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QM/MM studies of mono-zinc β -lactamase CphA suggest that the crystal structure of an enzyme-intermediate complex represents a minor pathway

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Abstract

QM/MM studies of the hydrolysis of a β -lactam antibiotic molecule (biapenem) catalyzed by a mono-zinc β -lactamase (CphA) reveal the complete reaction mechanism and show that an experimentally determined enzyme-intermediate complex is a stable intermediate or product in a minor pathway.

Bacterial resistance to β -lactam antibiotics such as penicillin, cephalosporins, and carbapenems are conferred by β -lactamases, which deactivate the antibiotics by hydrolyzing the common β -lactam ring.^{1–2} While the mechanism of serine-based β -lactamases is well established,² the mode of catalysis for metallo- β -lactamases (M β LS) is still not completely resolved.^{3–4} The rapid proliferation of M β LS genes from innocuous species to pathogenic ones via plasmid and/or integron-borne pathways, combined with the lack of clinically useful inhibitors, renders M β LS a more potent threat than their serine-based counterparts.^{5–6} Our incomplete understanding of the M β LS catalytic mechanism hampers the search for effective inhibitors.

Mechanistic insights can often be gained from X-ray structures of an enzyme and perhaps more importantly its complexes with substrate analogs. This is no exception for M β LS, where recently determined complex structures have shed valuable light on substrate binding modes and reaction pathways.^{7–9} However, the very existence of an enzyme complex underscores its thermodynamic and/or kinetic stability. In other words, structures determined by X-ray crystallography are associated with wells on the free-energy landscape that may or may not be associated with the kinetically favored reaction pathways. In this communication, we argue based on reliable quantum mechanical/molecular mechanical (QM/MM) calculations^{10–13} that a recently determined X-ray structure of a mono-zinc M β LS (CphA from an opportunistic pathogen *A. hydrophila*)⁷ represents a stable intermediate or a product in a minor pathway for the hydrolysis of a carbapenem antibiotic molecule (biapenem).

Our QM/MM simulations are based on a recently determined X-ray structure of CphA complexed with a hydrolysis intermediate of biapenem,⁷ thereafter denoted as the EI

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complex. As demonstrated in our earlier work,^{14–15} the putative mechanism (Scheme I) of CphA uses a non-zinc-bound water molecule as the nucleophile. In the initial nucleophilic addition (NA) step, the water nucleophile is assisted by the Asp120 general base to attack the carbonyl carbon of the substrate, leading to the cleavage of the C₇-N₄ bond in the lactam ring. The resulting anionic nitrogen is stabilized by the Zn(II) ion, forming an enzyme-intermediate complex. In the mean time, the zinc ion disengages the protonated Asp120 to maintain its tetrahedral coordination.

We report here the simulation of the complete catalysis mechanism for CphA. The details of our simulation methods are given in Supporting Information (SI). Briefly, the self-consistent charge-density tight binding (SCC-DFTB) method^{16–17} is used for the QM region while the surrounding MM region is described by the CHARMM all atom force field.¹⁸ The SCC-DFTB/MM method is extensively validated for this system by comparing with B3LYP/MM single point calculations, as described in SI. All simulations were carried out using CHARMM.¹⁹

Our simulations start by adding a H₂O molecule in the active site, which hydrogen bonds with both the protonated Asp120 and His118, shown as EI1 in Scheme I. This is justified by the X-ray structure of the EI complex,⁷ which has a H₂O molecule in the same position. The reaction proceeds with a rotation around the C₅-C₆ bond (ROT), as suggested by the X-ray structure.⁷ The resulting EI2 provides the branching point for two pathways.

Pathway I completes the reaction by transferring a proton from Asp120 to the zinc-bound nitrogen, via the water bridge (PT1). As shown in Fig. 1, the barriers for both the ROT and PT1 steps are much lower than the NA step (14.1 kcal/mol).¹⁵ The overall barrier for I is consistent with kinetic data on this⁷ and similar MβLs,²⁰ and it represents the kinetically favored pathway.

