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Molecular dynamics investigation of the active site dynamics of mycobacterial cyclopropane synthase during various stages of the cyclopropanation process

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ABSTRACT

Mycobacterial cyclopropane synthase 1 (CmaA1) is one of the most important drug targets in anti tuberculosis drug discovery as it is responsible for cis-cyclopropanation at the distal position of unsaturated mycolates, which is an essential step for the pathogenicity, persistence and drug resistance. Five representative models of CmaA1 which correspond to different stages in the cyclopropanation process have been studied using molecular dynamics (MD) simulations. The MD simulations and structural analyses provide a detailed account of the structural changes in the active sites of CmaA1. CmaA1 has two distinct binding sites, i.e., cofactor binding site (CBS) and acyl substrate binding site (ASBS). The apo state of CmaA1 corresponds to a closed conformation where the CBS is inaccessible due to the existence of H-bond between Pro202 of loop10 (L10) and Asn11 of N-terminal α1 helix. However, cofactor binding leads to the breaking of this H-bond and thus the H-bond is absent in the holo form. The hydrophobic side chains orient towards the inner side of the ASBS upon cofactor binding to create a hydrophobic environment for the substrate. The cofactor and substrate tend to come close to each other facilitated by opening of L10 to exchange the methyl group from the cofactor to the substrate. The MD study also revealed that the system tends to regain the apo conformation within 40 ns after releasing the product.

1. Introduction

Unique architecture of the thick waxy cell wall of *Micobacterium tuberculosis* (*M. tuberculosis*) is very crucial for the integrity of the bacteria. This cell wall prevents dehydration, offers protection against varying levels of pH and detrimental effects of free radicals, which is essential for the pathogenicity, persistence and drug resistance (Anand et al., 2008; Flynn and Chan, 2001; Wolfe et al., 2010). Thus, characterizing the proteome (Wolfe et al., 2010) and study of the dynamics and permeability of the cell wall (Hong and Hopfinger, 2004; Banerjee et al., 2011) is interesting in its own right. The main constituents which impart the above characteristics to the cell wall are mycolic acids, a group of highly hydrophobic long-chain α-alkyl-β-hydroxy fatty acids (Beken et al., 2011; Takayama and Qureshi, 1984; Minnikin and Polgar, 1967). Mycolic acids have been proposed to be biosynthesized via a diversion in normal fatty acid metabolism in which short chain fatty acids are extended and modified to form lipids of exceptional length (Kaneda et al., 1986). *M. tuberculosis* cell wall contains three types of mycolic acids, viz., α-mycolates and oxygenated keto- and methoxy-mycolates, out of which α-mycolates are the most abundant and important type of mycolic acids with an average length of 70–80 carbons (Kaneda et al., 1986; Yuan et al., 1998). It has been reported that in pathogenic *M. tuberculosis* majority of the usaturated α-mycolates undergo cyclopropanation of the double bonds using S-adenosyl-L-methionine (SAM) as the methyl donor by a family of enzymes called cyclopropane synthases, which catalyse the cyclopropanation selectively at different positions of the unsaturated mycolates (Ying et al., 1995; George et al., 1995; Grogan and Cronan, 1997; Glickman et al., 2000a,b; Glickman, 2003; Barkan et al., 2010; Boissier et al., 2006) and this cyclopropanation is essential for the proper functioning of the mycolic acids (Barkan et al., 2009). We have considered *M. tuberculosis* CmaA1 for our study which is responsible for cis-cyclopropanation at the distal position of α-mycolates. It has been experimentally shown that

