38 *Inorg. Chem.* **2010**, *49*, 38–46
DOI: 10.1021/ic901071v

Inorganic Chemistry
Article

Theoretical Insights into the Mechanism of Selective Peptide Bond Hydrolysis Catalyzed by $[Pd(H_2O)_4]^{2+}$

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Received June 3, 2009

In this study, mechanisms for the hydrolysis of the Gly-Pro bond in Gly-Pro-Met and Gly-Pro-His, the Gly-Sar bond in Gly-Sar-Met, and the Gly-Gly bond in the Gly-Gly-Met peptide catalyzed by $[Pd(H_2O)_4]^{2+}$ (**I**) have been investigated at the DFT level. In all cases, the optimized structure of the active bidentate complex, formed by the reaction of **I** with the substrate $[Pd(H_2O)_2\{(Gly)\text{-}(Pro)\text{-}(Met\text{-}\kappa S,\kappa N)\}]^{1+}$ complex for the Gly-Pro-Met peptide, was found to exist in the trans conformation. This structure is in agreement with the experimentally measured TOCSY and ROESY ^{1}H NMR spectra. After the formation of this complex, the following two mechanisms have been proposed experimentally: (1) external attack mechanism and (2) internal delivery mechanism. The DFT calculations suggest that in the external attack mechanism the calculated barriers are prohibitively high (i.e., 50−70 kcal/mol) for the cleavage of all the peptide bonds, and therefore, this mechanism is ruled out. However, in the internal delivery mechanism, the bidentate complex is first transformed from the trans to the cis conformation. Here, the overall barriers for the hydrolysis of the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met peptide bonds are 38.3, 41.4, 39.8, and 39.2 kcal/mol, respectively. These barriers are in much better agreement with the experimentally measured rate constants at pH 2.0 and at 60 °C. The substitution of Pd(II) with Pt(II) was found to make a negligibly small difference (0.53 kcal/mol) on the barrier for the cleavage of the Gly-Pro-His bond. These calculations indicate that after the creation of the active bidentate complex in the trans conformation the internal delivery mechanism is the most energetically feasible.

I. Introduction

In the last couple of decades, there has been a tremendous interest in designing artificial peptidases for protein sequencing[1,2] and footprinting,[3] proteomics,[4] bioanalytical analysis,[5] and bioengineering of proteins.[6] These applications require fragments of peptides derived either from residue-selective or sequence-specific hydrolytic cleavage processes. For instance, the cleavage of any X−Y bond in an X−Y−Met segment is residue-selective, while the splitting of the only X−Pro bond in an X−Pro-Met- fragment is sequence-specific (X and Y are amino acid residues and Met = methionine and Pro = proline).[7]

In the past few years, several transition metal (Pd, Pt, Zn, Cu, Co, and Fe) complexes have been synthesized to hydrolyze the amide bond under milder conditions.[1,8−20] In

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 Published on Web 12/03/2009

particular, in weakly acidic aqueous solutions, various Pd(II) ion-containing metal complexes, such as $[Pd(H_2O)_4]^{2+}$ (**I**), have been reported to anchor Met and His residues and to hydrolyze the proximal peptide bond.[2,21−25] In **I**, the Pd(II) ion has been shown to anchor Met and His residues in X−Y-Met and -His sequences, respectively, and to cleave the proximal X−Y (Gly-Gly, Gly-Pro, and Gly-Sar) peptide bond (Gly = glycine and Sar = sarcosine).[1,26] In this process, the selectivity of the cleavage is guided by the anchoring residues (Met or His), and the scissile bond-generating residues (X and Y) do not influence the coordination of the Pd(II) ion to Met or His.[27−36] Furthermore, the cleavage of the X−Y peptide bond is quite general and irrespective of the nature of X and Y (aliphatic or aromatic, polar or nonpolar, and charged or neutral) in every X−Y-Met and X−Y-His sequence the X−Y bond was activated.[37] It was found that the tertiary peptide bond X−Pro in X−Pro-Met and X−Pro-His segments at pH = 2.0 is cleaved much faster than the secondary peptide bond X−Gly in X−Gly-His and X−Gly-Met segments.[37] The adjustment in pH can transform this process from residue selective to sequence specific.[1] For instance, in acidic aqueous solutions, the cleavage is residue selective because it is directed by the anchoring residues (Met and His), while in mildly acidic and neutral solutions, due to the involvement of the Pro residue, the cleavage becomes specific to X−Pro-His and X−Pro-Met sequences.[1]

The hydrolysis of the Gly-Pro peptide bond of the Gly-Pro-Met sequence by **I** can be represented by the following overall reaction:

$$\text{Gly-Pro-Met} + H_2O \rightarrow \text{Gly}-\text{COOH} + NH_2-\text{Pro-Met} \quad (1)$$

The experimentally proposed mechanism of reaction 1 catalyzed by **I** is described in Figure 1.[1] In the first step, an aqua ligand of **I** is displaced, and the S atom of the anchoring Met residue of the substrate (R−Gly-Pro-Met-) interacts with the Pd(II) ion to generate a monodentate complex $[Pd(H_2O)_3\{(Gly)\text{-}(Pro)\text{-}(Met-\kappa S)\}]^{2+}$ (**II$_{PM}$**), where the subscript PM denotes the Pro-Met peptide. This binding is important to achieve the regioselectivity in the coordination of **I** to the R−Gly-Pro-Met peptide. In the second step, the nitrogen (N) atom in the amide backbone of Met is deprotonated by the anchored Pd(II) ion to form the Pd(II)−N bond, and a hydronium ion (H_3O^+) is released. The presence of the Pd(II) ion significantly lowers the pK_a for this deprotonation to less than 2.0, which in the absence of metal ions is estimated to be ca. 15.[29,31] Pd is known to be the most efficient transition metal ion in deprotonating the secondary amide group and the subsequent binding to the amidate anion.[27−32,38,39] This process leads to the generation of a reactive bidentate complex $[Pd(H_2O)_2\{(Gly)\text{-}(Pro)\text{-}(Met-\kappa S,\kappa N)\}]^{1+}$ (**III$_{PM}$**), Figure 1. This structure exists at pH values between 5 and 7.[1]

