Effects of Linkage Isomerism and of Acid−Base Equilibria on Reactivity and Catalytic Turnover in Hydrolytic Cleavage of Histidyl Peptides Coordinated to Palladium(II). Identification of the Active Complex between Palladium(II) and the Histidyl Residue

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Abstract: This is a quantitative study of hydrolysis of the His−Gly bond in the peptide AcHis−Gly catalyzed by *cis*-$[Pd(en)(H_2O)_2]^{2+}$. We exploit the diverse coordinating abilities and acid−base properties of histidyl residue to interpret the kinetics and explain the mechanism of this new reaction. We compare peptides selectively methylated at the N-1 or N-3 atom of imidazole and study effects of solution acidity on the abundance of different peptide−catalyst complexes and on the rate constant for hydrolysis. Only the catalyst bound to the N-3 atom of imidazole can effect this reaction; none of the four other modes of coordination is effective. The necessary approach of the palladium(II) aqua complex to the scissile peptide bond and the rate constant of hydrolysis are unaffected by the remote methyl group that merely controls the mode of peptide coordination to the catalyst. Acid in solution affects hydrolysis only by controlling the concentration of the reactive complex, not by catalyzing the reaction itself. Weakly acidic solution is required to suppress oligomerization of the catalyst. Hydrolytic cleavage occurs with a turnover greater than 4. With the halflife of 5.1 h at pH 5.0, the cleavage is fast enough at relatively mild conditions to be practical for various applications in biochemistry and structural biology. This study is an important step in our development of palladium(II) complexes as artificial metallopeptidases.

Introduction

Transition-metal complexes hold promise as reagents for hydrolytic cleavage of peptides and proteins[1−20] and may have some advantages over proteolytic enzymes in biochemical applications. Complexes of platinum(II)[21] and, especially, those of palladium(II)[22−28] spontaneously bind to heteroatoms in side chains and catalyze regioselective hydrolysis of the amide bond involving the adjacent carboxylic group. Kinetic studies with various oligopeptides revealed some aspects of the hydrolytic mechanisms,[21−27] and regioselective cleavage of cytochrome *c*[28] showed the applicability of the new palladium(II) reagents in biochemistry. These studies point the way toward new artificial metallopeptidases.

In most of the previous studies from this laboratory the transition-metal complexes were bound (anchored) to the methionine residue. Our latest study showed that histidine residue, too, can serve as an anchor.[26] Because of the importance of histidine in structural and mechanistic biochemistry and in structural biology this discovery opened several lines of research. We already showed that hydrogen bonds involving imidazole and certain hydroxyl-containing amino acids in peptides can affect kinetics of cleavage in interesting ways and result in partial selectivity of cleavage to amino-acid sequence. These findings, reminiscent of enzyme−substrate interactions, spurred our research.

In this study we exploit unique features of histidine in order to clarify the mechanism of the new reactions and to determine stereochemical requirements for peptide cleavage mediated by palladium(II). The imidazole group in the side chain can exist in various states of (de)protonation, and it can coordinate to transition metals in several interesting ways, alone or together with the donor groups in the main chain. We investigate kinetic

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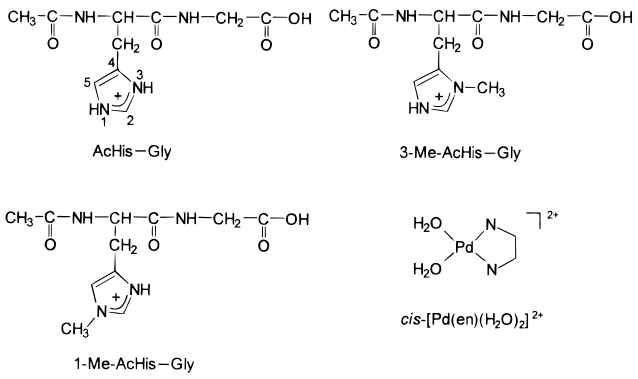
Chart 1



aspects of acid−base equilibria and stereochemical aspects of linkage isomerism. The results show what roles the weakly acidic medium does and does not play in cleavage reactions catalyzed by palladium(II) complexes, which of the several interconverting complexes is the active one, and why these reactions occur with turnover.

Experimental Procedures

Chemicals. Distilled water was demineralized and purified to a resistance greater than 10 MΩ·cm. The compounds D_2O, $DClO_4$, NaOD, and $K_2[PdCl_4]$ were obtained from Aldrich Chemical Co. Anhydrous $AgClO_4$ was obtained from G. Frederic Smith Chemical Co. Amino acids 1-methylhistidine (1-Me-His) and 3-methylhistidine (3-Me-His) and the dipeptide His−Gly were obtained from Sigma Chemical Co. All other chemicals were of reagent grade.

