizations and MD simulations were performed using the AMBER 7.0 package (Pearlman et al., 1995; Case et al., 2002) with a TIP3P water model (Jorgensen, 1982). The amber94 force field (Cornell et al., 1995) was used for both ligand bound and free states of the pseudolysin. All molecular systems (bound and free states) were solvated with a cubic box of water and neutralized by counter ions (Åqvist, 1996). The number of counter ions varied in the different systems. The particle mesh Ewald (PME) method was used for the treatment of long range electrostatic interactions (Darden et al., 1993). The non-bonded cutoff was set to 9.0 Å and the SHAKE option (Gunsteren and Berendsen, 1977) was used to constrain the bonds involving hydrogen atoms. The dielectric constant was set to 1 in all calculations. Generally all structures were minimized by the conjugate gradient method for 1000 cycles and equilibrated by 125 ps of MD. The temperature was gradually increased to 300 K during the first 25 ps of the equilibration, and kept constant for the rest of the equilibration. The time step during MD was 1 fs, and the non-bonded pair list was updated