Based on NMR studies, the active bidentate complex **III$_{PM}$** has been proposed to utilize the following two plausible mechanisms for the hydrolysis of the amide bond: (1) external

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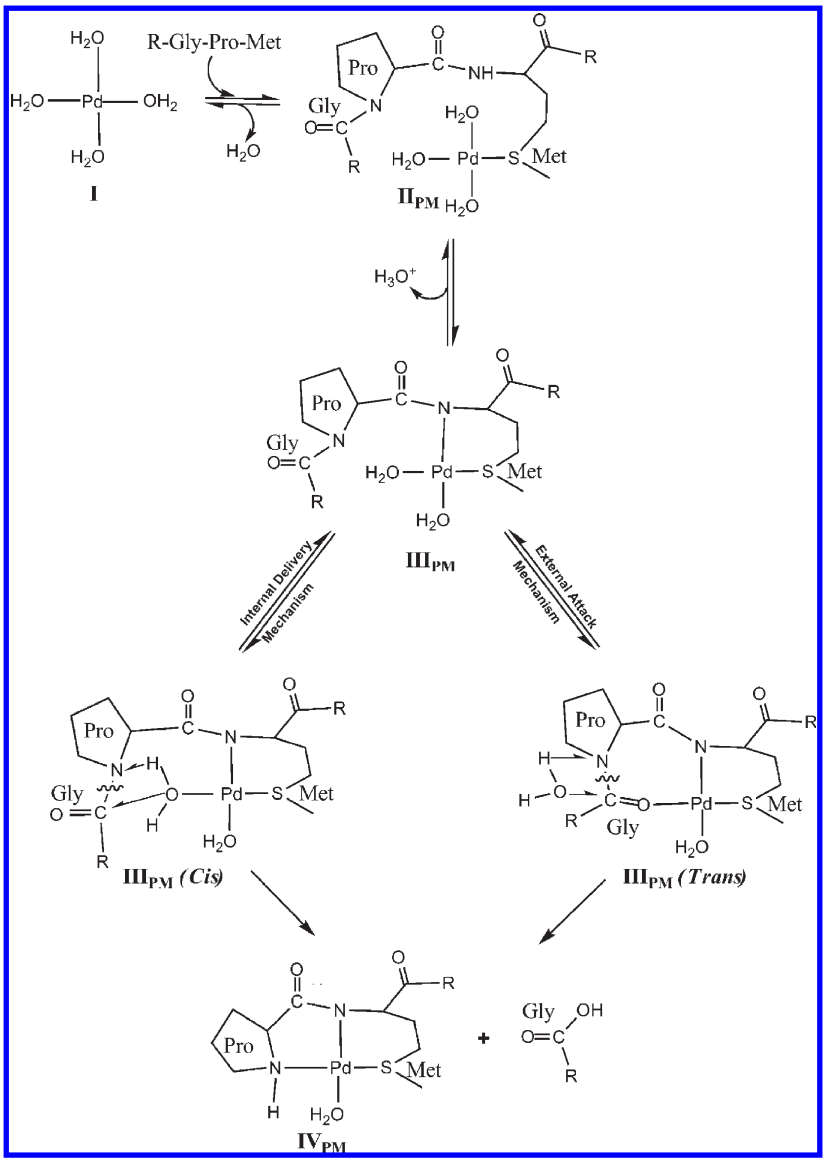

Figure 1. Experimentally suggested mechanism for the hydrolysis of the R−Gly-Pro-Met peptide bond catalyzed by $[Pd(H_2O)_4]^{2+}$. The arrows describe the movements of atoms.

attack and (2) internal delivery.[1] In the cleavage by the external attack mechanism (Figure 1), from **III$_{PM}$**, the oxygen atom (O) bound to the α-carbon atom (C^{α}) of the scissile bond interacts with the anchored Pd(II) ion, and the system adopts a trans conformation. In this conformation, the carbonyl oxygen atom is oriented toward the Pd(II) ion. The interaction between the Pd(II) ion and C^{α} atom increases the electrophilicity of the latter and activates it for an attack by an external water molecule.[31,40] In this mechanism, in a concerted manner, the proton and the hydroxyl group of the activated water molecule are abstracted by the N and C^{α}, respectively, to cleave the scissile peptide bond (Gly-Pro). Here, the Pd(II) ion functions as a Lewis acid. On the other hand, in the cleavage by the internal delivery mechanism, an aqua ligand coordinated to the Pd(II) ion is activated to hydrolyze the amide bond. In this mechanism, the anchored Pd(II) ion does not coordinate to the carbonyl oxygen atom, and the amide bond adopts the cis conformation. In this conformation, the carbonyl carbon atom is facing the Pd(II) ion. The measured rate constants at pH 2.0 and 60 °C for the cleavage of the tertiary peptide bonds, Gly-Pro bond in R−Gly-Pro-Met sequence, Gly-Pro bond in R−Gly-Pro-His sequence and Gly-Sar bond in R−Gly-Sar-Met peptide, and the secondary Gly-Gly peptide bond in R−Gly-Gly-Met peptide is 6.0×10^{-2}, 9.4×10^{-2}, 1.4×10^{-2}, and 2.8×10^{-3} min^{-1}, respectively, which corresponds to barriers of 24.0, 23.8, 25.0, and 26.1 kcal/mol, respectively.[1] The measured rate constants were converted into barriers using the Arrhenius equation ($k = Ae^{-Ea/RT}$, where A is the pre-exponential factor, Ea is the activation energy, R is the gas constant, and T is the temperature). The basicity of the amino group and the metal-binding ability of the amide oxygen atom have been suggested to contribute to the observed differences in the rate constants.[1,40] Among these peptides, the nitrogen atom of Pro has the most basic amino group (pK_a = 10.96) and exhibits the greatest propensity for the cleavage. The TOCSY and ROESY ^{1}H NMR spectra of the R−Gly-Pro-Met peptide (where the subscript GP denotes the Gly-Pro peptide) indicated that in **III$_{PM}$** the Gly-Pro sequence exists in

