# New Regioselectivity in the Cleavage of Histidine-Containing Peptides by Palladium(II) Complexes Studied by Kinetic Experiments and Molecular Dynamics Simulations

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**Abstract:** Palladium(II) complexes promote hydrolytic cleavage of amide bonds in *N*-acetylhistidylglycine (AcHis−Gly), *N*-acetylhistidine (AcHis), and their derivatives methylated at the N-1 or N-3 atom of imidazole. Methylation controls coordination of imidazole to palladium(II) and allows stereochemical analysis of the reactions. The complex $[PdCl_4]^{2-}$ regioselectively cleaves the amide bond involving the carboxylic group of histidine, the bond His−Gly; the rate constants of cleavage are virtually the same when the peptides coordinate to palladium(II) via the N-1 and the N-3 atom. The complex $[Pd(H_2O)_4]^{2+}$ cleaves, at comparable rates, the amide bonds involving both the carboxylic (His−Gly) and the amino (AcHis) groups of histidine in the acetylated dipeptide. This unprecedented reactivity is examined by theoretical calculations in which molecular dynamics and solution of Poisson−Boltzmann equation are combined in a new way. When the $Pd(H_2O)_3^{2+}$ group is attached to the N-1 atom, both scissile bonds can be cleaved by internal delivery of aqua ligands. When the $Pd(H_2O)_3^{2+}$ group is attached to the N-3 atom, both scissile bonds can be cleaved by internal delivery of aqua ligands and by external attack of water; in some conformers the two modes of cleavage may be combined in the reaction mechanism. In both N-1 and N-3 linkage isomers internal delivery seems to be assisted by weak hydrogen bonding. The rate constants for cleavage by $[Pd(H_2O)_4]^{2+}$ are approximately 10 times greater than those for cleavage by $[PdCl_4]^{2-}$. This difference is explained semiquantitatively by consideration of the aquation equilibria involving $[PdCl_4]^{2-}$. This study shows that kinetics and regioselectivity of peptide cleavage may be controlled simply by choosing ligands in palladium(II) complexes. This is another step in our development of simple metal complexes as artificial metallopeptidases.

## Introduction

Selective cleavage of proteins has long been one of the most important procedures in analytical biochemistry. The amide bond, however, is extremely unreactive. Uncatalyzed hydrolysis of peptides by water at pH around 7 occurs with the half-lives of 250−600 years.[1] Even the nonselective hydrolysis, under forcing conditions, is slow. For example, half-lives at room temperature of the dipeptide Gly-Gly are 2 days in 1.0 M NaOH and 150 days in 1.0 M HCl. In practical work, relatively concentrated solutions of strong bases as catalysts are applied under reflux overnight. Only several proteolytic enzymes are commonly used in determinations of amino acid sequence.[2] Moreover, these enzymes require narrow ranges of temperature and pH.

Transition-metal complexes have emerged as promising reagents for cleavage of peptides and proteins.[3−51] These new reagents can be attached to the substrate by a covalent tether[15−19,23,24,27−31] or simply by spontaneous coordination of amino acid side chains to the metal atom.[32−51] Transition-metal

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complexes are well suited for the traditional task of sequence determination and for new challenges, such as preparation of semisynthetic proteins, structural mapping and functional analysis of protein domains, analysis of protein interactions with other proteins and with nucleic acids, elucidation of protein folding, analysis of nonnative states, and development of new drugs. Because of their small size, transition-metal complexes can be applied in structural studies that require relatively high "resolution", at the level of functional groups and chemical bonds in proteins.

Studies in our laboratory showed that palladium(II) complexes spontaneously bind to the side chains of methionine and histidine residues and effect hydrolytic cleavage of short peptides with half-lives that range from several hours to several minutes.[35–51] Because palladium(II) complexes are diamagnetic, kinetic experiments are easily done by ^{1}H NMR spectroscopy. Because the cleavage reactions occur with turnover,[43,48,50] the simple palladium(II) complexes that catalyze them can be considered primitive artificial peptidases. Our successes in selective cleavage of cytochrome *c*,[38] myoglobin,[51] and other proteins[52] bode well for the general applicability of these new reagents in biochemistry and allied disciplines. Before palladium(II) complexes can become widely accepted, the basis for their selectivity must be explained in studies with peptides.

The present study shows, for the first time, that peptide bonds can be cleaved with commercially available salts of $[PdCl_4]^{2-}$ anion; no chemical derivatization or modification of this simple complex is required. Aquation of this complex, however, causes a remarkable difference in reactivity; the complex $[Pd(H_2O)_4]^{2+}$ cleaves peptides more rapidly and with different regioselectivity than its chloro precursor does. We combine experimental kinetics and theoretical molecular dynamics to study how simple substitution of ancillary ligands affects hydrolytic cleavage of peptides by palladium(II) complexes and to determine the stereochemical basis for the regioselectivity of cleavage.

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## Experimental Procedures

**Chemicals.** The deuterium-containing compounds $D_2O$, $DClO_4$, and NaOD and the salts $K_2[PdCl_4]$ and $AgClO_4$ monoxydrate (99.999% pure) were obtained from Aldrich Chemical Co. All other chemicals were of reagent grade. Dipeptide His-Gly and amino acids 1-Me-His and 3-Me-His were obtained from Sigma Chemical Co. The terminal amino group in each of these substrates was acetylated by a published procedure.[53] Dipeptides 1-Me-AcHis–Gly and 3-Me-AcHis–Gly were synthesized from 1-Me-AcHis and 3-Me-AcHis by a standard solid-state method, and their purity was checked by HPLC; this was done by the staff of the Protein Facility. The complex $[Pd(H_2O)_4]^{2+}$ was prepared by a published method.[36]

**Measurements.** Proton NMR spectra of solutions in $D_2O$, with DSS as an internal reference, were recorded with Bruker DRX 400 and Varian Unity 500 spectrometers. Temperature was kept within $\pm0.5$ °C. The pH was measured with Fisher 925 instrument and a Phoenix Ag/AgCl reference electrode. The pD values were calculated by the standard formula:[54] pD = pH + 0.41. Ultraviolet–visible spectra were recorded with a Perkin-Elmer $\lambda$-18 spectrophotometer.

