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Theoretical Insights into the Functioning of Metallopeptidases and Their Synthetic Analogues

Tingting Zhang,[†] Mehmet Ozbil,[‡] Arghya Barman,[§] Thomas J. Paul,[†] Ram Prasad Bora,[⊥] and Rajeev Prabhakar*,[†]

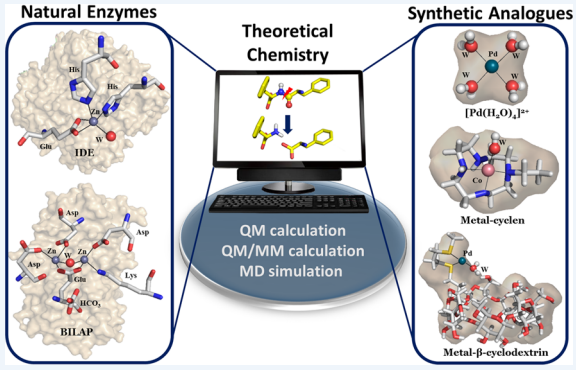
[†]Department of Chemistry, University of Miami, Coral Gables, Florida 33146, United States

[‡]Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107, United States

[§]Department of Chemistry, Georgia State University, Atlanta, Georgia 30302-3965, United States

[⊥]Department of Chemistry, University of Southern California, Los Angeles, California 90089-1062, United States

CONSPECTUS: The selective hydrolysis of a peptide or amide bond (−(O═)C−NH−) by a synthetic metallopeptidase is required in a wide range of biological, biotechnological, and industrial applications. In nature, highly specialized enzymes known as proteases and peptidases are used to accomplish this daunting task. Currently, many peptide bond cleaving enzymes and synthetic reagents have been utilized to achieve efficient peptide hydrolysis. However, they possess some serious limitations. To overcome these inadequacies, a variety of metal complexes have been developed that mimic the activities of natural enzymes (metallopeptidases). However, in comparison to metallopeptidases, the hydrolytic reactions facilitated by their existing synthetic analogues are considerably slower and occur with lower catalytic turnover. This could be due to the following reasons: (1) they lack chemical properties of amino acid residues found within enzyme active sites; (2) they contain a higher metal coordination number compared with naturally occurring enzymes; and (3) they do not have access to second coordination shell residues that provide substantial rate enhancements in enzymes. Additionally, the critical structural and mechanistic information required for the development of the next generation of synthetic metallopeptidases cannot be readily obtained through existing experimental techniques. This is because most experimental techniques cannot follow the individual chemical steps in the catalytic cycle due to the fast rate of enzymes. They are also limited by the fact that the diamagnetic d^{10} Zn(II) center is silent to electronic, electron spin resonance, and ^{67}Zn NMR spectroscopies. Therefore, we have employed molecular dynamics (MD), quantum mechanics (QM), and hybrid quantum mechanics/molecular mechanics (QM/MM) techniques to derive this information. In particular, the role of the metal ions, ligands, and microenvironment in the functioning of mono- and binuclear metal center containing enzymes such as insulin degrading enzyme (IDE) and bovine lens leucine aminopeptidase (BILAP), respectively, and their synthetic analogues have been investigated.



Our results suggested that in the functioning of IDE, the chemical nature of the peptide bond played a role in the energetics of the reaction and the peptide bond cleavage occurred in the rate-limiting step of the mechanism. In the cocatalytic mechanism used by BILAP, one metal center polarized the scissile peptide bond through the formation of a bond between the metal and the carbonyl group of the substrate, while the second metal center delivered the hydroxyl nucleophile. The $Zn(N_3)$ [Zn(His, His, His)] core of matrix metalloproteinase was better than the $Zn(N_2O)$ [Zn(His, His, Glu)] core of IDE for peptide hydrolysis. Due to the synergistic interaction between the two metal centers, the binuclear metal center containing $Pd_2(\mu\text{-OH})([18]aneN_6)]^{4+}$ complex was found to be ∼100 times faster than the mononuclear $[Pd(H_2O)_4]^{2+}$ complex. A successful small-molecule synthetic analogue of a mononuclear metallopeptidase must contain a metal with a strong Lewis acidity capable of reducing the pK_a of its water ligand to less than 7. Ideally, the metal center should include three ligands with low basicity. The steric effects or strain exerted by the microenvironment could be used to weaken the metal−ligand interactions and increase the activity of the metallopeptidase.

1. INTRODUCTION

The selective hydrolysis of a peptide or amide bond (−(O═)C−NH−) plays a critical role in a wide range of biological and biotechnological applications such as signal transduction,[1] cell death,[1] protein footprinting,[2] protein engineering,[3] and bioethanol production.[4] In industry, about 60% of all enzymes used are proteases or peptidases. These specialized enzymes are employed in a wide range of industries, such as textile, food, leather, paper, and ethanol production.[4] Peptide bonds are extremely stable and exhibit a half-life for hydrolysis of 350−600 years at room temperature and

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pH = 4−8.[5] In nature, this formidable task is accomplished by specialized enzymes known as proteases (cleave peptide bonds of proteins and large peptides) or peptidases (break peptide bonds of short peptides).[6−9] This suprafamily consists of the following five members: (1) serine proteases, (2) cysteine proteases, (3) threonine proteases, (4) aspartyl proteases, and (5) metallopeptidases. Enzymes that constitute the first four subfamilies utilize organic functional groups, while the enzymes belonging to the last subfamily commonly use either a mono- or binuclear metal center to hydrolyze their substrates.

Despite the availability of a wide range of peptide bond cleaving enzymes and synthetic reagents, currently only a handful of them have been used for the aforementioned biotechnological and industrial applications, but they possess several deficiencies.[10,11] Most of the enzymes employed are expensive, exhibit broad specificities, and function under narrow temperature and pH conditions. In comparison, the existing synthetic reagents such as cyanogen bromide require harsh conditions and a greater quantity of the starting material. Additionally, they are toxic and provide partial selectivity and low yields. Thus, the development of inexpensive, environmentally friendly, and efficient synthetic analogues of proteases and peptidases is highly desirable. There are very few examples of small molecule mimics of organic functional group containing proteases in literature. On the other hand, in the last few decades tremendous strides have been made in the design of metal (Pd, Pt, Zn, Cu, Co, Fe, Ni, etc.) containing complexes specialized for peptide hydrolysis.[12−19] The role of the metal center in this process has been extensively reviewed.[18,20−25]

The design of the next generation of synthetic analogues requires the understanding of the role metal ions and ligands (direct and indirect) play in the functioning of both natural and synthetic metallopeptidases. Despite the availability of a significant amount of experimental spectroscopic and kinetic data, the detailed structural and mechanistic information regarding the activities of natural enzymes and their existing synthetic analogues is not available. However, the existing data provide an ideal platform to employ theoretical and computational chemistry techniques to gain a deeper understanding of the functioning of these molecules.