(40) Martin, R. B. Peptide bond characteristics. *Met. Ions Biol. Syst* **2001**, *38*, 1−23.

trans conformation that supports the external delivery mechanism.[1]

Chin J. et al. reported that the hydrolysis of *p*-nitrophenyl acetate by the Zn-monoaqua complex could occur through either the external attack or the internal delivery mechanism, but they are kinetically indistinguishable.[41,42] Sutton et al. also investigated the hydrolysis of amino esters and peptides promoted by the Co(III) complexes through these mechanisms and concluded that they cannot be distinguished by purely kinetic methods.[17] In this aspect, theoretical calculations can make an important contribution in discriminating between these two reaction pathways. These calculations have been successfully applied to investigate reaction mechanisms of several complex organometallic systems.[43−46] Therefore, based on the experimental information, we have performed B3LYP calculations to elucidate the mechanisms of the cleavage of four different peptide bonds, i.e., the Gly-Pro bond in Gly-Pro-Met, the Gly-Sar bond in Gly-Sar-Met, the Gly-Gly bond in the Gly-Gly-Met, and Gly-Pro bond in the Gly-Pro-His peptide. Furthermore, in order to investigate the role of the metal ion, the energetics for the cleavage of the Gly-Pro bond in the Gly-Pro-His peptide were also computed by substituting the Pd(II) ion with the Pt(II) ion. The calculations performed in the present paper not only allow detailed study of individual steps of the mechanism but also provided energetics and structures of all short-lived intermediates and transition states. The specific mechanistic information gleaned from this study, such as the roles of the metal-bound water molecule, metal ion, and anchoring residues, will enhance our understanding of the functioning of the Pd metal center containing complexes and advance efforts in designing more efficient catalysts through modifications in the substrate, metal ion, and ligands.[47−49]

II. Computational Details

A. Methods. All calculations were performed using the Gaussian 03 program.[50] The geometries of reactants, intermediates, transition states and products were optimized without any symmetry constraints at the B3LYP/Lanl2dz level of theory with additional d polarization functions for the S atom ($\alpha = 0.55$) and the corresponding Hay−Wadt effective core potential (ECP) for Pd.[51−53] The final energies of the optimized structures were further improved by performing single-point calculations including additional d and p polarization functions for O ($\alpha = 0.96$), N ($\alpha = 0.74$), C ($\alpha = 0.59$), and H ($\alpha = 0.36$) atoms, respectively, (taken from the EMSL's Gaussian basis set library) in the basis set used for optimizations.

The dielectric effects from the surrounding environment were estimated using the self-consistent reaction field IEF-PCM method[54] at the B3LYP/Lanl2dz level with d polarization functions for the S atom. These calculations were performed at 298.15 K, and the dielectric constant of 78.39, corresponding to water, was used.[55] In general, when models with the same charge are used, the relative dielectric effects are not very sensitive to the methods used or to the value chosen for the dielectric constant.[43,56] However, due to the formation and dissociation of a hydronium ion (H_3O^+), the models with different charges have been used in the generation of the active bidenate complex, i.e., **I** → **III** process. In this process, the solvents were found to exert unusually large dielectric effects (ca. 40.0−60.0 kcal/mol) on the computed energies. Since the computed solvent effects are unreliable in the **I** → **III** process, B3LYP/{Lanl2dz + d(S,O) + p(H)} energies including zero point vibrational (unscaled), thermal (at 298.15 K and 1 atm.) and entropy corrections (at 298.15 K) are reported. However, in the **III** → **IV** process that represents both "external attack" and "internal delivery" mechanisms, models with the same charge have been used and the computed solvents effects are generally within the 2−5 kcal/mol range. Therefore, for this process the energies obtained at the B3LYP/{Lanl2dz + d(S,O) + p(H)} + zero-point energy (unscaled), thermal and entropy corrections (at 298.15 K and 1 atm.) + solvent effects in water are discussed, while the energies without solvent effects are provided in parentheses.

B. Models. The available experimental information was fully incorporated in building models for the calculations. In the $[Pd(H_2O)_4]^{2+}$ (**I**)−substrate complexes, tripeptide models of the substrates, including anchoring, and peptide bonds donating amino acid residues were utilized. For instance, to investigate the cleavage of the Gly-Pro peptide bond of the R−Gly-Pro-Met substrate, the Gly-Pro-Met tripeptide was used. Both Pro and Met residues including their backbones were utilized, while Gly was modeled as the acetyl ($-COCH_3$) group. The charge on all the systems is +1, and they exist in the singlet spin state.

III. Results and Discussion

The reaction 1 is calculated to be slightly endergonic (ΔG = 2.0 (0.29) kcal/mol). In the present B3LYP study, mechanisms for the hydrolytic cleavage of the Gly-Pro bond in the Gly-Pro-Met peptide, the Gly-Sar bond in the Gly-Sar-Met peptide, the Gly-Gly bond in the Gly-Gly-Met peptide, and the Gly-Pro bond in the Gly-Pro-His peptide catalyzed by **I** have been investigated. The entire mechanism is divided in the following two parts: (1) generation of the reactive