**Study of Hydrolysis.** Because all the solutions were made in $D_2O$, all the aqua ligands actually were $D_2O$. For simplicity and consistency with our previous publications, however, we show aqua ligands as $H_2O$. For the cleavage of peptides with $K_2[PdCl_4]$, 400 $\mu L$ of a 100 mM solution of $K_2[PdCl_4]$ in $D_2O$, 100 $\mu L$ of a 100 mM solution of peptide in $D_2O$, 50 $\mu L$ of a 100 mM solution of DSS in $D_2O$, and 150 $\mu L$ of $D_2O$ were mixed in the NMR tube. For the cleavage of peptides with $[Pd(H_2O)_4]^{2+}$, 2.00 mL of a 100 mM solution of this complex in $D_2O$ was mixed with 500 $\mu L$ of a 100 mM solution of the peptide or the histidine derivative in $D_2O$, in the Eppendorf vial. The pH was adjusted to 1.0 (i.e., pD to 1.4) with 2.0 M $DClO_4$ and was measured at the beginning and at the end of each experiment. The difference was always less than 0.10. Samples were kept at 40 °C for different periods of time. The reactions were followed by ^{1}H NMR spectroscopy, and the spectra were very similar to those that we published before.[40,43] The error in integration of the resonances was estimated at $\pm5\%$. In all the experiments with $[Pd(H_2O)_4]^{2+}$, and in the early experiments with $[PdCl_4]^{2-}$, the reactions were quenched by adding 400 $\mu L$ of a 200 mM solution of sodium diethyldithiocarbamate (Naddtc) in $D_2O$ to 200-$\mu L$ aliquots of the reaction mixture. The insoluble $[Pd(ddtc)_2]$ was removed by centrifugation, and ^{1}H NMR spectra of the clear supernatant were recorded. Free glycine and free acetic acid were identified by their ^{1}H NMR chemical shifts and by enhancement of the corresponding resonance upon addition of these compounds to the reaction mixture.

**Molecular Dynamics Simulation.** To sample the conformational space of the Pd(II)–peptide complex, we performed a Langevin molecular dynamics simulation in a vacuum (dielectric constant of 1.0), over a period of 3.00 ns, with an integration step of 1.00 fs; the energies were saved after each 10 fs, for further analysis. The system was coupled to a heat bath at 1000 K, and the friction coefficient $\beta$ was set to 2.0 ps$^{-1}$. In addition to the original set of CHARMM[55] parameters, the improper torsion centered at the $C_\gamma$ atom of histidine was constrained to keep this atom trigonal and therefore keep the $C_\beta$–$C_\gamma$ bond coplanar with the imidazole ring, as it must be. The palladium(II)–ligand torsion angles were left unconstrained. The parameters that are not included in the CHARMM set are given in Supporting Information, Table S1. The bonded energy parameters for the coordinated water were taken from the SPCE model for water.[56] Atomic charges in the palladium(II) complex were calculated by the extended Hückel method and are given in Supporting Information, Table S2. Nonbonded energies for all pairs of atoms were calculated without any cutoffs. The C-terminus of the peptide was reasonably assumed to be protonated.

The molecular dynamics trajectory was used to find conformations with the interatomic distances that are comparable to, or smaller than, the sum of the van der Waals radii of the two atoms involved. The

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pertinent distances are two: first, from the oxygen atom of an aqua ligand on palladium(II) to the carbon atom of an amide bond; and second, from the palladium(II) ion to the oxygen atom of an amide bond. The energy of each of these conformations was minimized in three steps. First, all non-hydrogen atoms were fixed in their position obtained by MD simulation, as mentioned above; only the hydrogen atoms were used in the minimization. In the second step, the non-hydrogen atoms were gently constrained in their position by a harmonic potential with the force constant of only 1.0 kcal mol$^{-1}$. In the final step of energy minimization all constraints were removed, and all degrees of freedom of the molecule were allowed to vary. This procedure is very likely to achieve the goal—find local energy minima close to the conformations with relatively small O—C and Pd—O distances, which were found in the trajectories.

**Energetics of Various Conformations.** In the molecules of interest a Pd(H$_2$O)$_3{}^{2+}$ group is attached to the N-1 or N-3 atom in the imidazole ring of AcHis—Gly. At least three factors contribute to the change in the energy of different conformations, $\Delta G_T$; see eq 1. Changes in

$$\Delta G_T = \Delta\Delta G_R + \Delta G_{NE} + \Delta G_C \tag{1}$$

solvation energy come from electrostatic, $\Delta G_R$, and nonelectrostatic, $\Delta G_{NE}$, interactions. There are also changes in Coulombic electrostatic energy, $\Delta G_C$. The G$_C$ term is energy calculated with the Coulomb law, using a single dielectric constant for a uniform medium. The $\Delta G_R$ term, which is calculated with the Poisson—Boltzmann equation, includes $\Delta G_C$ but contains an additional term if more than one dielectric medium is present. In calculations of solvation energy with a thermodynamic cycle, the Coulombic terms cancel out in the difference; see the next subsection. We neglect contributions to the change of the conformational energy that arise from the bonded-energy terms. The three terms in eq 1 can be estimated under various approximations.

Electrostatic contribution to the solvation energy ($\Delta G_R$) arises from polarization of the medium by the charges in the (H$_2$O)$_3$Pd—peptide complex. This contribution can be calculated by solving numerically the Poisson—Boltzmann equation.[57–59] The energy required to bring a molecule with the dielectric constant $\epsilon_s$ from a medium with the dielectric constant $\epsilon_m$ to a medium with the dielectric constant $\epsilon_s$ is given in eq 2. The energy of a molecule within its reaction field is defined in eq 3. In it, $q_i$ are the atomic charges in the molecule, and $\phi_i$

$$\Delta G_R = G_R(\epsilon_s, \epsilon_m) - G_R(\epsilon_s, \epsilon_s) \tag{2}$$

$$G_R(\epsilon_s, \epsilon_i) = {}^1/{}_2 \sum q_i \phi_i(\epsilon_s, \epsilon_i) \tag{3}$$

is the electrostatic potential at the position of the charge $i$ inside a dielectric cavity with a dielectric constant $\epsilon_s$ in a medium with a dielectric constant $\epsilon_i$ such that $\epsilon_i$ is $\epsilon_m$ or $\epsilon_s$. The cavity is defined by the water-accessible surface of the molecule. The potential $\phi_i$ can be obtained by solving numerically the Poisson—Boltzmann equation or its linearized form.

Nonelectrostatic contribution to the solvation energy ($G_{NE}$) is caused by van der Waals interactions between the solute and the solvent and also by creation or enlargement of the cavity for the solute against the solvent pressure. This, the second term in eq 1, can be estimated by the empirical eq 4, in which $\Delta A$ is the change in the water-accessible

$$\Delta G_{NE} = a + b\Delta A \tag{4}$$

surface during a conformational transition. The water-accessible surface ($A$) is defined by rolling a sphere with a diameter of 1.4 Å over the (H$_2$O)$_3$Pd—peptide molecule. The parameters $a$ and $b$ are empirically fitted.[59,60] Since we are interested in relative, not absolute, energies of various conformations, we justifiably neglected the parameter $a$, which

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**Scheme 1**
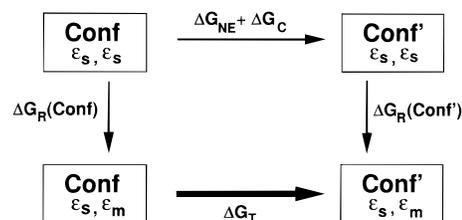


is common to all of them. The parameter $b$ was set at 5.0 cal mol$^{-1}$ A$^{-2}$.[59]

Coulombic energy ($G_C$) arises from pairwise interactions among all the atoms and can be calculated according to eq 5, in which $i$ and $j$ run

$$G_C(\epsilon_s) = {}^1/{}_2 \sum_{j \neq i} (q_i q_j / \epsilon_s r_{ij}) \tag{5}$$

over all the atoms. In eqs 2, 3, and 5, $\epsilon_m$ was set at 80.0 for water, and $\epsilon_s$ was set at 4.0 for the peptide interior. In eqs 2 and 3, ionic strength was set at zero, and a Stern layer of 4.0 Å was used. In the finite-difference algorithm for numerically solving the Poisson—Boltzmann equation, an 81 × 81 × 81 grid with a 0.5-Å spacing was used.