In this Account, we will discuss the theoretical studies regarding the functioning of mono- and binuclear metal core containing enzymes, insulin degrading enzyme (IDE) and bovine lens leucine aminopeptidase (BILAP), respectively, and synthetic analogues of mononuclear metal center containing metallopeptidases with particular emphasis on our work in the field. Several state-of-the-art theoretical and computational chemistry techniques involving molecular dynamics (MD), quantum mechanics (QM), and hybrid quantum mechanics/molecular mechanics (QM/MM) have been employed in these studies.

2. METALLOPEPTIDASES

A mononuclear $Zn(N_2O)$ [Zn(His, His, Glu)] catalytic core containing insulin degrading enzyme (IDE) and a binuclear $Zn1(O_4)$−$Zn2(NO_3)$ [Zn1(Asp, Glu, Asp)−Zn2(Lys, Glu, Asp, Asp)] core comprising bovine lens aminopeptidase (BILAP) are currently the well-studied enzymes of this family. The X-ray structures of human IDE[26] and BILAP[27,28] suggest that these enzymes possess the most common active site motifs and key structural features of metalloproteases. Due to their structural and catalytic properties, they serve as model systems to study metallopeptidases.[7,29,30] IDE is a metalloendopeptidase that catalyzes the hydrolysis of a wide range of peptide bonds such as Val−His, His−Gln, Phe−Phe, and Lys−Gly present within amyloidogenic substrates like insulin, amyloid β (Aβ) peptides, amylin, and glucagon.[26] This enzyme is also of great biological relevance. Its inhibitors and activators are potential targets for the treatment of diabetes and Alzheimer's disease (AD), respectively.[31] In contrast, binuclear BILAP is a metalloexopeptidase that prefers to hydrolyze a leucine residue located at the N-terminus in a di- or tripeptide sequence, but it is also capable of hydrolyzing other amino acids as well.[29] This enzyme has been implicated in HIV, cancer, cataracts, and cystic fibrosis.[30]

2.1. Insulin Degrading Enzyme (IDE)

Based on the available experimental and theoretical information, the most plausible mechanism utilized by N_2O core containing IDE is shown in Figure 1.[32−34] In the reactant (**I**), due to the binding of the water molecule to the Zn^{2+} ion and polarization by the negatively charged Glu111 residue, the pK_a value of the water was reduced from ∼14 in solution to ∼7 or even lower.[35] From **I**, Glu111 functioned as a base and accepted a proton from the metal bound water molecule to create a hydroxyl ion ($-O^1H^3$) containing intermediate (**II**). In the next step, the Zn^{2+} bound hydroxyl ion made a nucleophilic attack on the electrophilic carbon atom (C^6) of the scissile peptide bond concomitantly with the donation of a proton from Glu111 to the nitrogen (N^7) atom. As a result a tetrahederal, gem-diol intermediate (**III**) was generated. In the last step, an abstraction of the proton (H^3) from the Zn^{2+} bound hydroxyl group ($-O^1H^3$) by Glu111 steered the cleavage of the C^6−N^7 peptide bond, and a product (**IV**) containing separated carboxyl ($R\text{-}C^6O^1O^{8-}$) and amine ($R\text{-}N^7H_2$) termini was created.

Despite the availability of the aforementioned data, the structures and energies of minima and saddle points for the cleavage of chemically diverse peptide bonds catalyzed by this enzyme were not known.

To obtain this information, the cleavage of three distinct peptide bonds, Lys−Gly (polar−nonpolar), Phe−Phe (nonpolar−nonpolar), and His−Gln (polar−polar), were studied through DFT calculations using pruned models of the active site of IDE.[33] The PES diagram of the cleavage of the Lys−Gly bond is shown in Figure 1. The computed barrier for the formation of the hydroxyl nucleophile in the first step was 14.3, 18.8, and 22.3 kcal/mol for the Lys−Gly, Phe−Phe, and His−Gln substrates, respectively. Two layer QM/MM (B3LYP/Amber) calculations using the ONIOM method[36] showed that the presence of the surrounding enzyme significantly lowered the barrier for this step to 11.1 kcal/mol for the Phe−Phe substrate. In the next step, the formation of the gem-diolate intermediate (**III**) occurred through a barrier of 4.9, 7.8, and 10.2 kcal/mol for Lys−Gly, Phe−Phe, and His−Gln, respectively. Site directed mutagenesis experiments showed that the two outer sphere residues (Arg824 and Tyr831) played important roles in the functioning of the enzyme.[26] Our QM/MM calculations suggested that these two residues facilitate the formation of **III** by reducing the barrier by 2.5 kcal/mol for the Phe−Phe case. The computed energetics of the mechanism explicitly showed that the nature of the peptide bond played an important role in the cleavage process and the hydrolysis of the Lys−Gly peptide bond by IDE was the most favorable, followed by Phe−Phe and His−Gln.

