Mechanism of the Cell Wall DD-Carboxypeptidase Reaction of Penicillin-Binding Protein 5 of *Escherichia coli*

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TITLE RUNNING HEAD Mechanism of PBP 5 Hydrolysis

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Formation of tetrahedral species of the *cis*-Amide Conformer. The preparation of the *cis*-D-Ala-D-Ala conformer used the structure of the optimized *trans*-*N*-acetyl-D-Ala-D-Ala–PBP 5 Michaelis complex (d_0 = 1.0 Å and d_2 = 2.5 Å). Rotation of the terminal D-Ala about the N–C bond gave the *cis* conformer. The conformation sampling using the 2ns MD simulation was carried out and followed by the QM/MM geometry optimization as explained in the context. The generated *cis*-*N*-acetyl-D-Ala-D-Ala–PBP 5 Michaelis complex is shown in Figure 1S.

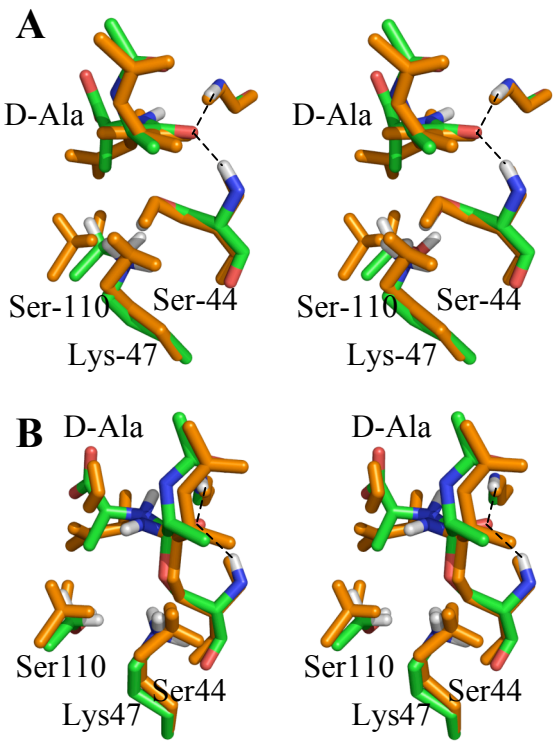

Figure 1S. Stereo representation of superimposed *trans*- and *cis*-*N*-acetyl-D-Ala-D-Ala conformers with side chains of PBP 5 residues, Ser44, Lys47, Ser110, Gly215, His216, and water molecule for energy-minimized Michaelis complex (A) and tetrahedral intermediate (B). For *cis* conformer carbon atoms are colored in green, nitrogen in blue, oxygen in red, and hydrogen in grey. For *trans* conformer atoms are colored in orange except substrate amide NH atoms (N in blue and H in grey). Two hydrogen bonds of the oxyanion hole for *cis* conformers are shown in dash lines (black). The *rmsd* values for superimposing are 0.177 Å for the Michaelis complex and 0.188 Å for the tetrahedral intermediate.

The stereo representation includes the Michaelis complex (A) and tetrahedral intermediate (B). The *cis*-conformer (red-green-blue) was superimposed to the *trans*-conformer (orange) by superimposing the backbone portion of Lys47 and Ser44. The superimposing quality are given by *rmsd* = 0.18 Å for the Michaelis complex and *rmsd* = 0.19 Å for the tetrahedral intermediate. For the Michaelis complex (A) the energy minimum of the structures on the MP2 potential energy surface occurs at d_1 = 1.0 Å and d_2 = 2.5 Å for the *trans*-conformer and at d_1 = 1.0 Å and d_2 = 2.7 Å for the *cis*-conformer. The carboxylate of the *trans*-conformer is anchored through an ion pair with the guanidinium group of Arg198. The distances of the ion interactions are nearly equal to

2.8 Å. For *cis*-conformer, however, one ion interaction (the oxygen is upper in Figure 1S) is substituted by a 2.6 Å hydrogen bond of a water molecule. This occurs due to the rotation of the carboxylate by 150° with respect to the carboxylate plane in the *trans*-conformer. The rotation of the carboxylate of the *cis*-conformer in the tetrahedral intermediate (B) was also observed as shown in Figure 1S.