**The Thermodynamic Cycle.** Numerical solution of the Poisson—Boltzmann equation requires discrete redistribution of charge on the grid. The artifact resulting from this redistribution is termed grid energy. It precludes direct calculation of the change of the conformational energy $\Delta G_T$, along the thick horizontal line in Scheme 1. This artifact can be avoided by calculating the other three steps in the thermodynamic cycle.[61] We calculated the energy required to transfer the (H$_2$O)$_3$Pd—peptide complex in an arbitrary reference conformation from a medium with the dielectric constant of water to a medium with the dielectric constant of the peptide (the vertical on the left) and the energy required to transfer the complex in a different conformation in the same direction (the vertical on the right). The grid energies canceled out in the "vertical" calculations, and we obtained reliable quantities for the two "vertical" processes in Scheme 1. Because in the calculation of $\Delta G_C$ the dielectric constants of the solute and of the medium are equal ($\epsilon_s$, $\epsilon_s$ in the upper portion of Scheme 1), this term was calculated with the Coulomb law and a single dielectric constant. Estimation of $\Delta G_{NE}$ completed the cycle and allowed an estimation of $\Delta G_T$. These calculations were done with the program package MEAD 1.1.3.[62]

## Results and Discussion

**Substrates for Hydrolysis.** In all the substrates the terminal amino group was acetylated, so that it cannot be the first one to coordinate to palladium(II). In four of the substrates the N-1 or N-3 atom of the imidazole ring was methylated, to enforce palladium(II) binding to the other nitrogen atom. The six substrates are AcHis, AcHis—Gly, 1-Me-AcHis, 1-Me-AcHis—Gly, 3-Me-AcHis, and 3-Me-AcHis—Gly. See Chart 1. For the sake of brevity, the amide bonds involving the amino and carboxylic groups of histidine, respectively, are designated "left" and "right." (In biochemical parlance, these bonds are designated as lying "upstream" and "downstream" from the histidine residue.) The "left" amide bond exists in all the substrates; the "right" one exists only in the dipeptide and its two methyl derivatives.

**Palladium(II) Complexes.** Although the reactions were run in D$_2$O and therefore the aqua complexes contain D$_2$O, we write H$_2$O for the sake of clarity and consistency with our previous publications. The complex [Pd(H$_2$O)$_4$]$^{2+}$ was made by the

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**Chart 1**



**Ac-His**



**Ac-His-Gly**
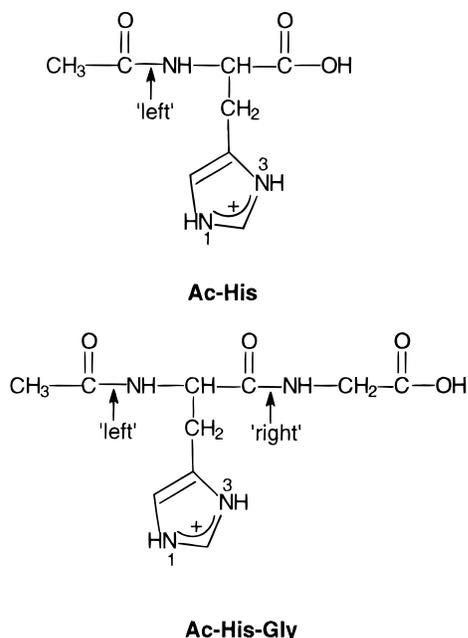
aquation reaction in acidic aqueous solution, as shown in eq 6.

$$[PdCl_4]^{2-} + 4Ag^+ + 4H_2O \rightarrow$$
$$[Pd(H_2O)_4]^{2+} + 4AgCl(s) \quad (6)$$
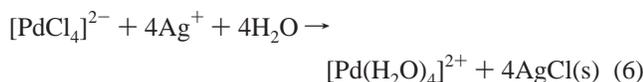
The ultraviolet absorption maximum occurs at 390 nm, as reported previously.[36] Control experiments showed that $AgClO_4$ in the absence of palladium(II) complexes does not promote cleavage of peptides.[36] The aqua complex was freshly prepared before the kinetic experiments and kept at pD 1.4, to suppress formation of hydroxo-bridged polynuclear complexes.

**Regioslective Cleavage of AcHis−Gly Catalyzed by $[PdCl_4]^{2-}$.** Upon mixing AcHis−Gly with $[PdCl_4]^{2-}$, three complexes detectable by NMR spectroscopy formed rapidly. The known modes of coordination, shown in Chart 2, were defined on the basis of the characteristic chemical shifts of the imidazole H-2 and H-5 protons.[40] The amide proton can be displaced by the palladium(II) ion, as in the complex designated **2**, even at pD < 2.0.[63−69] The unspecified ligands on the palladium(II) ion are $Cl^-$ anions, at least initially. (Their aquation on standing will be discussed below.) The major species is the complex designated **3**.

As Figure 1 shows, in the presence of $[PdCl_4]^{2-}$ glycine was completely released from the dipeptide AcHis−Gly. Two derivatives of AcHis−Gly, containing a methyl group at either nitrogen atom in the imidazole ring, also undergo this cleavage reaction. It was followed by the decline of the $^1H$ NMR resonance for the glycyl residue in the coordinated dipeptide and the growth of the resonance for the free glycine. The decline and growth occur with the same rate constant, which is listed in Table 1. Under the same conditions free acetic acid was not

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**Figure 1.** Hydrolytic cleavage at pD 1.4 and 60 °C of the histidine−glycine bond in the peptide AcHis−Gly. The solution in $D_2O$ was initially 32 mM in AcHis−Gly and 128 mM in $K_2[PdCl_4]$.