2.2. Bovine Lens Leucine Aminopeptidase (BILAP)

BILAP also followed the general acid/base mechanism utilized by IDE but with a binuclear metal center. The experimentally proposed mechanism for the catalytic cycle of BILAP is shown in Figure 2.[29] In the first step of the mechanism, a bicarbonate ion

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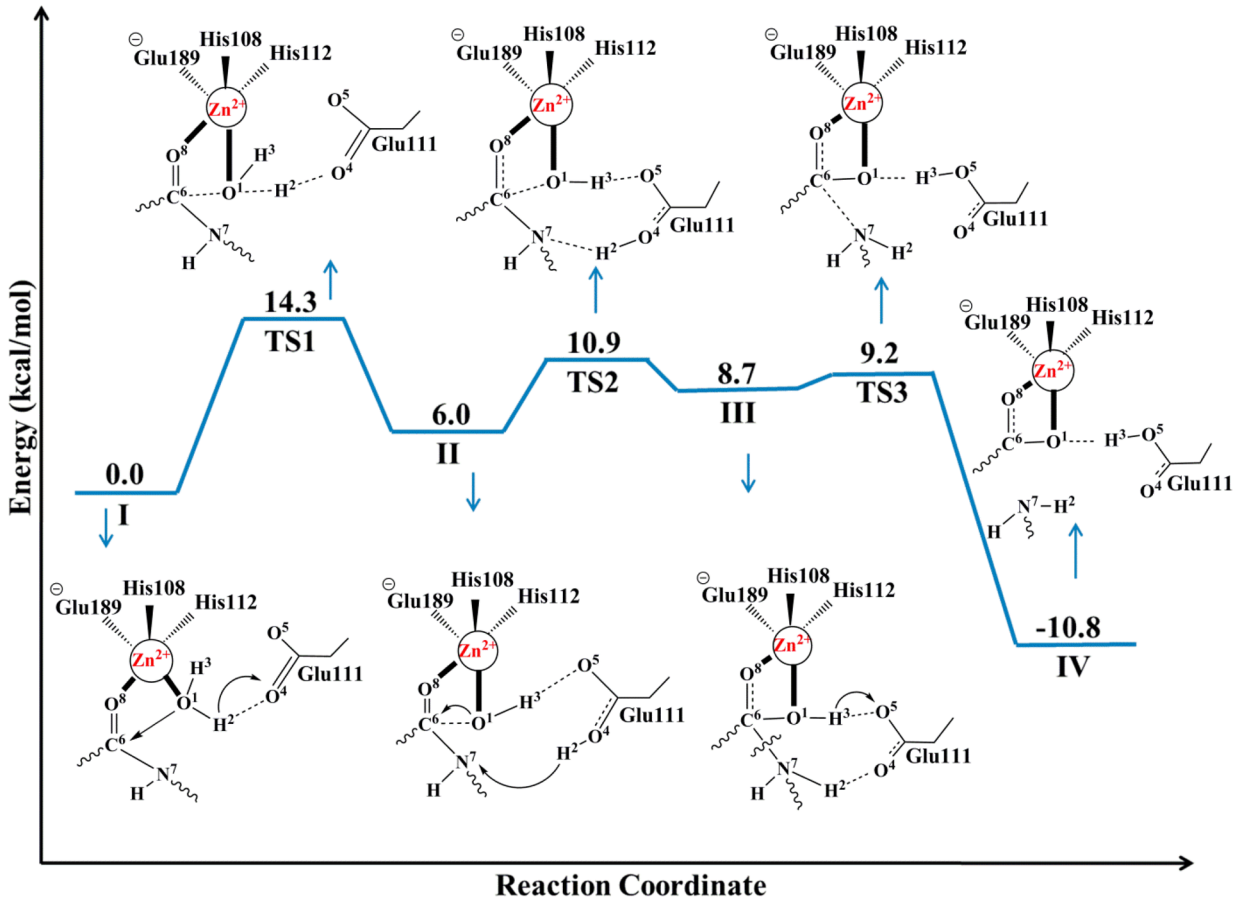

Figure 1. Mechanism and energetics for the hydrolysis of the Lys-Gly bond by IDE.

acted as a base by abstracting a proton from the water molecule that was bridging both (Zn1 and Zn2) metal ions. The coordination to both metal centers is likely to reduce the pK_a of this water molecule from 14 to almost 7.[37] The hydroxide ion created in this process simultaneously made a nucleophilic attack on the electrophilic carbon atom (C^1) of the peptide bond (C^1–N^2). In the *gem*-diolate intermediate (**II**) formed in this step, the Zn1 ion polarized the peptide bond through interaction with the oxygen atom (O^3) of the carbonyl (C^1═O^3) group of the substrate. In the last step, a proton is transferred from the bicarbonate ion to the nitrogen atom (N^2) to cleave the amide bond. The measured k_{cat} for the cleavage of the L-leucine-*p*-nitroanilide and L-leucyl-*p*-anisidine substrates are 0.13 and 0.22 μmol min^{-1} mg^{-1}, respectively.[38]

Despite the substantial experimental information, several issues regarding the mechanism of this enzyme remained unresolved. For instance, the exact protonation state of the nucleophile (H_2O or −OH) was not known. The effect of the electronic nature of the substrate (an electron withdrawing nitro group (-NO_2) in the L-leucine-*p*-nitroanilide and an electron donating methoxy group (−OCH_3) of the L-leucyl-*p*-anisidine) on the energetics was also not available. In addition, the structural modifications upon the substitutions of the Zn^{2+} ions with Mg^{2+} and Co^{2+} were not known.

To address these outstanding questions, the hydrolysis of the L-Leu-*p*-nitroanilide substrate by BILAP was investigated.[39] In the reactant (**I**), a water molecule was coordinated to both metal centers. From **I**, the formation of the gem-diolate intermediate (**II**), which used the bicarbonate molecule as a base, underwent a barrier of 18.6 kcal/mol (Figure 2). Unlike the mechanism of IDE in which the generation of **II** occurred in the rate-limiting step, here the collapse of this intermediate took place in the rate-determining step with a barrier of 25.5 kcal/mol. For the L-leucine-*p*-nitroanilide substrate, the cleavage of the peptide bond occurred in the rate-determining step, while for L-leucyl-*p*-anisidine, all three steps took place with similar energy barriers. For the Mg1−Zn2 and Mg1−Co2 metallovariants, the overall barrier increased by 0.2 and 6.4 kcal/mol, respectively. The metal ion was found to influence only the formation of **II**, and after that, all three variants followed basically the same energetics.

Our mechanistic studies on IDE and BILAP suggested that, despite the considerable differences in the catalytic sites and substrate specificities, some aspects of the mechanism utilized by binuclear enzymes like BILAP (Figure 2) were similar to the mechanism used by mononuclear N_2O core containing IDE (Figure 1). In the reactant (**I**), the water molecule was coordinated to both the Zn1 and Zn2 ions (Figure 2), while in the reactant of IDE, it was bound to the only Zn ion present at the active site. Both in BILAP and IDE, the catalytic base bicarbonate ion and Glu111, respectively, were located in the second coordination shell. Moreover, in both enzymes only one Zn ion polarized the scissile peptide bond by coordinating with the carbonyl (C═O) group of the scissile peptide bond. In **I**, the second metal (Zn2) in BILAP participated in positioning the substrate through its interaction with the N-terminus. Similar to IDE, the hydroxyl nucleophile created in the first step was coordinated to one Zn ion (Zn2) and the carbonyl carbon atom of the peptide bond in **II** (Figure 2). In the next step, a bicarbonate ion functioned as a proton shuttle in BILAP, whereas in IDE, the second coordination shell residue Glu111 directly transferred the proton to the nitrogen atom of the scissile peptide bond.

