Inhibitor and Substrate Binding by Angiotensin-Converting Enzyme: Quantum Mechanical/Molecular Mechanical Dynamics Studies

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ABSTRACT: Angiotensin-converting enzyme (ACE) is an important zinc-dependent hydrolase responsible for converting the inactive angiotensin I to the vasoconstrictor angiotensin II and for inactivating the vasodilator bradykinin. However, the substrate binding mode of ACE has not been completely understood. In this work, we propose a model for an ACE Michaelis complex based on two known X-ray structures of inhibitor–enzyme complexes. Specifically, the human testis angiotensin-converting enzyme (tACE) complexed with two clinic drugs were first investigated using a combined quantum mechanical and molecular mechanical (QM/MM) approach. The structural parameters obtained from the 550 ps molecular dynamics simulations are in excellent agreement with the X-ray structures, validating the QM/MM approach. Based on these structures, a model for the Michaelis complex was proposed and simulated using the same computational protocol. Implications to ACE catalysis are discussed.

1. INTRODUCTION

The angiotensin-converting enzyme (ACE) is a zinc-dependent dipeptidase, which was discovered more than half a century ago.1 This enzyme exhibits an important biological function in regulating the conversion of the biologically inactive angiotensin I to angiotensin II, a powerful vasoconstrictor. It is also involved in the inactivation of bradykinin, a potent vasodilator. The dual functionality is now known to play a key role in the blood pressure regulating renin-angiotensin system (RAS). As a result, ACE is a prominent target for treating hypertension and cardiovascular diseases.2,3 Although several FDA approved ACE inhibitors are already available for clinic use, the substrate binding and catalysis of ACE are still not completely understood. Interestingly, the first few ACE inhibitors were identified using carboxypeptidase A (CPA) or thermolysin (TLN) as a model,4,5 which was believed to have a similar active-site architecture. It is only recently that the three-dimensional structure of ACE was determined via X-ray diffraction.6 While confirming the similarities of the active sites among these enzymes, ACE was shown to have a vastly different overall fold from CPA and TLN.

Two types of ACE are known. The human somatic angiotensin-converting enzyme (sACE) has two domains, namely the N domain and the C domain. They have about 55% sequence similarity,7 but both domains contain the same zinc binding motif, HEXXH, and a downstream E residue.8 This phenomenon is thought to be a result of gene duplication. The C domain was found to be the dominant angiotensin-converting site in controlling blood pressure and cardiovascular functions, based on the observation that the inhibition of the N domain has little effect on these functions.9 Another form of ACE is found in testis, which plays a role in fertilization. The testicular ACE (tACE) shows an identical active site with the C domain of sACE but has no N domain.10 Recently, three-dimensional structures of various ACEs have been determined.6,11–16

An interesting structural characteristic of ACE is the presence of two chloride ions outside the active site. Experiments indicated that they are essential to maintain the binding structure and catalytic activity.17–19 Based on X-ray structures of ACE, the Cl− ion at the first binding position (I) is about 21 Å away from the zinc ion. It is in hydrogen bonding distances with Arg186 and Arg489 and exhibits van der Waals interactions with a shell formed by Trp485, Trp486 side chain groups and the Asp507 backbone. These interactions are thought to be very important for the stabilization of the enzyme–substrate complex.20 The chloride ion at the second binding position (II) is about 10 Å away from the zinc ion and is in hydrogen bonding distances with Arg522, Tyr224, and a water. In addition, a hydrophobic shell formed by residues of Pro407, Pro519, and Ile521 was found to surround Cl− (II). Kinetic experiments suggested that Cl− (II) is critical for enzyme catalysis.17,19 In our simulations, both anions were placed in their experimentally determined positions.