**Chart 2**



**1**



**2**



**3**
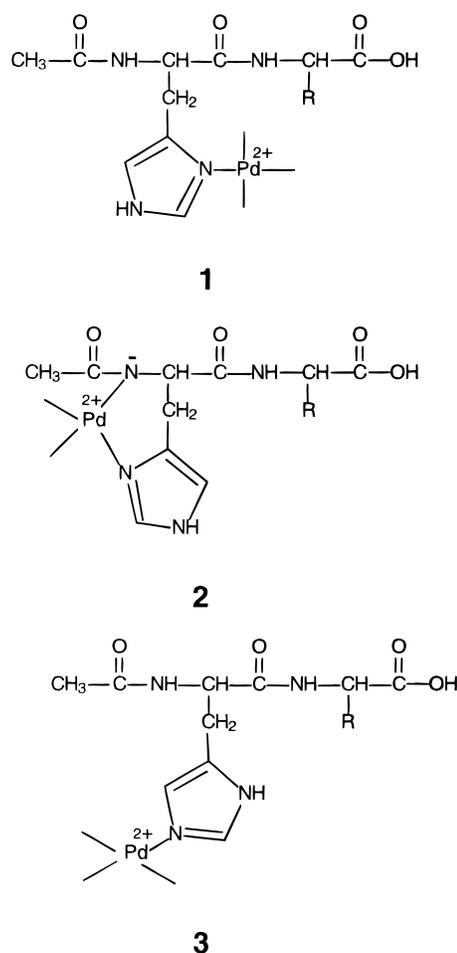
detected by NMR spectroscopy even after 1 week. Clearly, only the "right", not the "left", amide bond was cleaved. This regioselectivity was further examined in the reaction of $[PdCl_4]^{2-}$ with AcHis, which lacks the "right" amide bond. Although the complexes **1** and **3** formed immediately upon mixing of the reactants, the "left" amide bond was not cleaved to a detectable extent. As Table 1 shows, $[PdCl_4]^{2-}$ did not promote cleavage of methylated AcHis, either. This unreactivity confirms that the $PdCl_3^-$ group bound to either nitrogen atom in the histidine side chain does not promote cleavage of the "left" amide bond.

That the substrate is consumed at the same rate at which the products are formed indicates that in the cleavage reaction

**Table 1.** Rate Constants ($10^4 k_{obs}$, in $min^{-1}$)[a] for the Hydrolysis of Histidine-Containing Substrates Catalyzed by Two Palladium(II) Complexes, at pD 1.4 and 40 °C[b]

| substrate[c] | $[PdCl_4]^{2-}$ "right" | $[Pd(H_2O)_4]^{2+}$ | |
|---|---|---|---|
| | | "left" | "right" |
| AcHis−Gly | 5.0 | 29 | 49 |
| AcHis | | 21 | |
| 1-Me-AcHis−Gly | 5.2 | 22 | 43 |
| 1-Me-AcHis | | 20 | |
| 3-Me-AcHis−Gly | 4.1 | 11 | 49 |
| 3-Me-AcHis | | 10 | |

[a] To obtain $k_{obs}$, divide the number by $10^4$. Estimated error is ±10%. [b] Blank spaces correspond to reactions that cannot occur with a given substrate. [c] The "left" and "right" amide bonds are shown in Chart 1.

intermediates do not accumulate. Indeed, $^1$H NMR spectra did not contain any resonances attributable to intermediates.

The substrate 1-Me-AcHis−Gly forms complexes **1** and **2**, which are interconverted by (de)protonation of the "left" amide nitrogen atom. Our previous study showed that the chelate complex **2** is inactive in hydrolysis.[43] In it the "left" amide bond is stabilized by the nitrogen coordination to palladium(II), whereas the "right" amide bond is remote from the palladium-(II) that is firmly held on the "left" side. Complex **3**, however, is flexible enough to allow the approach of the $PdCl_3^-$ group to the "right" amide bond but, judging from Table 1, not to the "left" amide bond. Because the substrate 3-Me-AcHis−Gly forms only the complex **3**, this linkage isomer must be responsible for the substrate cleavage.

In cleavage reactions promoted by *cis*-$[Pd(en)(H_2O)_2]^{2+}$, the unspecified ligands in Chart 2 are ethylenediamine and $H_2O$. In this previous case,[40,43] as in the present one, only the "right" amide bond was cleaved. In the previous case, however, the linkage isomer **3** was at least 50 times more reactive than the linkage isomer **1**,[43] whereas in the present case the two isomers are nearly identical in reactivity, as Table 1 shows. The ancillary ligands—bidentate ethylenediamine versus two unidentate $Cl^-$ ions—apparently exert decisive influence on the cleavage reaction.

**Catalytic Turnover.** One equivalent of the $[PdCl_4]^{2-}$ complex cleaves as many as five equivalents of AcHis−Gly. This is a modest turnover number, but it proves the principle of catalysis. There are two "connected" catalytic cycles in Scheme 2, for the linkage isomers **1** and **3**. All the species shown in Scheme 2 were detected by $^1$H NMR spectroscopy. So was the unreactive complex **2**, which yielded the active complex **1** upon protonation; this process and free chloride ions are omitted from Scheme 2, for the sake of clarity.

Turnover is assisted by the relative lability of the palladium-(II)−imidazole bond and by the acidic solvent. Because hydronium ions displace palladium(II) from AcHis, the cleavage reaction is not inhibited by this product of cleavage. The palladium(II) complex detaches from AcHis and attaches to the AcHis−Gly molecule to be cleaved next.

**Cleavage of AcHis−Gly Promoted by $[Pd(H_2O)_4]^{2+}$.** Because the acidic solvent protonates the imidazole ring in the peptide and lowers its affinity for palladium(II), the $[Pd(H_2O)_4]^{2+}$ complex was always added in a 4-fold molar excess over the dipeptide, to enhance coordination. Thorough control experiments showed that in the acidic solution of pD 1.4, in the absence of palladium(II) reagents, the peptide is not cleaved to a significant degree.

The reaction of interest is shown in Scheme 3. Dipeptide AcHis−Gly forms all three complexes shown in Chart 2; 1-Me-AcHis−Gly forms complexes **1** and **2**; and 3-Me-AcHis−Gly

forms complex **3**. Cleavage of the "right" amide bond is easily followed by $^1$H NMR spectroscopy. The singlets at 3.89 and 3.48 ppm correspond to free glycine and to the complex *cis*-$[Pd(N,O\text{-}Gly)(H_2O)_2]^+$, in which glycine is coordinated via its amino and carboxylate groups. This mode of glycine chelation is well known.[70] Indeed, an equimolar mixture of $[Pd(H_2O)_4]^{2+}$ and glycine showed the same singlet in the $^1$H NMR spectrum.