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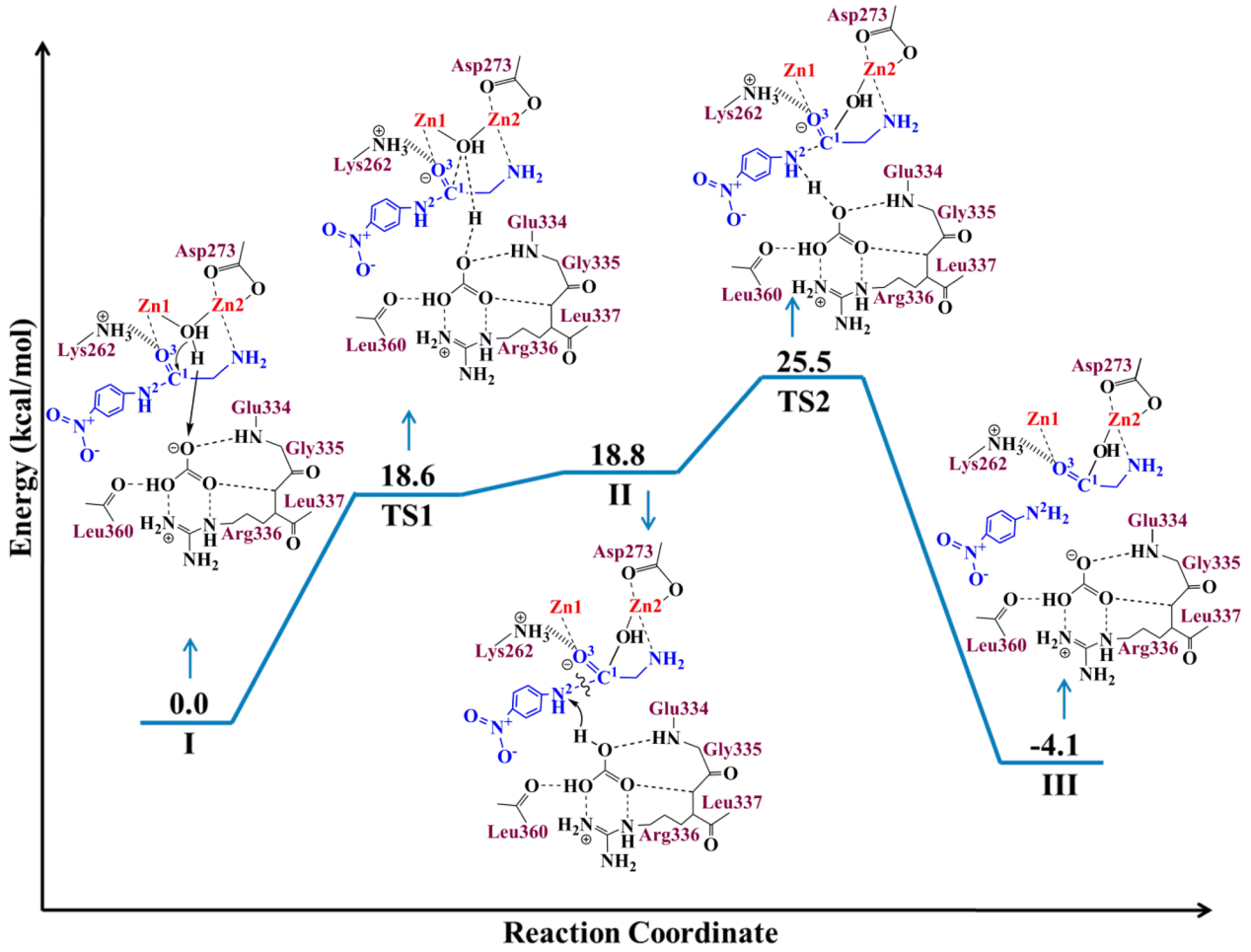

Figure 2. Mechanism and energetics for the hydrolysis of the L-Leu-*p*-nitroanilide substrate by BILAP.

3. SYNTHETIC ANALOGUES OF METALLOPEPTIDASES

Small molecule mimics of metallopeptidases can offer the following advantages over enzymes and synthetic reagents: (1) they are inexpensive and may be recyclable, (2) being smaller in size they impose little or no steric constraints, (3) they can cleave peptides either terminally or internally, and (4) their properties can be tuned for specific applications. In this section, we will discuss the role of the metal ions, ligands, and microenvironment of the reaction center in the functioning of different synthetic analogues of metallopeptidases. Based on available experimental information (discussed below), they can be considered as good model systems to gain deeper insights into mechanisms of peptide hydrolysis.