Starting from the QM/MM structure of the *cis*-conformer, a potential energy scan was done using reaction coordinate d_1, the distance between Ser44 OH atoms, and reaction coordinate d_2, the distance between the substrate carbonyl carbon and Ser44 Oγ. The resulting potential energy surface is shown in Figure 2S. The surface reveals interesting features that are distinct from the surface obtained for the *trans*-conformer. Three potential energy minima are found. Minimum I is a shallow but well defined minimum and is assigned to the Michaelis complex. Minimum II occurs for the species resulting from proton transfer from Ser44 to Lys47. Minimum III corresponds to the tetrahedral species. Hence the *cis*-conformer has two paths to the tetrahedral species from the Michaelis complex. The first path is stepwise (I to II, followed by II to III). Proton transfer from Ser44 to Lys47 (I to II) occurs over a kcal·mol^{-1} energy barrier, with the resulting zwitterion (II) at an energy that is 8 kcal·mol^{-1} lower than (I). Nucleophilic addition of the Ser44 side chain oxyanoion (II to III) corresponds to a 35 kcal·mol^{-1} barrier. The second path from I to III involves proton transfer from Ser44 to Lys47, concerted with Ser44-Oγ addition to the amide carbonyl. The barrier for this process is 29 kcal·mol^{-1}. The energy separation between I and III is 20 kcal·mol^{-1}.

Inspecting carefully the structure of the tetrahedral intermediate in Figure 1S, we noted that the 150° rotation of the carboxylate anchoring to Arg198 dramatically changes the positions of the substrate amide NH group and the methyl moiety of the terminal D-Ala. The former is locked within the electron clouds of both carboxylate oxygen and carbonyl oxygen. In contrast, the later repels the methylene group of Ser110 side chain, separating those proton donators such as Ser110 and Lys47 from approaching the amide nitrogen as discussed in the *trans*-conformer. This separation is unlikely overcome by simple conformational adjustments between the donator and the substrate amide.