Since the $^1$H NMR resonance of the acetyl group in AcHis−Gly and its methyl derivatives is broadened upon addition of $[Pd(H_2O)_4]^{2+}$, this resonance overlaps with that of free acetic acid, and cleavage of the "left" amide bond could not be observed directly. Fortunately, upon removal of the palladium-(II) species as the insoluble complex $[Pd(ddtc)_2]$, acetic acid, a product of the cleavage of the "left" amide bond, was easily detected and quantitated. As Table 1 shows, the "right" bond is cleaved more rapidly than the "left" one. The respective yields of cleavage are 100% and 85% with AcHis−Gly and 100% and 75% with 3-Me-AcHis−Gly. Partial oligomerization of $[Pd(H_2O)_4]^{2+}$ into polynuclear, hydroxo-bridged complexes may be slow enough not to affect the faster cleavage, but it may compete somewhat with the slower one. Moreover, 3-Me-AcHis tends to form polynuclear complexes.[71]

AcHis and its methyl derivatives lack the "right" amide bond when they are treated with $[Pd(H_2O)_4]^{2+}$, acetic acid and histidine (or its methyl derivative) are formed. Indeed, this aqua complex promotes the cleavage of the "left" amide bond. In this respect, it differs from *cis*-$[Pd(en)(H_2O)_2]^{2+}$, which cleaves only the "right" amide bond in AcHis−aa dipeptides, in which aa designates glycine and several other amino acids.[40]

Table 1 shows several interesting results. First, both amide bonds are cleaved in AcHis−Gly and also in both methyl derivatives of it. *Evidently, attachment of the $Pd(H_2O)_3^{2+}$ group to either nitrogen atom in the imidazole ring results in cleavage of both amide bonds in the dipeptide.* Second, the adjacent numbers in the "left" column form three pairs. Within each pair the numbers are nearly the same. Clearly, the rate constant for the cleavage of the one amide bond is only slightly affected by the presence (and cleavage) of the other. Cleavage of the "right" bond is 1.7 to 4.5 times faster than cleavage of the "left" bond. This difference in the rate constants exceeds the margins of error, and we consider it real. But the difference is too small to be explained with certainty. The main purpose of this study is to explain in stereochemical terms our qualitative finding that attachment of the $Pd(H_2O)_3^{2+}$ group to either nitrogen atom in the imidazole ring results in cleavage of both amide bonds in the peptide.

**Mechanism of Peptide Hydrolysis Promoted by Transition-Metal Complexes.** Transition-metal complexes bind to a nucleophilic side chain—in this study, the imidazole ring—and effect cleavage of a proximate peptide bond by two limiting mechanisms;[7,8] see Chart 3. In one mechanism, the metal cation acts as a Lewis acid, interacts with the amide oxygen atom, further polarizes the carbonyl group, and makes the carbon atom more electrophilic and therefore susceptible to external attack by water from the solvent. This mechanism requires close approach of the amide oxygen atom and the metal cation in an orientation favorable for a bonding interaction. In the case of the square-planar palladium(II) complex, axial approach of the oxygen atom allows partial donation of the lone pair of electrons into the vacant $4d_{z^2}$ orbital.

(70) Appleton, T. G.; Bailey, A. J.; Bedgood, D. R., Jr.; Hall, J. R. *Inorg. Chem.* **1994**, *33*, 217.

(71) Remelli, M.; Munerato, C.; Pulidon, F. *J. Chem. Soc., Dalton Trans.* **1994**, 2050.

**Scheme 2**
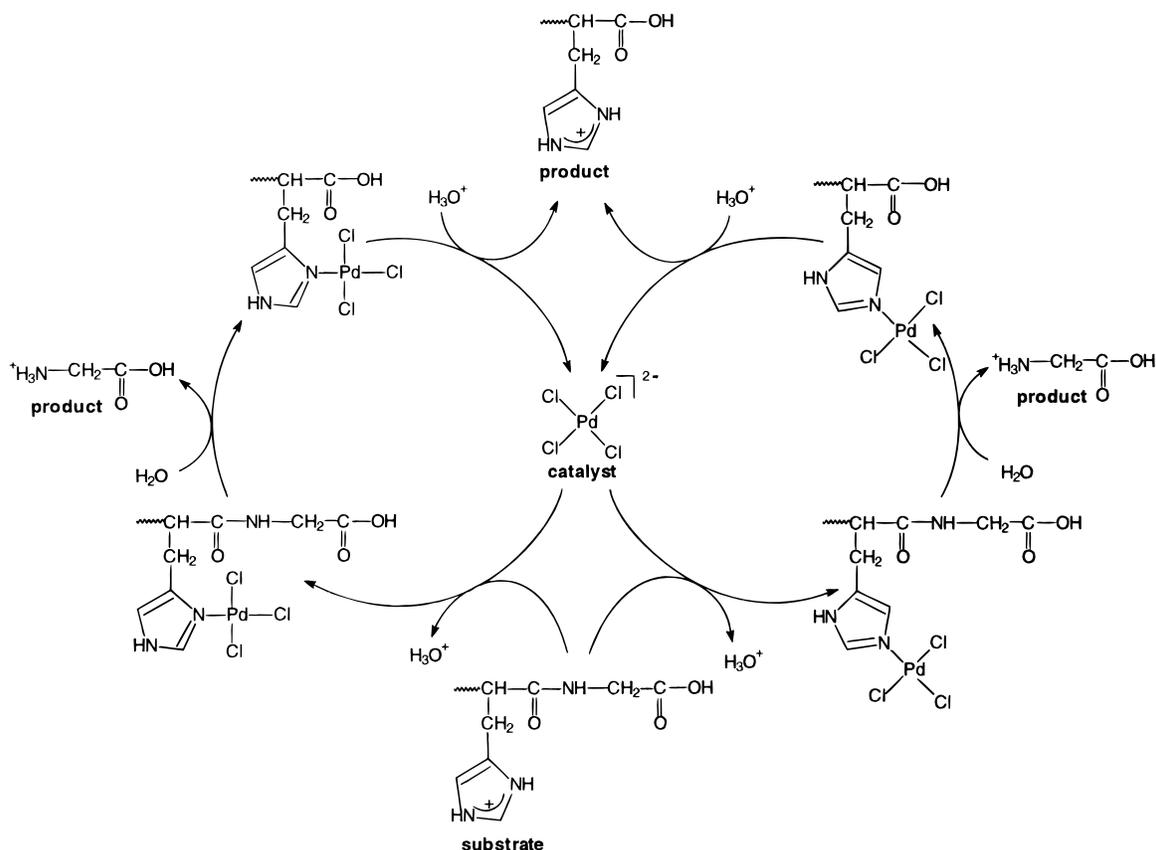


In the other mechanism, the metal aqua complex internally delivers one of its ligands to the scissile amide bond. If the aqua complex is sufficiently acidic to be deprotonated, cleavage is enhanced because hydroxo ligand is more nucleophilic than aqua ligand. Because the p$K_a$ value of the aqua ligand bound to palladium(II) is by several units greater than the pH of our reaction mixture, the concentration of the palladium(II) hydroxo species must be very low. In our case, the cleavage reaction may be assisted by the proximity of the aqua ligand to the amide carbon atom. This is the main requirement for the second mechanism.

Also possible is a combination of these two limiting mechanisms, in which the metal ion simultaneously polarizes the amide bond and delivers a nucleophilic ligand to that same bond.[8] None of the mechanisms in Chart 3 requires breaking of a bond between a hydrogen atom and another atom. Consequently, primary isotope effect H/D is not expected. The isotope effect of the solvent D$_2$O, if it exists, would change the kinetics so little that it would be undetectable in our experiments.