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Table 1. Electronic Charges on N^1 and C^2 Atoms and Peptide-Bond (N^1-C^2) Lengths of the Reactants Formed in Trans and Cis Conformations

substrate	N^1	C^2	N^1-C^2 (Å)
	External Mechanism (trans)		
Pro-Met	−0.21	0.38	1.34
Pro-His	−0.15	0.43	1.33
Sar-Met	−0.08	0.41	1.34
Gly-Met	0.07	0.43	1.34
	Internal Mechanism (cis)		
Pro-Met	−0.15	0.19	1.36
Pro-His	−0.16	0.23	1.37
Sar-Met	0.25	0.31	1.38
Gly-Met	0.08	0.21	1.36

bidentate complex and (2) cleavage of the peptide bond. In the first part, from **I**, generation of the active bidentate complex, through the monodentate complex, for all the aforementioned peptides have been studied (see Supporting Information). It was found that the nature of the substrate influences the energetics of this complex (Table 1 in the Supporting Information). The effects of the electronic nature of the Pro, Sar, and Met residues on the computed bond length of the scissile peptide bond were also investigated. In the presence of the Pro residue, the N^1 atom becomes more electronegative, and there is a large charge separation of 0.59e and 0.57e in the N^1-C^2 peptide bonds for the Gly-Pro-Met and the Gly-Pro-His peptides, respectively, in the trans conformation (Table 1). The substitution of Pro with the electron-donating Sar residue in the Gly-Sar-Met sequence reduces it by 0.10e. Furthermore, the existence of Gly in the Gly-Gly-Met peptide significantly decreases it by 0.24e. Since the abstraction of a proton from the Pd(II)-bound water molecule by the N^1 atom initiates the cleavage of the peptide bond, the excess of negative charge on this atom in the presence of Pro may contribute to the faster splitting of the tertiary peptide bonds. Interestingly, these charge differences were not found to influence the N^1-C^2 bond length, which remained around 1.34 Å for all the peptides (Table 1).

After the creation of the bidentate complex, the hydrolysis of all the peptide bonds has been studied. Throughout the paper, first the energies of the Gly-Pro-Met sequence are discussed followed by the comparisons with the remaining peptides.

IIIa. Cleavage of the Peptide Bond. After the generation of the active bidentate complex, the peptide bonds can be cleaved through the following two mechanisms: (1) the external attack and (2) internal delivery.

IIIa1. External Attack Mechanism. In this mechanism, the Pd(II) ion functions as a Lewis acid and activates the peptide bond for hydrolysis by an external water molecule. Here, the N^1-C^2 bond between the Gly and Pro residues in the Gly-Pro-Met sequence of $\mathbf{III_{PM\text{-}E}}$ (where subscript -E denotes the structures formed in the external attack mechanism) is activated through the coordination of the O^5 atom of the Gly residue with the Pd(II) ion ($Pd-O^5$ = 2.14 Å), Figure 2. A tetra-coordinated metal center containing $\mathbf{III_{PM\text{-}E}}$ was confirmed to be the energetically most stable conformation. The penta-coordinated structure in which the Pd(II) ion weakly interacts with the O^5 atom at the axial position was found to be 17.6 and 17.8 kcal/mol (in gas phase) more endothermic for the Gly-Pro-Met and Gly-Sar-Met peptides, respectively. From $\mathbf{III_{PM\text{-}E}}$, in a concerted fashion, the N^1 atom of the side chain of the Pro residue abstracts a proton (H^4) from the external water molecule (H_2O^3), and the hydroxyl group ($-O^3H^-$) makes a nucleophilic attack on the C^2 atom of the Gly residue. This process leads to the cleavage of the N^1-C^2 peptide bond. The optimized transition state ($\mathbf{TS_{PM\text{-}E}}$) is shown in Figure 2. In $\mathbf{TS_{PM\text{-}E}}$, all the relevant bond distances indicate that this process is concerted (O^3-H^4 = 1.20, N^1-H^4 = 1.35, O^3-C^2 = 1.77, and N^1-C^2 = 1.50 Å). In comparison to $\mathbf{III_{PM\text{-}E}}$, the charge on the Pd(II) ion is reduced by 0.04e in $\mathbf{TS_{PM\text{-}E}}$. However, the computed barrier for this process is extremely high at 70.2 (79.4) kcal/mol. In comparison to the cleavage of the Gly-Pro-Met, the barrier for the splitting of Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met peptide bonds are lower by 17.3 (19.9), 19.2 (23.0), and 22.0 (26.8) kcal/mol respectively. The optimized transition states ($\mathbf{TS_{PH\text{-}E}}$, $\mathbf{TS_{SM\text{-}E}}$, and $\mathbf{TS_{GM\text{-}E}}$) for the cleavage of Gly-Sar-Met, Gly-Gly-Met, and Gly-Pro-His bonds, respectively, are quite similar. It is noteworthy that the measured barrier for the activation of the Gly-Pro-Met (24.0 kcal/mol), Gly-Pro-His (23.8 kcal/mol), Gly-Sar-Met (25.0 kcal/mol), and Gly-Gly-Met (26.1 kcal/mol) are significantly lower by 46.2, 29.1, 26.0, and 22.1 kcal/mol, respectively, than that of the computed values. Due to the large difference in the measured and computed values, this mechanism for the peptide-bond cleavage is ruled out.