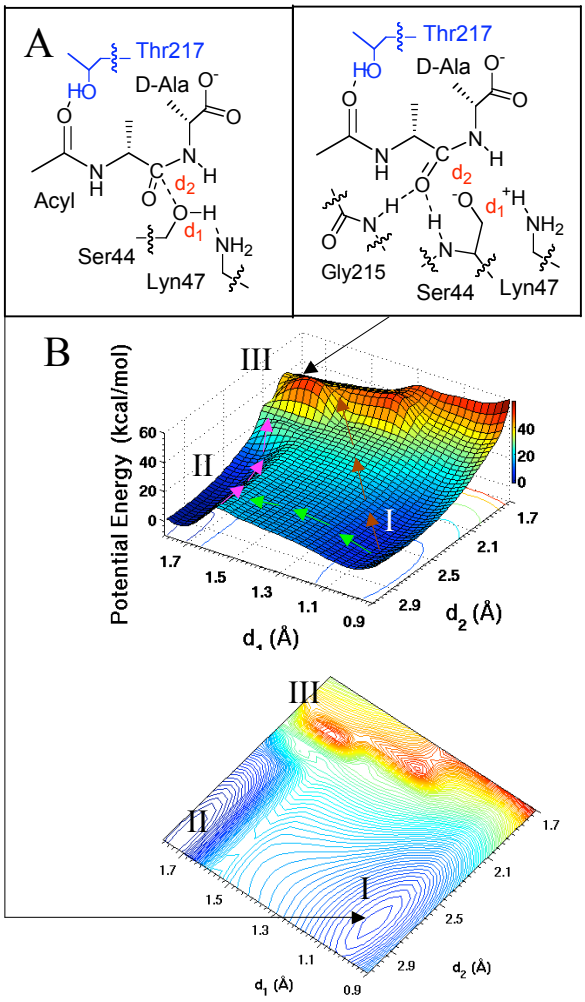

Figure 2S. QM/MM calculations of acylation reaction for PBP 5 enzyme and *cis-N*-acetyl-D-Ala-D-Ala substrate. (A) Diagrams of Michaelis complex (left) and tetrahedral intermediate (right) with reaction coordinates d_1 (Å) and d_2 (Å) (see definition in context), in which only residues colored in black are included in the QM layer. (B) QM/MM potential energy surface (upper) and contour (bottom) with Michaelis complex I, zwitterion II, and tetrahedral intermediate (III). Reaction paths are shown in colorful arrows. Thin guiding lines are in black.

Dual proton transfer in the water-mediated Lys47 protonation. The event of water molecule intervening the pathway of proton transfer complicates the conformation of the direct Lys47 protonation to the substrate amide nitrogen. Examinations were made on energetics for the two proton transfer processes, Lys47 proton to the water oxygen and

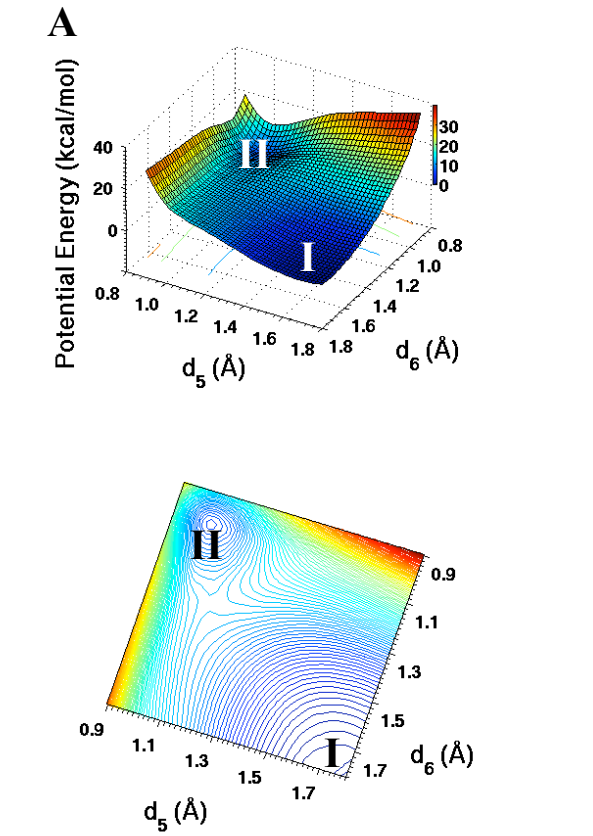

Figure 3S. (A), QM/MM potential energy surface (top) and contour for examining the dual proton transfer process in the water-mediated proton transfer of Lys47 at distance of beginning of the peptide bond cleavage, d_4 = 1.55 Å; (B), reaction coordinate diagrams of the tetrahedral intermediate (I) to the acyl-enzyme species (II). The coordinate d_5 (Å) describes the distance between the water proton and the amide nitrogen of the substrate and d_6 the distance between Lys47 ammonium η-proton and water oxygen.

one of water protons to the substrate amide nitrogen. Two protonation coordinates are defined, d_5 the distance between the water proton and the nitrogen and d_6 the distance

between the Lys47 proton and water oxygen. The N-C peptide bond of the substrate was fixed at distance d_4 = 1.6 Å. The potential energy surface is given in Figure 3S. Results show that the dual proton transfer reaction takes place in an energy optimized pathway that requires an approximately symmetric conformation of $d_5 = d_6$.

References. *Full Citation to Reference (25):*

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