Since the aforementioned mechanisms are indistinguishable by kinetic methods, we applied molecular-dynamics calculations in several steps to analyze possible interactions between the Pd(H$_2$O)$_3{}^{2+}$ group bound to the imidazole ring and the adjacent amide bonds in AcHis−Gly. The composite molecule is relatively large but still amenable to thorough calculations without debilitating assumptions and constraints. Fortunately, this molecule was also amenable to kinetic investigations discussed above. Because the theoretical and the experimental parts of this study were done with the same system, the analyses of conformations in the next three subsections explain the kinetic results well.

**Molecular-Dynamics Calculations.** To our knowledge, high-temperature molecular dynamics (for conformation sampling)

and the solution of Poisson−Boltzmann equation (for treatment of the solvation effects) have not been combined in previous studies. We did the calculations with complexes designated **1** and **3** in Chart 2 containing water as the three unspecified ligands in the coordination sphere. The simulated peptide molecules, like the actual chemicals used in the experiments, contain hydrogen. Even though the actual ligands are D$_2$O, the difference in isotopes has no effect on our calculations and on their relevance to the experiments. Atomic mass affects vibrational frequencies, which we do not calculate. In classical mechanics and statistical thermodynamics, energy of the system does not depend on the mass (if gravity is negelected). Energies of the complexes are calculated by using the thermodynamic cycle in Scheme 1 and are shown in Table 2; a similar cycle was successfully used in our recent study of metalloprotein complexes.[61] Because the two linkage isomers are different compounds, their energies cannot be compared. Consequently, differences between the rate constants in the same column in Table 1 cannot be explained quantitatively. But the regioselectivity of peptide cleavage is explained below. The calculations provided stereochemical information that would be difficult or impossible to obtain by experimental methods.

We examined the trajectories obtained by the simulations and sought conformers in which the atoms of interest approach each other. We took the sum of van der Waals radii as the contact distance between two atoms:[72,73] 3.6 Å between oxygen of an aqua ligand and amide carbon (for the internal-delivery mechanism) and 3.2 Å between palladium(II) and amide oxygen (for the external-attack mechanism). We also considered the spatial orientation of the groups that potentially are involved in the

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(73) Mayo, S. L.; Olafson, B. D.; Goddard, W. A., III *J. Phys. Chem.* **1990**, *94*, 8897.

*Peptide Cleavage by Palladium(II)*

*J. Am. Chem. Soc., Vol. 121, No. 13, 1999* 3133

**Scheme 3**
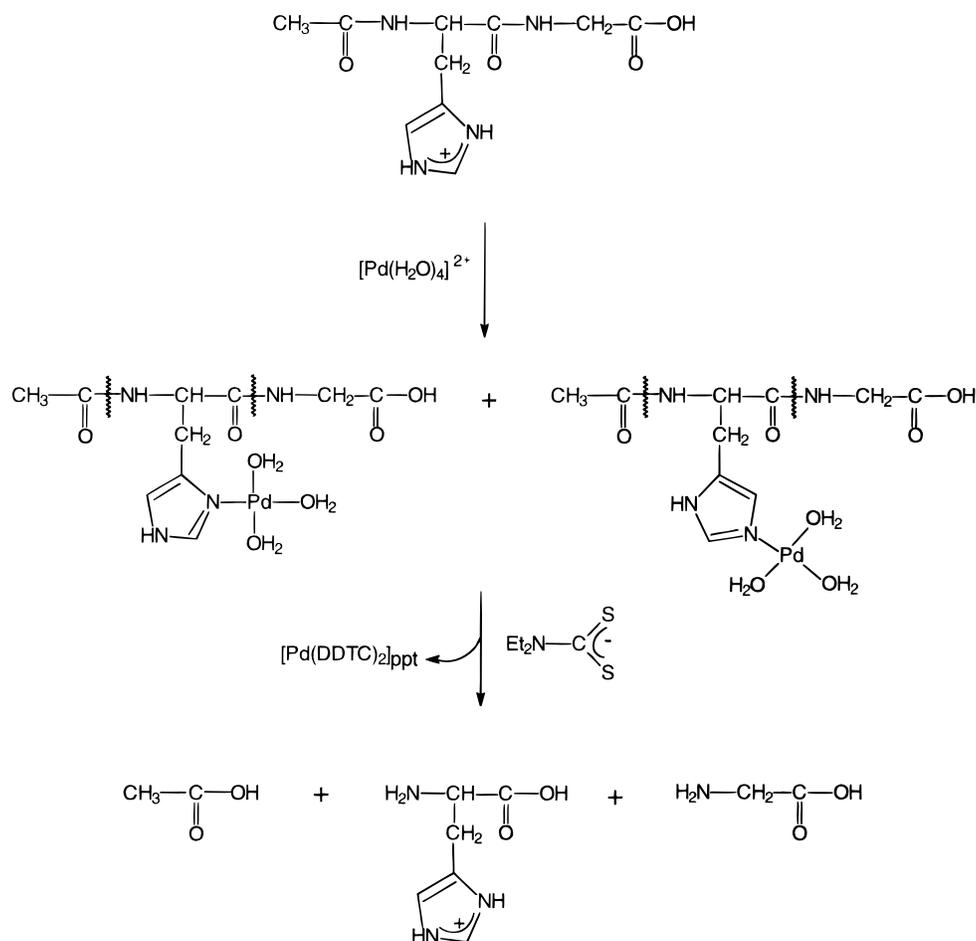


**Table 2.** Energies (in kcal·mol$^{-1}$) for Selected Conformers of the Dipeptide AcHis−Gly Bearing a Pd(H$_2$O)$_3{}^{2+}$ Group at the N-1 Atom (the upper four rows) or at the N-3 Atom (the lower four rows) of the Imidazole Ring

| conformer | total $\Delta G_T$ | solvation | | Coulombic $\Delta G_C$ |
|---|---|---|---|---|
| | | $\Delta\Delta G_R$ | $\Delta G_{NE}$ | |
| **A1** | −724.54 | −124.06 | 2.81 | −603.29 |
| **B1** | −722.72 | −123.32 | 2.86 | −602.26 |
| **C1** | −725.87 | −123.99 | 2.95 | −604.83 |
| **D1** | −723.55 | −117.87 | 2.83 | −608.52 |
| **A3** | −718.53 | −107.30 | 2.60 | −613.82 |
| **B3** | −722.58 | −113.23 | 2.68 | −612.03 |
| **C3** | −722.50 | −108.78 | 2.64 | −616.37 |
| **D3** | −728.12 | −115.43 | 2.65 | −616.33 |

cleavage. The dihedral angle between the planes of the amide bond and of the aqua ligand is relevant to internal delivery, whereas the angle formed by the amide carbonyl group and the palladium(II) ion is relevant to external attack. The structures having minimal energies in their respective simulations may not correspond to the respective global minima in solution, because the simulations were performed in a vacuum. But contributions of solvation were included in the calculation of the conformational energy via the thermodynamic cycle, as explained above. Each conformational family (a set of similar conformers) is designated with a capital letter. In each family we highlight the conformer having the shortest interatomic distances that are relevant to the mechanisms of cleavage shown in Chart 3. Eight such conformers, four for each of the two linkage isomers, are included in Tables 2 and 3. Six of them are shown in Figure 2.