IIIa2. Internal Delivery Mechanism. In the first step of the mechanism, the reactant $\mathbf{III_{PM\text{-}E}}$ is transformed from the initial trans to the cis conformation ($\mathbf{III_{PM\text{-}I}}$), where subscript -I denotes the structures formed in the internal delivery mechanism. In $\mathbf{III_{PM\text{-}I}}$, the scissile peptide bond (N^1-C^2) does not interact with the Pd(II) ion. The $\mathbf{III_{PM\text{-}E}}$ (trans) → $\mathbf{III_{PM\text{-}I}}$ (cis) transformation is caused by the substitution of a water molecule (H_2O^3), the dissociation of the $Pd-O^5$ bond, and the rotation of the N^1-C^2 bond. The potential energy surface for this transformation is so complex that it is not possible to locate the corresponding transition state(s). In comparison to the trans conformation, all the N^1-C^2 bonds are longer by 0.02−0.04 Å in the cis structure (Table 1). In $\mathbf{III_{PM\text{-}I}}$, the N^1-C^2, $Pd-O^3$ and $Pd-N^6$ bond distances are 1.36, 2.13, and 1.97 Å, respectively, and it is computed to be 21.0 (24.4) kcal/mol more endergonic than that of $\mathbf{III_{PM\text{-}E}}$. Similarly, the generation of $\mathbf{III_{PH\text{-}I}}$, $\mathbf{III_{SM\text{-}I}}$, and $\mathbf{III_{GM\text{-}I}}$ complexes are 14.2 (19.6), 18.1 (27.7), and 16.2 (19.1) kcal/mol higher in energy than those of $\mathbf{III_{PH\text{-}E}}$, $\mathbf{III_{SM\text{-}E}}$, and $\mathbf{III_{GM\text{-}E}}$, respectively.

In this mechanism, from $\mathbf{III_{PM\text{-}I}}$, a water molecule ($H_2O^3$) bound to the Pd(II) ion is activated. Here, the O^3-H^4 bond of the water molecule is broken, and the proton is transferred to the N^1 atom of the Pro residue. This process occurs concomitant with the transfer of the hydroxyl group ($-O^3H$) to the C^2 atom of the Gly residue and cleaves the Gly-Pro peptide bond. From $\mathbf{III_{PM\text{-}I}}$, this process undergoes a barrier of 17.3 (21.7) kcal/mol, and the optimized transition state ($\mathbf{TS_{PM\text{-}I}}$) is shown in Figure 2. The key bond distances ($Pd-O^3$ = 2.05, O^3-H^4 = 2.12, N^1-H^4 = 1.04, O^3-C^2 = 1.97, and N^1-C^2 = 1.62 Å) indicate that this process is concerted. In contrast to the external delivery mechanism, here the charge on the

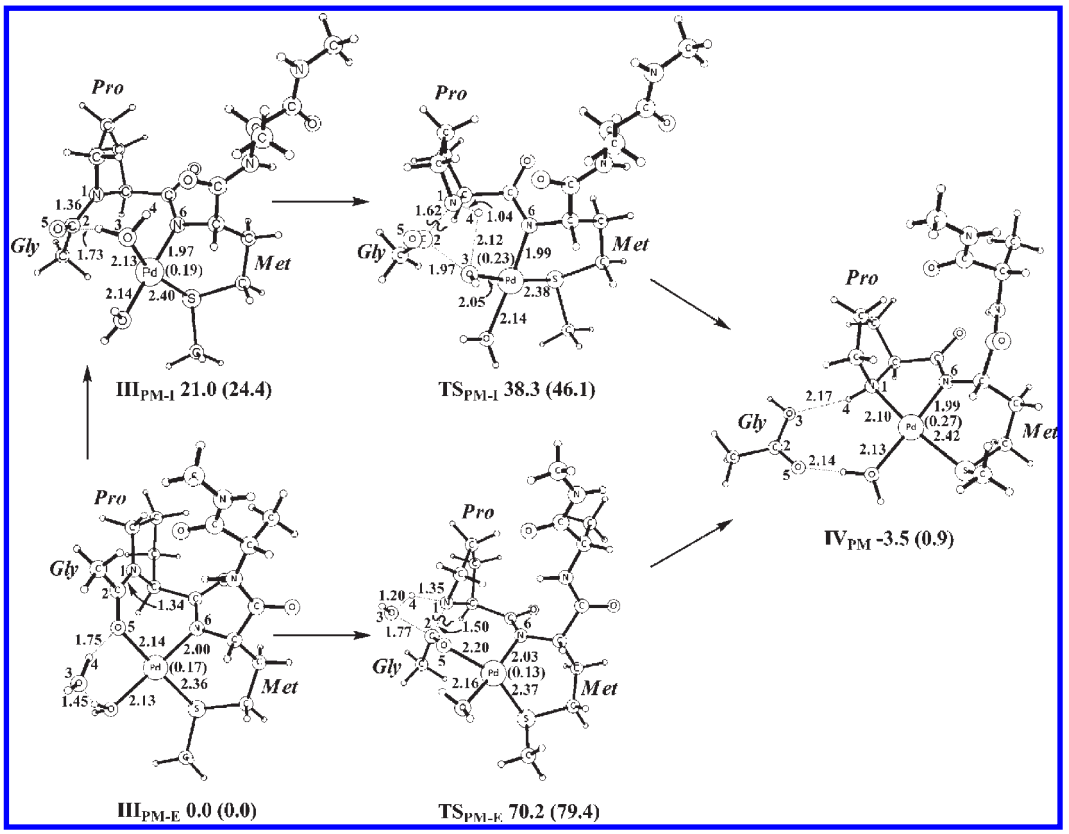

Figure 2. Structures (in Å) and energies [with and without (in parentheses) solvent effects in kcal/mol] of the reactant, intermediate, transition states (optimized), and product for the peptide-bond hydrolysis catalyzed by $[Pd(H_2O)_4]^{2+}$. The arrows describe the movements of atoms, and the charge on the Pd(II) ion is shown in parentheses.