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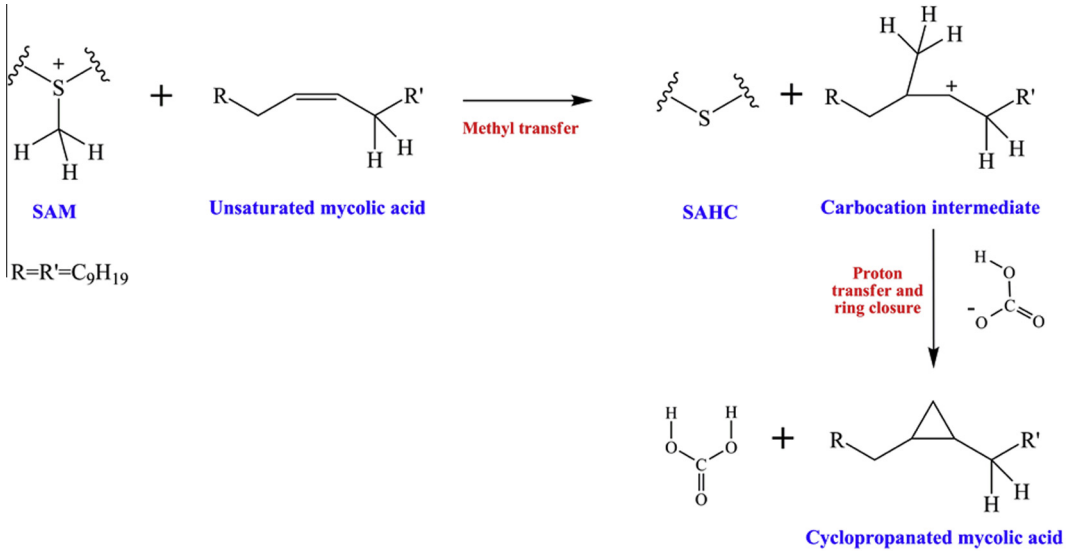
over expression of the protein CmaA1 makes the bacteria resistant to hydrogen peroxide, suggesting that the cyclopropanation at the distal positions may be an important adaptation of *M. tuberculosis* against oxidative stress (Takayama and Qureshi, 1984; Ying et al., 1995). Hence CmaA1 is an important target for anti-tuberculosis therapy (Arcus et al., 2006; Lamichhane, 2011). The crystal structures of apo and holo forms of CmaA1 reveal the existence of two distinct binding sites viz., a CBS containing S-adenosyl-L-homocysteine (SAHC) and an ASBS containing a lipid inhibitor DDDMAB (Huang et al., 2002). The lipid inhibitor mimics the carbocation intermediate in the cyclopropanation reaction and points to be the possible site of binding for the hydrophobic mycolic acids (Huang et al., 2002), which provide valuable information on the ligand orientation in the active site. CmaA1 has several additions to the core methyl transferase fold such as, a short helix inserted between β4 and α4, 2 helices inserted between β5 and α5, 3 helices inserted between β6 and β7 and additions that form active site cover (Martin and McMillan, 2002). CmaA1 shows >50% sequence identity with the other proteins of the family CmaA2, and PcaA, which may point to the similarity of reaction mechanism (Kozbial and Mushegian, 2005). The mechanism of cyclopropanation as proposed by earlier studies (Umbarger, 1978; Molitor et al., 2003; Courtois et al., 2004; Iwig et al., 2004, 2005; Churlet et al., 2005; Liao et al., 2011) is illustrated in Scheme 1. In the first step, the transfer of a methyl group from SAM to the substrate double bond occurs to form a carbocation intermediate and SAM converts to SAHC after the methyl transfer. Then the bicarbonate ion acts as a base to take a proton from the methyl group, resulting in the ring closure (Iwig et al., 2005). DFT studies by Liao et al. (2011) have shown that the rearrangement of the carbocation intermediate after methyl transfer has a slightly lower barrier than the proton transfer supporting this mechanism.

CmaA1 belongs to the class methyl transferases and mixed α/β fold. As studied from the crystal structure, this protein consists of 7β strands (12%) and 14α helices (51%). The core region comprises of the β strands arranged in a parallel fashion (except for β6 which is antiparallel to its neighbouring strands β5 and β7) as β3–β2–β1–β4–β5–β7–β6 and α helices situated at both sides of each strand. The CBS consists of 4 structural motifs viz., the loop between β1 and α4 (motif I), the loop between β2 and α5 (motif II), the loop between β3 and α6 (motif III), α7 (motif IV), α1 of the N-terminus and the loop between α1 and α2. The major difference in the apo and the holo structures of CmaA1 lies in residue range 137–144 which is a two turn helix in the holo form while is a loop in the apo form. This constitutes a major part of motif IV involved in cofactor binding. Also the residues 170–210 of ASBS are situated towards the surface of the apo protein (Fig. 1).

Here, we aim to study the structural properties, energetics and dynamics of the active sites of CmaA1 in detail as understanding the rigidity and flexibility of the binding sites is essentially a first step in rational inhibitor design (Carlson, 2002). The apo and holo model systems of CmaA1 were taken to elucidate the precise changes in the conformations of the binding pockets using MD simulation and to correlate the structure with the catalytic and mechano-chemical functions of the enzymes (Yang and Bahar, 2005; McGeagh et al., 2011). The stabilities and roles of various molecular interactions in the active sites have been thoroughly studied using the MD simulations. Particular attention has been paid to the way in which non bonded interactions such as H-bonds modulate the active site dynamics. The cation–π and the π–π interactions also play major roles in maintenance of the protein structure and also facilitate various enzyme catalyses (Chourasia et al., 2011; Mahadevi and Sastry, 2013). CmA1 has been recognized as an important drug target, and inhibitors of this enzyme have been reported in the literature including thiacetazone and its analogues (Alahari et al., 2007) and dideoxy nucleosides (Rai et al., 2007). Upon CmaA1 inhibition by thiacetazone and its chemical analogues, Alahari et al. have observed significant reduction in the mycolic acid contents in various mycobacterial strains. Rai et al. have synthesized and experimentally tested various classes of dideoxy nucleosides as potent and selective inhibitors of *Mycobacterium bovis*, *M. tuberculosis* and drug-resistant *M. tuberculosis*. A better understanding of the conformational changes of the active site of CmA1 is expected to immensely help in structure based drug design. With respect to the several stages in the reaction cycle, the geometric dynamic properties are quite likely to change with respect to each other, and hence one needs to consider all these conformations/states during ligand design.

We have considered 5 model systems of CmaA1 representing various stages of cyclopropanation, and we propose Scheme 2 for the cyclopropanation cycle based on the previous studies (Umbarger, 1978; Molitor et al., 2003; Courtois et al., 2004; Iwig et al., 2004, 2005; Churlet et al., 2005; Liao et al., 2011).



Scheme 1. Mechanism of cyclopropanation of unsaturated mycolates in mycobacteria.