3.1. Pd-Containing Complexes

In weakly acidic aqueous solutions, the $[Pd(H_2O)_4]^{2+}$ (I_{MPC}) complex had been reported to anchor Met and His residues in X-Y-Met and X-Y-His sequences, respectively, and cleaved the proximal X-Y (Gly-Gly, Gly-Pro, and Gly-Sar) peptide bond (Sar = sarcosine).[10] The cleavage of the X-Y peptide bond was found to be rather general, and in all X-Y-Met and X-Y-His sequences, this bond was cleaved irrespective of the nature of X or Y (aliphatic and aromatic, polar or nonpolar, and charged and neutral). The measured rate constants at pH 2.0 and 60 °C for the cleavage of the peptide bonds in R-Gly-Pro-Met, R-Gly-Pro-His, R-Gly-Sar-Met, and R-Gly-Gly-Met peptide are 6.0×10^{-2}, 9.4×10^{-2}, 1.4×10^{-2}, and 2.8×10^{-3} min^{-1}, respectively. The mechanism for the cleavage of the Gly-Pro-Met sequence is shown in Figure 3. According to the mechanism, an aqua ligand of I_{MPC} was substituted by the sulfur atom of the anchoring Met residue of the substrate to generate a monodentate complex $[Pd(H_2O)_3\{(Gly)\text{-}(Pro)\text{-}(Met\text{-}\kappa S)\}]^{2+}$ ($\mathbf{II}_{PM}$), where the subscript PM denotes the Pro-Met peptide (Figure 3a). In the second step, the Pd(II) ion deprotonated the nitrogen (N) atom in the amide backbone of the Met residue to create a Pd(II)−N bond and a hydronium ion (H_3O^+) was released. This process created a NSO core containing reactive bidentate complex $[Pd(H_2O)_2\{(Gly)\text{-}(Pro)\text{-}(Met\text{-}\kappa S,\kappa N)\}]^{1+}$ ($\mathbf{III}_{PM\text{-}E}$), Figure 3a. The measured TOCSY and ROESY ^1H NMR spectra suggested that $\mathbf{III}_{PM\text{-}E}$ existed in the *trans* conformation.[10] After the formation of this complex, the following two mechanisms for the peptide bond cleavage were possible: (1) Cleavage by external attack. In this mechanism, the Pd^{2+} ion functioned as a Lewis acid and coordinated with a carbonyl oxygen atom. This binding polarized the amide bond for cleavage by a solvent water molecule. (2) Cleavage by internal delivery. In this mechanism, a Pd^{2+} bound water molecule was activated to cleave the peptide bond.[10] These mechanisms cannot be distinguished by purely kinetic methods. The specific mechanistic information such as the roles of the metal bound water molecule, metal ion, and anchoring residues were also not available. In addition, the details of individual steps of the mechanism, energetics, and structures of all short-lived intermediates and transition states were not known.

To address these unresolved issues, in a DFT study, the mechanisms for the hydrolysis of the Gly−Pro bond in

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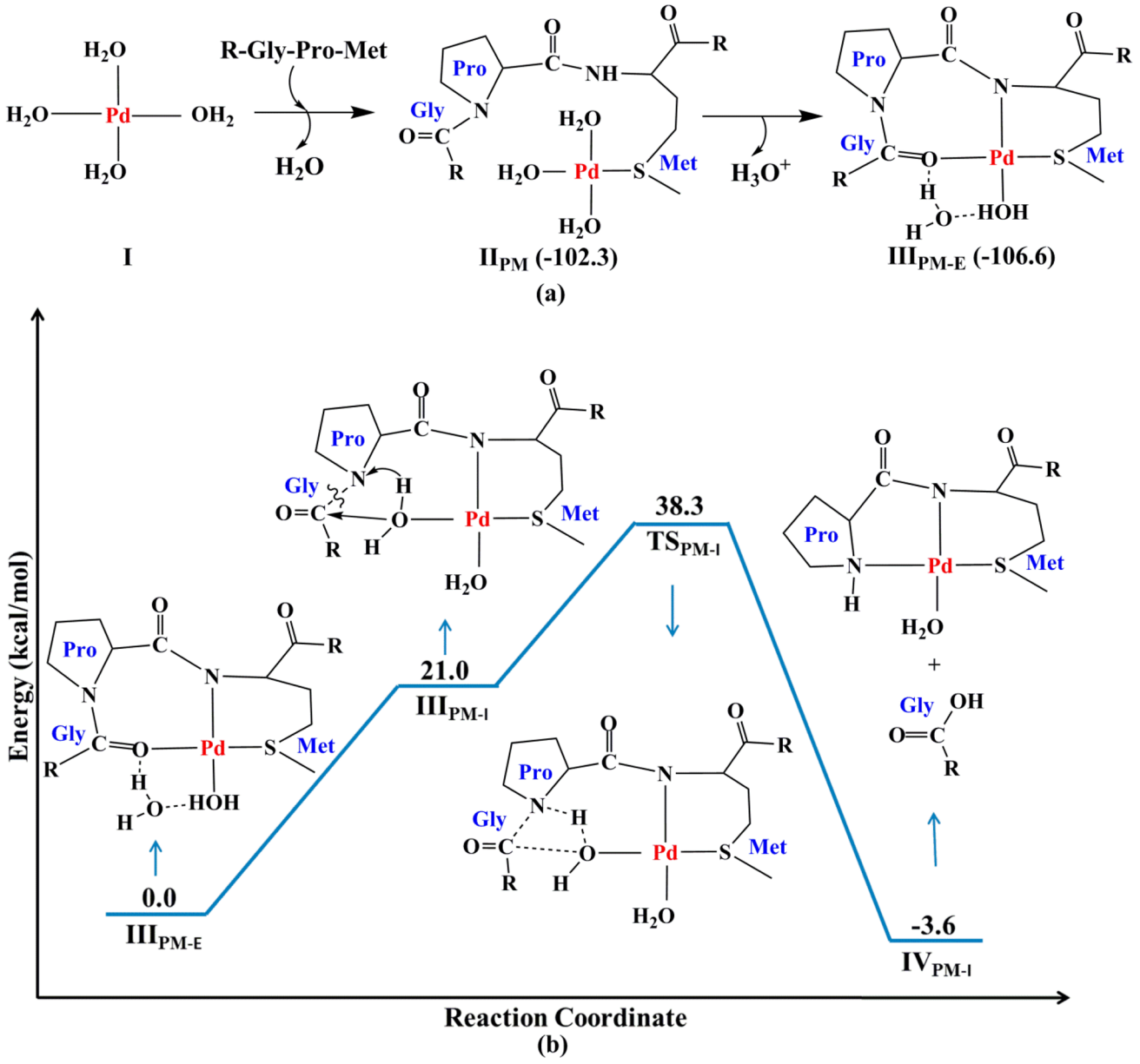

Figure 3. Mechanism and energetics for the cleavage of the Gly-Pro-Met sequence by $[Pd(H_2O)_4]^{2+}$.