Pd(II) ion is increased by 0.04e in **TS$_{PM-I}$**. The reason for such an easy activation of the water molecule is its binding with the Pd(II) ion and its polarization by the negatively charged O^5 atom of Gly and the metal cation. This situation is similar to that of the Zn metal center containing metallopeptidases, such as thermolysin, in which the metal-bound water molecule has been reported to strongly acidify, and its pK_a value goes down from ~14 in solution to ~7.[57] In this enzyme, the water molecule is strongly polarized between the negatively charged glutamate and the Zn cation.[58] Since this step is followed by a step (**III$_{PM-E}$** → **III$_{PM-I}$**) that is endergonic by 21.0 (24.4) kcal/mol, the overall barrier from **III$_{PM-E}$** becomes 38.3 (46.1) kcal/mol. In this mechanism, the overall barriers from **III$_{PH-E}$**, **III$_{SM-E}$**, and **III$_{GM-E}$** are 41.4 (49.0), 39.8 (51.1) and 39.2 (49.9) kcal/mol, respectively. The corresponding optimized transition states (**TS$_{PH-I}$**, **TS$_{SM-I}$**, and **TS$_{GM-I}$**) for the Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met sequences are quite similar (Figure 3). It is noteworthy that the computed barriers for this mechanism are significantly lower by 31.9 (33.3), 11.5 (10.5), 11.2 (5.3), and 9.0 (2.7) kcal/mol for the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met bonds, respectively, than in the external delivery mechanism. These results clearly suggest that after the generation of the reactant in the trans conformation, the internal delivery mechanism is energetically more feasible. However, in comparison to experiments, in this mechanism the computed barriers for the cleavage of Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met bonds are higher by 14.3, 17.6, 14.8, and 13.1 kcal/mol, respectively. This overestimation could be partially contributed by the measurement of the rate constants at a much higher temperature (60 °C), whereas our calculations were performed at room temperature (25 °C). Due to the temperature dependence of the pre-exponential constant in the Arrhenius equation, it is not possible to accurately estimate the measured barrier at 25 °C.

The measured rate constants at 60 °C and pH = 2.0 suggest that the cleavage of the tertiary Gly-Pro and Gly-Sar peptide bonds is faster than the secondary Gly-Gly bond.[1] The measured barriers for the cleavage of these bonds are quite close, i.e., within 0.2−2.3 kcal/mol. The computed barriers for these processes are also found to be in the same range, i.e., 0.5−3.1 kcal/mol. However, the accuracy of the B3LYP method (3−5 kcal/mol in computing reaction barrier) employed in this study does not allow for discrimination between the hydrolysis of these two different types (secondary and tertiary) of peptide bonds.

The cleavage of the Gly-Pro-His bond was also investigated through the activation of an external water molecule trapped between the Pd-bound water molecule (H^4O^3H) and the peptide bond (N^1-C^2) through hydrogen bonds. In comparison to hydrolysis by the metal-coordinated H^4O^3H molecule discussed above, the computed barrier is significantly higher by 16.5 kcal/mol

(57) Glades, A.; Vallee, B. L. *Metal Ions in Biological Systems*. Dekker: New York, 1983; Vol. *15*, p 6−7.
(58) Pelmenschikov, V.; Blomberg, M. R. A.; Siegbahn, P. E. M. Theoretical study of the mechanism for peptide hydrolysis by thermolysin. *J. Biol. Inorg. Chem.* **2002**, *7*, 284−298.