However, it is clear that Pathway I cannot explain the existence of the experimentally observed EI complex,⁷ which features an additional ring structure formed by adding the hydroxyl group at C₈ position to the C₂=C₃ double bond. To reach the experimental structure, we have computed the free-energy profile for a second pathway (II), which starts with the addition (A) reaction which converts EI2 to EI3, as shown in Scheme I. This is followed by the water-bridged proton transfer from the protonated Asp120 to the zinc-bound nitrogen (PT2), which is essentially barrierless, as shown in Fig. 1. The resulting EI4 complex is structurally identical to the experimental EI complex. Specifically, the protonated N₄ atom is 2.18±0.16 Å away from the zinc ion, which compares favorably with the experimental value of 2.22 Å,⁷ but quite different from either EI1 (1.99±0.11 Å) or EP (2.93±0.33 Å). In Fig. 2, the crystal structure is compared with a snapshot of the EI4 complex, and the overlap is quite satisfactory. A detailed comparison of the geometric parameters can be found in SI.

As shown in Fig. 1, the EI4 complex resides in a deep free-energy well in Pathway II, flanked by large barriers to EI3 and EP. It is clear that Pathway II is kinetically uncompetitive with Pathway I, but the thermodynamic stability of EI4 provides a conceivable explanation for its entrapment and observation in the X-ray diffraction experiment.⁷ Interestingly, the CphA enzyme is inhibited by its product,⁷ which might very well be the stable double-ring structure in EI4, due to the high barrier to EP.

It should be pointed out that there are other mechanistic proposals for CphA catalysis. Specifically, Simona et al. has recently advanced a mechanism with concerted NA and PT steps.²¹ However, this mechanism was based on an initial ES configuration in which the substrate binds indirectly with zinc through a water molecule. This model is thus inconsistent with a large body of experimental data suggesting a direct substrate-zinc contact

through the functionally conserved carboxylate of β -lactam antibiotics.⁴ These data includes X-ray absorption,²² EPR,²³ and X-ray structures of M β Ls complexed with various substrate analogs.^{7–9} The mechanism proposed by Simona et al. also precludes the existence of an anionic nitrogen intermediate,^{24–28} which is considered as a common determinant in M β L catalysis.³ In particular, such an intermediate has recently been observed in a mono-zinc M β L (GOB) with a very similar active-site arrangement.²⁹ In contrast, the mechanism outlined in Scheme I is consistent with all known experimental observations and consistent with earlier theoretical studies.^{30–31}

The demonstration that the crystal structure of EI represents a stable intermediate/product in a minor pathway in CphA catalysis is probably not an isolated case. Numerous examples can be found where crystal structures depict non-productive configurations for binding and reactions. Thus, sole reliance on X-ray structures could lead to incorrect or incomplete mechanisms. However, we also want to emphasize that it is not our intention to discount the paramount importance of crystal structures in elucidating catalytic mechanism. Indeed, the proposed mechanism owes largely to the X-ray structure of CphA. However, caution must be exercised in such endeavors. To this end, reliable theoretical models will play an increasingly indispensable role in checking the viability of various mechanistic proposals.

To summarize, detailed QM/MM free-energy simulations have allowed us to map out the entire reaction pathway for the hydrolysis of a carbapenem antibiotic molecule catalyzed by a mono-zinc M β L. In addition, it is demonstrated that the experimentally observed enzyme-intermediate complex is involved in a minor pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Free-energy profiles of the catalytic reaction. The two pathways are represented by green (I) and red (II) lines. Only forward barriers are given.

Fig. 2. Overlap of active-site moieties between the calculated EI4 complex (cyan for the carbon atom, red for water, purple for the zinc ion) and the X-ray structure of the EI complex (orange for the carbon atoms, yellow for water, green for the zinc ion).⁷ All hydrogen atoms are removed.

Scheme I.

Proposed mechanism for the hydrolysis of biapenem catalyzed by CphA. Two pathways are depicted for the second step of the hydrolysis. The purple ball represents the zinc ion.