Gly-Pro-Met, Gly−Pro bond in Gly-Pro-His, Gly−Sar bond in Gly-Sar-Met, and Gly−Gly bond in the Gly-Gly-Met peptides catalyzed by I_{MPC} were investigated.[40] From I_{MPC}, the formation of the reactive bidentate complex for the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met sequences was found to be significantly exothermic by 106.6, 94.9, 95.6, and 83.6 kcal/mol, respectively (in gas phase). According to our proposed mechanism, the bidentate complex first transformed from the *trans* ($\mathbf{III}_{PM\text{-}E}$) to *cis* ($\mathbf{III}_{PM\text{-}I}$) conformation, Figure 3b. From this conformation, the barriers for the hydrolysis of the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met peptide bonds were 38.3, 41.4, 39.8, and 39.2 kcal/mol, respectively. These barriers were in good agreement with the experimentally measured rate constants at pH 2.0 and 60 °C.[10] This study proposed that after the creation of the active bidentate complex in the *trans* conformation, the internal delivery mechanism is the most energetically feasible.

I_{MPC} can be conjugated to the hydrophobic moiety of *β*-cyclodextrin (CD) to create an artificial enzyme (I_{MPC}−CD).[41] Since little entropy and conformational enthalpy are spent in approaching the transition state, such complexes can provide enormous rate accelerations in comparison to the uncatalyzed reaction.[42] The I_{MPC}−CD complex, 6-*S*-2-(2-mercaptomethyl)-propane-6-deoxy-*β*-cyclodextrin diaqua palladium(II), was shown to sequence-specifically cleave the unactivated tertiary Ser−Pro peptide bond in the sequence Ser-Pro-Phe of the bradykinin substrate at pH 7.0 and 60 °C (Figure 4).[41] According to the experimentally proposed mechanism, in the first step an active enzyme−substrate complex was formed through weak hydrophobic interactions between the aromatic side chain of the Phe residue of the substrate and the cavity of the CD. In this complex, the Pd^{2+} metal center was placed adjacent to the Ser−Pro peptide bond. In the next step, the Pd^{2+} ion activated the amide group toward nucleophilic attack by a water molecule through the formation of a bond with the carbonyl oxygen atom (O^S) of the substrate. This reactive species could cleave the peptide using either an external solvent water (W1) molecule (cleavage by external attack) or a Pd^{2+} bound water (W2) molecule

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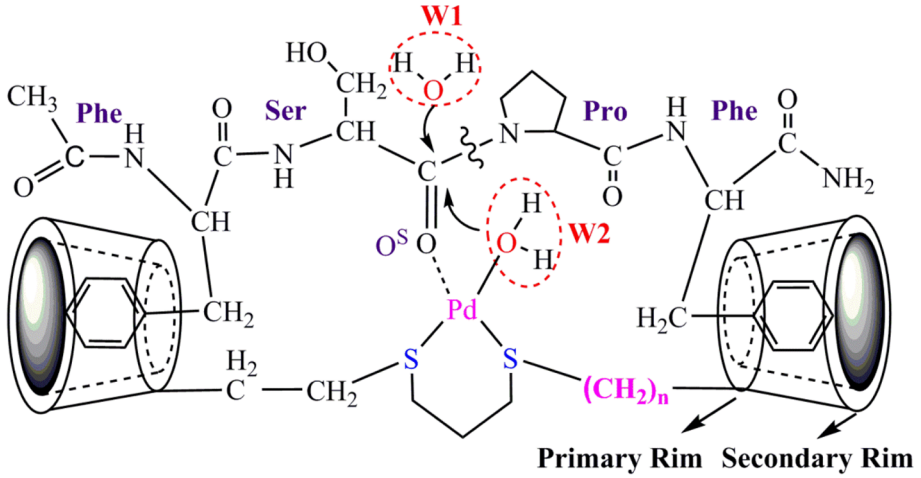

Figure 4. Experimentally proposed mechanisms for a conjugate of $[Pd(H_2O)_4]^{2+}$–β-cyclodextrin.

(cleavage by internal delivery). However, the exact reaction mechanism and the information regarding the location of the CD (number (n) of $-CH_2$ groups downstream from the S atom), conformation of the CD (primary or secondary rim of CD facing the substrate), the number of CDs (one or two), and optimum metal ion (Pd^{2+}, Co^{2+}, or Zn^{2+}) in the I_{MPC}-CD complex were not available (Figure 4).

A hybrid quantum mechanics/molecular mechanics (QM/MM: B3LYP/Amber) approach was employed to address these outstanding questions.[43] Our calculations showed that the internal delivery mechanism was the most energetically feasible for the peptide hydrolysis and both concerted and stepwise pathways occurred through similar barriers. The ideal position for the inclusion of the CD ring was found to be n = 2. At that position, the activity of I_{MPC}–CD could increase by as much as 3×10^5 times. The substrate should associate through the primary rim of the CD. For two CD ring containing systems, no rate acceleration was attained after the formation of the substrate–catalyst complex. Co^{2+} was the best substitution for Pd^{2+}, which could further improve the rate by 3.7×10^4 times.

3.2. Metal–Cyclen Complexes

In a different type of synthetic metallopeptidase, transition metals were attached to a macrocycle ring of 1,4,7,10-tetraazacyclododecane (cyclen) and 1-oxa-4,7,10-triazacyclododecane (oxacyclen), Figure 5a. Suh and co-workers showed that metal (M = Co(III) and Cu(II)) containing complexes of cyclen (1-M) and oxacyclen (2-M) can selectively cleave a wide range of biomolecules such as lysozyme, albumin, myoglobin, and Aβ peptide.[44−47] To provide specificity, an organic group or an aromatic chain (pendant) was attached to these complexes (B in Figure 5a).[48] This pendant acted as a recognition site and, based on its chemical structure, interacted with either a specific group or region of the substrate and placed the metal center next to the scissile peptide bond. It is noteworthy that the pendant was not required for the activities of these complexes and, even in its absence, the metal (M) complexes of cyclen (1′-M in Figure 5a) and oxacyclen (2′-M in Figure 5a) were reported to hydrolyze a wide range of biomolecules.[44,47,49]

The most plausible mechanism proposed for the Co(III)–cyclen (1-Co) complex is shown in Figure 5b.[46,50] In the reactant, the 1-Co complex was coordinated to a hydroxyl nucleophile and water molecule. In the first step, the substrate replaced the water molecule and covalently linked to the metal complex through a bond between its carbonyl oxygen atom and the metal ion