**Conformations when the N-1 Atom Is Coordinated to the Pd(H$_2$O)$_3{}^{2+}$ Group.** The results of simulations for this linkage isomer, designated **1**, are given in the upper half of Tables 2 and 3; see also Figure 2. Judging by the Pd$^{II}$−O distances that are never shorter than 3.5 Å, this interaction, and consequently the external attack, is unlikely.

In conformer **A1** an aqua ligand sits at 3.3 Å from the carbon atom of the "left" amide bond, whereas in conformer **B1** an aqua ligand sits at 3.6 Å from the carbon atom of the "right" amide bond. In both cases the spatial orientation of the oxygen lone pair and the C−N antibonding orbital of the amide group is favorable for cleavage by internal delivery. Interestingly, in both of these conformers the oxygen atom of the scissile amide bond and the oxygen atom of the aqua ligand poised to cleave it sit at distances that suggests O−H−O hydrogen bonding: 2.7 Å in **A1** and 2.6 Å in **B1**. Although weak, these hydrogen bonds may further polarize the amide bonds and render them more electrophilic and therefore more reactive toward the internally delivered aqua ligand. Conformers **A1** and **B1** have similar total energies and therefore similar probabilities of existence. The similarity of the rate constants (in Table 1) for cleavage of the two amide bonds may be attributed to these probabilities.

**Conformations When the N-3 Atom Is Coordinated to the Pd(H$_2$O)$_3{}^{2+}$ Group.** The results of simulations for this linkage isomer, designated **3**, are given in the lower half of Tables 2 and 3; see also Figure 2. In an interesting family, represented by conformer **A3**, one aqua ligand is proximate to the "left" amide bond, another aqua ligand is proximate to the "right" amide bond, and the palladium(II) ion is proximate to oxygen atoms in both amide bonds! Moreover, the aqua ligands are

**Table 3.** Interatomic Distances (in Å) between the Amide Carbon Atom and the Oxygen Atom of the Aqua Ligand and between the Amide Oxygen Atom and the Palladium Atom; Dihedral Angles (in deg) between the Plane of the Amide Bond and the Plane of the Aqua Ligand; and Angles (in deg) Formed by the Amide Carbonyl Group and the Palladium(II) Ion for Selected Conformers of the Dipeptide AcHis−Gly Bearing a Pd(H$_2$O)$_3^{2+}$ Group at the N-1 Atom (the upper four rows) or at the N-3 Atom (the lower four rows) of the Imidazole Ring[a]

| conformer | distance | | | | dihedral angle | | angle | |
|---|---|---|---|---|---|---|---|---|
| | C$_{left}$−OH$_2$ | C$_{right}$−OH$_2$ | O$_{left}$−Pd | O$_{right}$−Pd | left | right | left | right |
| **A1** | 3.3 | 5.8 | 4.1 | 6.6 | 52 | −79 | 60 | 23 |
| **B1** | 4.4 | 3.6 | 4.3 | 4.2 | −129 | −128 | 10 | 35 |
| **C1** | 3.7 | 6.1 | 3.5 | 7.6 | 175 | −34 | 19 | 64 |
| **D1** | 4.3 | 3.8 | 4.8 | 3.8 | 142 | −100 | 26 | 10 |
| **A3** | 3.2 | 3.4 | 3.1 | 3.1 | 105 | −73 | 30 | 42 |
| **B3** | 4.0 | 3.2 | 4.2 | 3.0 | −173 | −88 | 53 | 26 |
| **C3** | 3.7 | 3.9 | 3.0 | 3.0 | 141 | 126 | 71 | 27 |
| **D3** | 4.4 | 3.6 | 2.9 | 3.0 | −135 | 122 | 76 | 30 |

[a] The "left" and "right" amide groups are shown in Chart 1.

**Chart 3**



external attack

internal delivery

combined mechanism



A1        B1

A3        B3

C3        D3
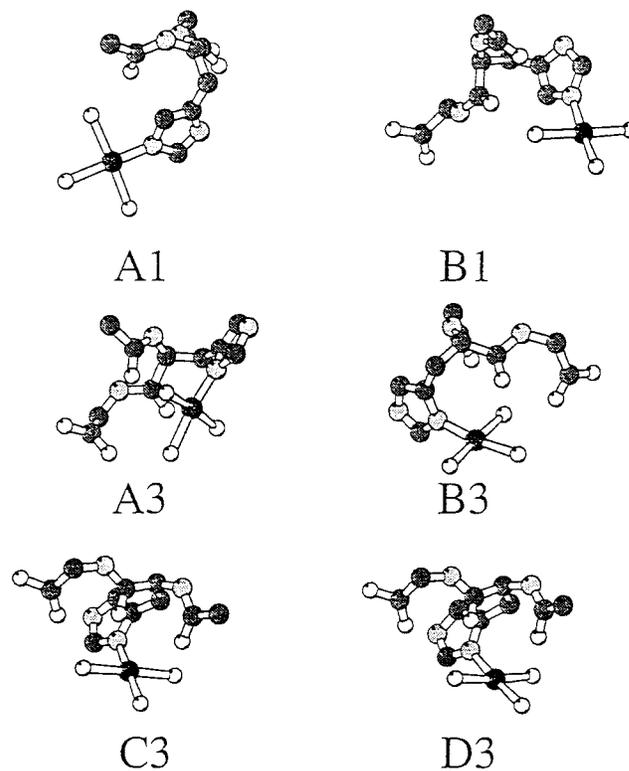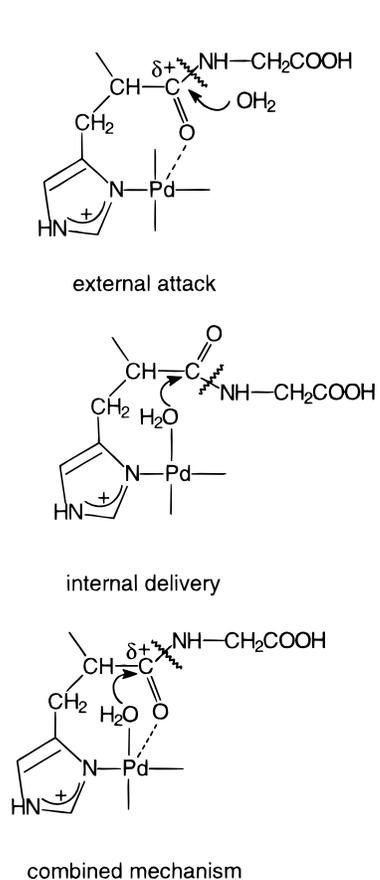
**Figure 2.** Selected conformers of the dipeptide AcHis−Gly bearing a Pd(H$_2$O)$_3^{2+}$ group at the N-1 atom (the upper two pictures) or at the N-3 atom (the lower four pictures) of the imidazole ring. These conformers were identified by thorough molecular-dynamics simulations, as described in the text.

nearly perpendicular to the respective amide planes, and the amide oxygen atoms sit above the palladium(II) coordination plane. Naturally, the oxygen atoms from both amide groups cannot simultaneously occupy the axial or nearly axial positions. This family is well suited to cleavage of both amide bonds by the mechanism that combines internal delivery of aqua ligands and external attack by water from the solvent.