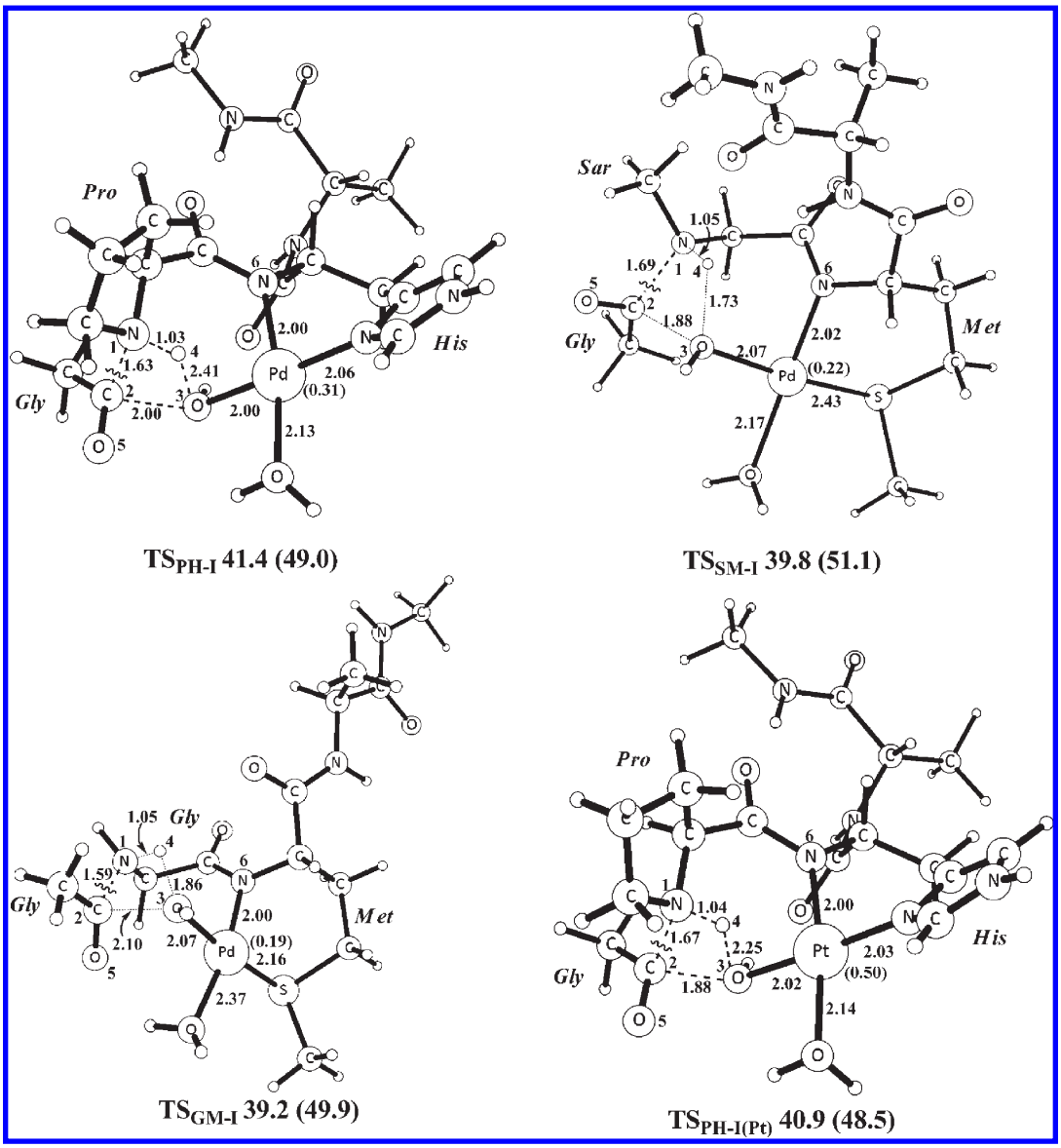

Figure 3. Optimized structures (in Å) and energies [with and without (in parentheses) solvent effects in kcal/mol] of transition states for the internal delivery mechanism of the peptide-bond hydrolysis catalyzed by $[Pd(H_2O)_4]^{2+}$. The charge on the Pd(II) ion is shown in parentheses.

(in gas phase) for this mechanism. Furthermore, in order to study the role of the metal ion, the cleavage of this peptide was also studied by substituting Pd(II) with Pt(II) in the bidentate complex. The presence of Pt was found to slightly reduce the barrier by 0.53 (0.50) kcal/mol. The optimized transition state ($\mathbf{TS_{PH-I(Pt)}}$) for this process is shown in Figure 3. The cleavage of the Gly-Pro bond in the Gly-Pro-Met sequence leads to the carboxyl (−COOH) and amine ($-NH_2$) terminals of the Gly-Pro peptide ($\mathbf{IV_{PM}}$), Figure 2. In $\mathbf{IV_{PM}}$, the N^1 atom of the Pro side chain occupies the vacant location in the Pd(II) metal center. The generation of $\mathbf{IV_{PM}}$ is exergonic by 3.5 (0.9) kcal/mol from $\mathbf{III_{PM-E}}$. The corresponding $\mathbf{IV_{PH}}$, $\mathbf{IV_{SM}}$, and $\mathbf{IV_{GM}}$ products are also structurally similar to $\mathbf{IV_{PM}}$ and exergonic by 8.1 (2.6), 8.6 (3.4) and 12.6 (0.3) kcal/mol from $\mathbf{III_{PH-E}}$, $\mathbf{III_{SM-E}}$, and $\mathbf{III_{GM-E}}$, respectively.

These results explicitly indicate that, from the external bidentate complex, the internal delivery mechanism is the most energetically feasible for the hydrolysis of peptide bonds.

IV. Summary and Conclusions

In the present B3LYP study, mechanisms for the hydrolysis of the Gly-Pro bond in 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 peptide catalyzed by $[Pd(H_2O)_4]^{2+}$ (**I**) have been elucidated. The entire mechanism is divided into the following two parts: (1) generation of the reactive bidentate complex and (2) cleavage of the peptide bond. The potential energy diagram for the most plausible mechanism is shown in Figure 4.

In the first step of the mechanism, a water molecule bound to the Pd(II) ion of **I** is replaced by the anchoring Met residue of the Gly-Pro-Met peptide to generate a monodentate complex $[Pd(H_2O)_3\{(Gly)\text{-}(Pro)\text{-}(Met{-}\kappa S)\}]^{2+}$ ($\mathbf{II_{PM}}$), Figure 1 in the Supporting Information. The formation of $\mathbf{II_{PM}}$ is highly exergonic by 102.3 kcal/mol. In comparison to $\mathbf{II_{PM}}$, the generation of the corresponding complexes, $\mathbf{II_{PH}}$ (for the Gly-Pro-His peptide), $\mathbf{II_{SM}}$ (for the Gly-Sar-Met peptide), and $\mathbf{II_{GM}}$ (for the Gly-Gly-Met peptide) were found to be energetically less favorable by 18.2, 5.2, and 17.9 kcal/mol (in

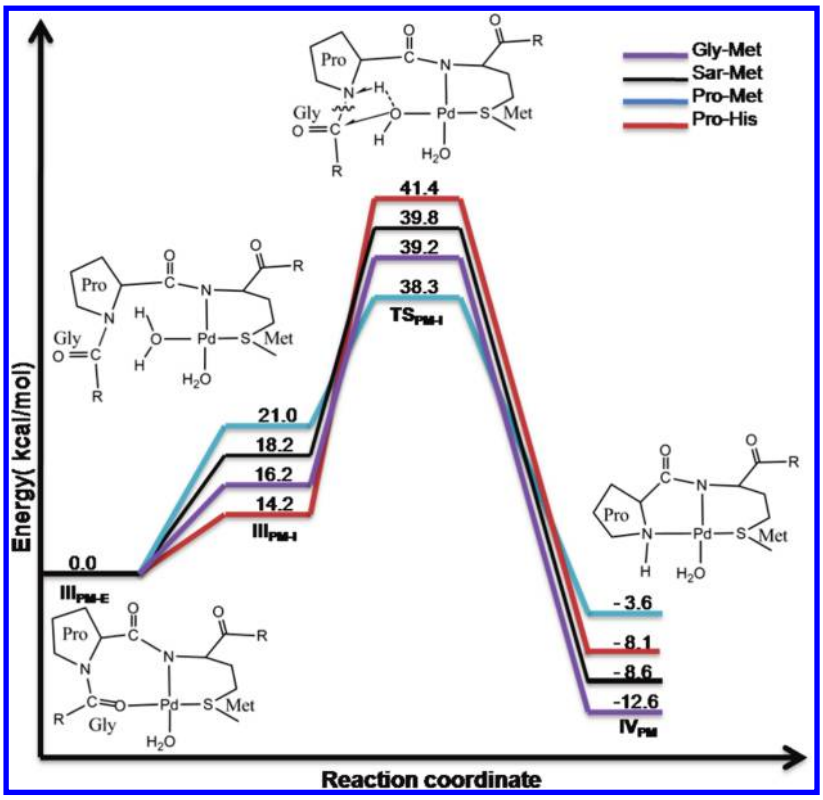

Figure 4. Potential energy diagram of the mechanism of the peptide-bond hydrolysis catalyzed by $[Pd(H_2O)_4]^{2+}$.