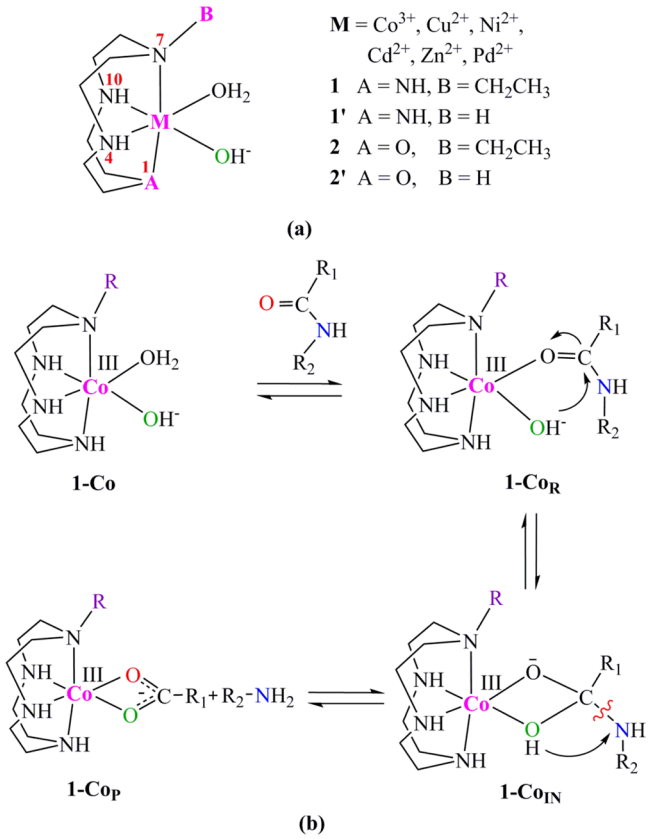

Figure 5. (a) Structures of metal–cyclen complexes. (b) The experimentally proposed mechanism for peptide hydrolysis.

(**1-Co_R**). This bond polarized the scissile peptide bond. In the second step, the metal bound hydroxyl group attacked the amide carbonyl carbon and formed a tetrahedral intermediate (**1-Co_{IN}**) consisting of a four-membered ring. The rate of the reaction was suggested to be determined by the stability of the transition state of this process, which was influenced by the Lewis acidity of the metal ion and the structure of the complex.[46] In the last step, a proton transfer to the amine group led to the splitting of the peptide bond, and separate amine ($-NH_2$) and carboxyl ($-COO$) termini (**1-Co_P**) were created. However, information concerning the mechanisms, structures and roles of the metal ion, ligand environment, and pendant on the energetics of this

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reaction was not consistently available. For example, on the basis of the direction of the hydrogen atoms coordinated to nitrogen atoms, the reactant can adopt the following four conformations: (1) *anti*−*anti* (N^4H and $N^{10}H$ hydrogen atoms are on opposite sides of the metal), (2) *syn*−*syn* (N^4H and $N^{10}H$ hydrogen atoms are on the same side of the metal), (3) *syn*−*anti* (N^4H is on the same and $N^{10}H$ is on the opposite side of the metal), and (4) *anti*−*syn* (N^4H is on the opposite and $N^{10}H$ is on the same side of the metal), Figure 5a. The structures of the reactants had not been established experimentally. The mechanism of the reaction had also not been elucidated. The coordination site (1, 4, 7, or 10 position of the cyclen ring) of the pendant and its influence on the energetics of the reaction were also not known. Moreover, the effect of diverse metals on the energetics using the same substrate was not available.

Our B3LYP calculations using the Phe20−Ala21 substrate suggested that the reactant of the 1-Co and 1-Cu complexes adopted the *syn*−*anti* and *syn*−*syn* conformations, respectively.[51] For divalent metal ions, the strain imposed by the cyclen ring was found to affect the stability of the reactants. In the Co(III) case, the N^{10} atom of the cyclen ring needed to be substituted with oxygen to form oxacyclen, while in the Cu(II) case the N^4 atom should be replaced. 1-Co and 2-Co cleaved the peptide bond with similar barriers of 39.8 and 40.1 kcal/mol, respectively, from the common *syn*−*anti* conformation. These barriers were in agreement with the experimental result that indicated that the cyclen complex exhibited only four times lower activity than the oxacyclen complex.[44] The barriers of the reaction using the Co(III)− and Cu(II)−cyclen complexes without the pendant (1′-Co and 1′-Cu, respectively) were lowered by 9.3 and 3.0 kcal/mol, respectively. The barrier of 30.5 kcal/mol for 1′-Co was found to be in good agreement with the experimentally measured value of 25.9 kcal/mol for the cleavage of myoglobin at pH 9.0 and 50 °C.[49] The reaction occurred with the lowest barrier of 27.2 kcal/mol for 1′-Ni (triplet) and with the highest barrier of 41.5 kcal/mol for 1′-Pd (singlet), Table 1. These results showed that the electronic state of the metal ion and binding mode of the macrocycle ring played critical roles in their activities.

Table 1. Energy Barriers for Hydrolysis of the Phe20−Ala21 Substrate for Different Metal−Cyclen Complexes

catalyst	barriers (kcal/mol)	conformation
1′-Ni (triplet)	27.2	*syn*−*anti*
1′-Cu (doublet)	29.7	*syn*−*syn*
1′-Cd (singlet)	30.5	*syn*−*syn*
1′-Co (singlet)	30.5	*syn*−*anti*
1′-Zn (singlet)	31.9	*syn*−*syn*
1′-Pd (triplet)	35.0	*syn*−*syn*
1′-Pd (singlet)	41.5	*syn*−*syn*

4. ROLE OF MICROENVIRONMENT OF THE ACTIVE SITE IN PEPTIDE HYDROLYSIS

To explore the precise role of the active site microenvironments of different peptidases, the hydrolysis of a single peptide bond (Phe1−Phe2) by the following three types of catalysts was studied: (1) aspartyl protease [*β*-secretase (BACE2)], (2) metallopeptidase [matrix metalloproteinase (MMP) and IDE], and (3) artificial metallopeptidases [I_{MPC}] and [$Pd_2(\mu$-OH)-([18]$aneN_6$)]$^{4+}$ (where [18]$aneN_6$ is 1,4,7,10,13,16-hexaazacyclooctadecane), I_{DPC}], Figure 6.[52] This study allowed us to make