In the conformational family represented by **B3**, an aqua ligand approaches the carbon atom of the "right" amide bond at the dihedral angle favorable for internal delivery, and the palladium(II) ion approaches the oxygen atom of the same bond. Conditions exist for cleavage of the "right" amide bond by the combined mechanism.

In the conformational family represented by **C3**, the "left" amide bond may be cleaved by the combined mechanism; because the amide oxygen atom sits in an axial position with respect to the palladium(II) ion, external attack may be an important component in this mechanism. In this family, the "right" amide bond may be cleaved by the external attack.

In the conformational family represented by **D3**, conditions exist for the cleavage of the "right" amide group by a combined mechanism and of the "left" amide group by external attack; again, the oxygen atom of the "left" amide bond sits in an axial position above the palladium(II) ion.

Interestingly, in all of the conformers discussed in this subsection, the oxygen atoms of an aqua ligand and of the proximate amide group are separated by a distance consistent with (weak) hydrogen bonding. These interactions may activate the amide group by both electronic and steric effects—by polarizing this group and by holding it in the orientation favorable for nucleophilic cleavage.

Clearly, the conformational space of the linkage isomer **3** is rich in conformers suitable for hydrolytic cleavage of both amide groups in the dipeptide. This finding explains the similarity of corresponding rate constants from the last two columns in Table 1.

**Importance of the Aqua Ligands on Palladium(II) for Hydrolysis of the Amide Bond.** We can only surmise why the $[PdCl_4]^{2-}$ complex does not cleave the "left" amide bond. Under reasonable assumption that both amide bonds in the same peptide are cleaved by the same mechanism, the difference between $[Pd(H_2O)_4]^{2+}$, which cleaves both bonds, and $[PdCl_4]^{2-}$, which cleaves only the "right" one, may be due to steric factors. The former complex is smaller than the latter and presumably may approach the scissile bonds more closely. This explanation is consistent with our previous findings that chelate aqua complexes, which are relatively bulky, cleave only the "right" amide bond, i.e., the one downstream from the anchoring methionine or histidine residue in short peptides.[36,37,39,40,43]

We can, however, explain the different rates of cleavage by the two palladium(II) complexes. As Table 1 shows, the dipeptide is cleaved about 10 times more rapidly by $[Pd(H_2O)_4]^{2+}$ than by $[PdCl_4]^{2-}$. We examine this finding at two levels. In a simple analysis, we consider displacement of only one of the four identical ligands by the imidazole ring (the peptide). The $Pd(H_2O)_3^{2+}$ group is more effective than the $PdCl_3^-$ group in both limiting mechanisms for hydrolysis—external attack and internal delivery. Palladium(II) ion in the cationic complex is a stronger Lewis acid, and therefore better able to activate the amide group for external attack, than that ion in the anionic complex. The aqua complex, obviously, is better suited than the chloro complex for internal delivery of water. Because the $Pd(H_2O)_3^{2+}$ group attached to the imidazole ring in the dipeptide contains several aqua ligands, conformations that brings one of them near the scissile amide bond are likely to exist. Indeed, our molecular-dynamics simulations confirm this intuitive expectation.

In a more detailed analysis, we take into consideration the aquation of the $PdCl_3^-$ group attached to the dipeptide. In eq 7

$$[PdCl_4]^{2-} \overset{K_1}{\rightleftharpoons} [PdCl_3(H_2O)]^- \overset{K_2}{\rightleftharpoons} [PdCl_2(H_2O)_2] \overset{K_3}{\rightleftharpoons}$$
$$[PdCl(H_2O)_3]^+ \overset{K_4}{\rightleftharpoons} [Pd(H_2O)_4]^{2+} \quad (7)$$
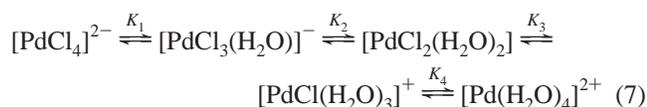
the entering aqua ligands and leaving chloro ligands are implied; showing them explicitly would have needlessly complicated the equation. The equilibrium constants $K_1$, $K_2$, $K_3$, and $K_4$ are 4.2 $\times\ 10^{-2}$, 3.8 $\times\ 10^{-3}$, 5.2 $\times\ 10^{-4}$, and 3.3 $\times\ 10^{-5}$ at 25 °C.[74,75] As expected, every substitution step is less favorable than the previous one. In the cleavage experiments with $[PdCl_4]^{2-}$ the

(74) Elding, L. I. *Inorg. Chim. Acta* **1972**, *6*, 647.
(75) Elding, L. I. *Inorg. Chim. Acta* **1972**, *6*, 683.

initial concentration of this complex was 75 mM. Although it attaches to the imidazole ring in the peptide and aquates simultaneously, we separated these two reactions and applied equilibrium constants in eq 7 to estimate the composition of the mixture. The solution of $[PdCl_4]^{2-}$ at equilibrium would be approximately 37 mM in $[PdCl_4]^{2-}$, 28 mM in $[PdCl_3(H_2O)]^-$, 8.0 mM in $[PdCl_2(H_2O)_2]$, 1.8 mM in $[PdCl(H_2O)_3]^+$, and 0.20 mM in $[Pd(H_2O)_4]^{2+}$. The imidazole ring of the peptide is much more likely to displace an aqua than a chloro ligand. Therefore the total concentration of the complexes bearing both the imidazole ligand and at least one aqua ligand is approximately 10 mM, a sum of $8.0 + 1.8 + 0.20$ mM. If we consider the requirement that the aqua ligand for internal delivery to the amide bond needs to be in a cis position to the imidazole ring, the concentration of the complexes suitable for cleavage becomes even smaller than 10 mM. Clearly, $[Pd(H_2O)_4]^{2+}$ is more reactive than $[PdCl_4]^{2-}$ in promoting hydrolytic cleavage. The semiquantitative argument in this subsection explains the approximately 10-fold difference between the rate constants for these two complexes in Table 1.

## Conclusions

This study shows that kinetics and regioselectivity of cleavage of histidine-containing peptides can be controlled by the choice of ligands in palladium(II) complexes. Whereas selectivity of enzymes depends on the structural fit between the active site and the substrate, selectivity of the new artificial dipeptidases depends on the proximity of the anchored metal complex to the scissile amide bonds. This study shows, for the first time, that stereochemical requirements for efficient cleavage by palladium(II) complexes can be explained by thorough molecular dynamics calculations. Although the new reagents so far achieved only modest turnover of substrate, their proven catalytic ability justifies their designation as artificial peptidases.

**Supporting Information Available:** Table S1, energy parameters not included in the CHARMM force field ($K_i$ and $\zeta_o$), and Table S2, charges used in the molecular dynamics simulation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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