gas phase), respectively, (Table 1 in the Supporting Information). In the second step, the amide group of the Pro residue (in the Gly-Pro-Met sequence) is deprotonated by the Pd(II) ion to generate the active bidentate complex $[Pd(H_2O)_2\{(Gly)\text{-}(Pro)\text{-}(Met{-}\kappa S,\kappa N)\}]^{1+}$ (**III$_{PM-E}$**). The **III$_{PM-E}$** complex exists in the trans conformation that is also supported by experimentally measured TOCSY and ROESY ^{1}H NMR spectra.[1] The formation of **III$_{PM-E}$** is exergonic by 4.3 kcal/mol (in gas phase) from **II$_{PM}$**. The generation of **III$_{PH-E}$**, III$_{SM-E}$, and **III$_{GM-E}$** are exergonic by 10.8, −1.5, and 0.8 kcal/mol (in gas-phase), respectively (Table 1 in the Supporting Information). It was found that the creation of the bidentate complex is energetically more favorable in the presence of the tertiary peptide bond generating Pro residue.

After the formation of the active bidentate complex, the hydrolysis of the peptide bond can proceed through the following two pathways: (1) the external attack and (2) internal delivery mechanisms. In the external attack mechanism, an external water molecule is utilized for the hydrolysis of the peptide bond. However, this process was computed to undergo extremely high barriers of 70.2 (79.4), 52.9 (59.5), 51.0 (56.4), and 48.2 (52.6) kcal/mol from **III$_{PM-E}$**, **III$_{PH-E}$**, **III$_{SM-E}$**, and **III$_{GM-E}$**, respectively. Since the computed barriers in this mechanism are prohibitively higher than the experimental values (measured at pH 2.0 and 60 °C) of 24.0, 23.8, 25.0, and 26.1 kcal/mol for the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met peptides, respectively,[1] it is ruled out.

In the first step of the internal delivery mechanism, **III$_{PM-E}$** is converted from the trans to the cis conformation (**III$_{PM-I}$**). From **III$_{PM-E}$**, the generation of **III$_{PM-I}$** is computed to be endergonic (ΔG = 21.0 (24.4) kcal/mol). The formation of **III$_{PH-I}$**, **III$_{SM-I}$**, and **III$_{GM-I}$** complexes are also endergonic by 14.2 (19.6), 18.1 (27.7), and 16.2 (19.1) kcal/mol from **III$_{PH-E}$**, **III$_{SM-E}$**, and **III$_{GM-E}$**, respectively. From **III$_{PM-I}$**, the $O^3{-}H^4$ bond of the Pd(II)-bound water molecule is cleaved, and the proton is transferred to the N^1 atom of Pro concomitantly with the nucleophilic attack of the hydroxyl group ($-O^3H$) to the C^2 atom of the scissile peptide bond ($N^1{-}C^2$). This concerted process leads to the splitting of the $N^1{-}C^2$ peptide bond. From **III$_{PM-I}$**, this process climbs a barrier of 17.3 (21.7) kcal/mol, and the formation of the product (**IV$_{PM}$**) that contains the separate carboxyl (COO^-) and amine ($-NH_2$) terminals is exergonic by 3.5 (0.9) kcal/mol. Since this step follows a 21.0 (24.4) kcal/mol endergonic process, the overall barrier from **III$_{PM-E}$** becomes 38.3 (46.1) kcal/mol. Similarly, the corresponding overall barriers from **III$_{PH-E}$**, **III$_{SM-E}$**, and **III$_{GM-E}$** are 41.4 (49.0), 39.8 (51.1), and 39.2 (49.9) kcal/mol, respectively, and the formation of the **IV$_{PH}$**, **IV$_{SM}$** and **IV$_{GM}$** products are exergonic by 8.1 (2.6), 8.6 (3.4), and 12.6 (0.3) kcal/mol, respectively. The computed barriers in this mechanism are significantly lower by 31.9 (33.3), 11.5 (10.5), 11.2 (5.3), and 9.0 (2.7) kcal/mol for the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met bonds, respectively, than those of the external delivery mechanism and in much better agreement with the measured barriers at pH 2.0 and 60 °C. This discrepancy between the computed and measured values could be due to the significantly high temperature used in the experiments.[1] The cleavage of the Gly-Pro-His bond through an external water molecule, located between the Pd(II)-bound water molecule and the scissile peptide, was found to undergo a barrier that is higher by 16.5 kcal/mol (in gas phase). Furthermore, to investigate the role of the metal ion, the cleavage of this bond was also studied with the Pt(II)-bound bidentate complex. This substitution was found to make a little difference (0.53 kcal/mol) on the computed barrier.

These results explicitly indicate that after the formation of the bidentate complex in the trans conformation, the internal delivery mechanism is the most energetically feasible for the hydrolysis of the Gly-Pro-Met, Gly-Pro-His, Gly-Sar-Met, and Gly-Gly-Met peptide bonds. Our calculations have provided deeper insights into the complex mechanism of peptide-bond hydrolysis by the metal-containing $[Pd(H_2O)_4]^{2+}$ complex. The results reported in this study will advance our efforts to design efficient artificial peptidases for advanced bioengineering and bioanalytical applications.

Acknowledgment. A funding grant (DOH grant number 08KN-11) to R.P. from the James and Esther King Biomedical Research Program of the Florida State Health Department is acknowledged.

Supporting Information Available: Complete ref 50, Generation of the reactive bidentate complex, and Tables S1−S30: Cartesian coordinates (in Å) of all the optimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.