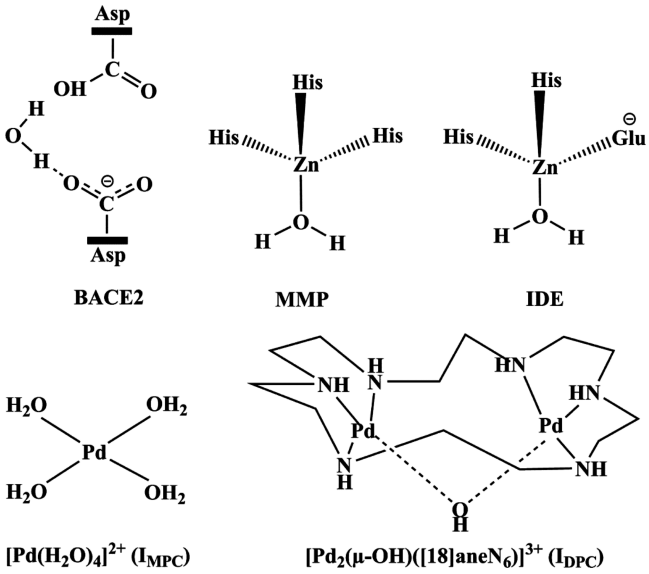

Figure 6. Structures of different peptidases.

the following interesting comparisons: (1) the effect of the active site in two aspartate residues containing aspartyl protease (BACE2) and metallopeptidases (MMP and IDE); (2) the influence of the ligand environment of the Zn^{2+} center in MMP (His, His, His (N_3)) and IDE (His, His, Glu (N_2O)); (3) the impact of the nature of the metal center in natural mononuclear Zn^{2+} containing metallopeptidases (MMP and IDE) and artificial mononuclear Pd^{2+} containing metallopeptidase (I_{MPC}); (4) the effect of the nature and number of the Pd metal centers in mononuclear (I_{MPC}) and binuclear (I_{DPC}) synthetic metallopeptidases. The computed energetics predicted that among these catalysts MMP with [N_3] moiety was the most efficient in catalyzing this reaction (Table 2). BACE2 and IDE catalyzed this

Table 2. A Comparison of the Rate-Limiting Barriers for the Phe−Phe Substrate Using Different Peptidases

catalyst	barriers (kcal/mol)
MMP	0.0
BACE2	+5.0
IDE	+6.9
[$Pd_2(\mu$-OH)([18]$aneN_6$)]$^{3+}$ (I_{DPC})	+13.5
[$Pd(H_2O)_4$]$^{2+}$ (I_{MPC})	+17.9

reaction with 5.0 and 6.9 kcal/mol higher barriers than MMP, respectively. The difference between MMP and IDE could be attributed to the reduction in acidity of the Zn ion in the [N_2O] moiety of IDE. However, the reduction of the barrier for BACE2 in comparison to IDE was quite surprising. Both artificial peptidases I_{MPC} and I_{DPC} catalyze this reaction with significantly high barriers of 35.4 and 31.0 kcal/mol, respectively. A binuclear metal center containing I_{DPC} was found to be ∼100 times faster than mononuclear I_{MPC}.

5. CONCLUDING REMARKS

The results gleaned from our theoretical studies provided intricate details regarding the role of metal ion(s), ligands, and the microenvironment of the reaction center and structures of short-lived intermediates and transition states and energetics in the functioning of the IDE and BILAP enzymes and their synthetic analogues. Understanding the guiding principles of

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peptide hydrolysis by metallopeptidases and designing the next generation of synthetic analogues will require a great amount of interdisciplinary cooperation. To accomplish these goals theoretical models need to be further improved to simulate experimental conditions as accurately as possible. The effect of dynamics in computed energetics using free energy calculations of reaction pathways also needs to be included. In this regard, methods such as orthogonal space random walk (OSRW) would be very useful.[53] The knowledge gained from the studies of peptide hydrolysis may also be useful in designing metal complexes that catalyze the hydrolysis of esters, nitriles, and phosphates and other organic reactions including epoxide opening, aldol condensation, Michael addition, and Diels–Alder reactions. However, significant challenges remain in designing small molecule catalysts with better activities, higher turnover numbers, and multiple reaction sites.

AUTHOR INFORMATION

Corresponding Author

*E-mail: rpr@miami.edu. Tel: 305-284-9372. Fax: 305-284-4571.

Notes

The authors declare no competing financial interest.

Biographies

Tingting Zhang obtained her B.S. from Southwest University, Chongqing, China, in 2008. Her research in the lab of Dr. Rajeev Prabhakar is mainly focused on the design of synthetic analogues of metallopeptidases.

Mehmet Ozbil received his B.S. from Istanbul Technical University, Istanbul, Turkey, in 2007. He received his Ph.D. in the laboratory of Dr. Rajeev Prabhakar at the University of Miami, FL, U.S.A., in 2013. He is currently a postdoctoral fellow in Dr. Victor Batista's lab at Yale University. His current research interests include determination of pK_a values and odor compound selectivity.

Arghya Barman received a M.S. in Biochemistry from the University of Calcutta, India, in 2005. He received his Ph.D. in the laboratory of Dr. Rajeev Prabhakar at the University of Miami, FL, U.S.A., in 2012. He is currently a postdoctoral fellow in Dr. Donald Hamelberg's lab at Georgia State University. His current research interests include investigations of molecular interactions and information transfer in complex biomolecular systems.

Thomas J. Paul received a B.A. in chemistry from Florida Gulf Coast University, Fort Myers, Florida, in 2013. His research in the laboratory of Dr. Rajeev Prabhakar at the University of Miami, FL, U.S.A., is focused on the mechanistic and structural aspects of proteases and peptidases.

Ram Prasad Bora received his M.S. in physical chemistry from Andhra University, Andhra Pradesh, India. From 2008 to 2010, he did postdoctoral research training in the laboratory of Dr. Rajeev Prabhakar at the University of Miami, Miami, Florida, U.S.A. He is currently a postdoctoral fellow in Dr. Arieh Warshel's lab at the University of Southern California, U.S.A. His current research interests include exploring and understanding enzyme evolutionary aspects.

Rajeev Prabhakar is an associate professor at the University of Miami, FL, U.S.A., in the department of chemistry. His main research interests are applications of innovative theoretical and computational approaches to study mechanisms of chemical and biochemical reactions, the design of catalysts, computer aided drug design, and chemistry associated with neurological disorders.

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