Dipeptides 1-Me-AcHis−Gly and 3-Me-AcHis−Gly were synthesized by a standard solid-state method, and their purity was checked by HPLC; this was done by the staff of the Protein Facility. The terminal amino group in amino acids and dipeptides was acetylated by a standard procedure.[29] The complex *cis*-$[Pd(en)(H_2O)_2]^{2+}$ was prepared by treating the corresponding dichloro complex with 2 equiv of $AgClO_4$ and removing AgCl by centrifugation, all in the dark.[30]

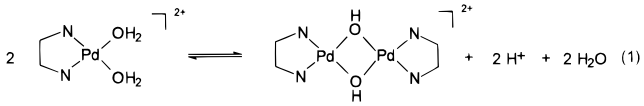
Measurements. Proton NMR spectra of D_2O solutions containing DSS as internal reference were recorded at 300 and 500 MHz, with Nicolet NT 300, Varian VXR 300, and Varian Unity 500 spectrometers. Temperature was kept at 60 ± 0.5 °C. The pH values were measured with a Fisher 925 instrument and a Phoenix Ag/AgCl reference electrode and converted into hydrogen-ion concentrations. Neither the pH nor the $[H^+]$ values were corrected for the deuterium effect. Ultraviolet−visible spectra were recorded with a IBM 9420 spectrophotometer.

Study of Hydrolysis. The following D_2O solutions were mixed in an NMR tube: 150 μL of 100 mM freshly-prepared *cis*-$[Pd(en)(H_2O)_2]^{2+}$, 150 μL of 100 mM peptide, and 50 μL of 100 mM DSS. After addition of 150 μL of D_2O the pH was adjusted with a 2.0 M solution of $DClO_4$. The complete solution was 26−30 mM in the peptide, and pH was in the range of 0.80−5.0. The pH value never changed by more than 0.10 between the beginning and the end of the experiment. Acquisition of ^1H NMR spectra, at 60 ± 0.5 °C, began as soon as possible, and 16 scans were taken each time. The error in integrating the resonances was estimated at ± 5%. A typical first-order plot of $\ln[C_0/(C_0 - C_t)]$ versus time, based on concentrations of glycine, consisted of 20 points spanning at least three half-lives. At the end of hydrolysis 2 equiv of solid sodium diethyldithiocarbamate (Naddtc) were added for each equivalent of *cis*-$[Pd(en)(H_2O)_2]^{2+}$, and the precipitate of $[Pd(ddtc)_2]$ was removed by centrifugation.[26]

Stability of Peptides without Palladium. A solution of AcHis−Gly at pH 1.0 was prepared as described above except that the complex *cis*-$[Pd(en)(H_2O)_2]^{2+}$ was missing. The solution was kept at 60 ± 0.5 °C, and its ^1H NMR spectra were recorded occasionally.

Results and Discussion

The Catalyst. The palladium(II) complex shown in Chart 1 had the UV absorption maximum at 340−345 nm, the correct value.[31] It was always prepared fresh, and the reaction mixtures were kept acidic in order to suppress or minimize the dimerization shown in eq 1, which occurs at pH >4.0.[32]



Stability of the Substrates. In the absence of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ the peptide AcHis−Gly did not detectably hydrolyze in the time that, in the presence of this catalyst, was sufficient for complete hydrolysis. Monitoring over a long period of time yielded an estimate of 5×10^{-6} min^{-1} for the rate constant of the uncatalyzed reaction at pH 1.0. Clearly, the fast hydrolytic cleavage of the peptide bond is caused by the palladium(II) complex, not by the acidic solvent. Further evidence for this conclusion will be given below.

Binding of the Catalyst to the Substrates. As we recently reported,[26] a reaction between equimolar amounts of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and AcHis−Gly spontaneously yields five complexes, designated A through E in Chart 2. In this study we clarify linkage isomerism in these complexes by methylating N-1 and N-3 atoms of the imidazole ring. The atom numbering and the methylated dipeptides are shown in Chart 1. The new complexes in Chart 2 are designated with letters and subscripts Me. The ^1H NMR chemical shifts of the methylated substrates and complexes, given in Table 1, differ only slightly from the shifts of the corresponding unmethylated species.

Mixing of equimolar amounts of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and 1-Me-AcHis−Gly in solutions having $0.80 \leq pH \leq 5.0$ results in spontaneous, fast formation of the complexes $\mathbf{A}_{Me}$ and $\mathbf{D}_{Me}$. The unidentate coordination is favored at lower pH values, and bidentate at higher, at which the amide nitrogen atom is deprotonated. Mixing of equimolar amounts of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and 3-Me-AcHis−Gly in solutions having pH 1.2 or 3.0 yields complexes $\mathbf{B}_{Me}$ and $\mathbf{E}_{Me}$ as the major and the minor product, respectively. These modes of coordination are known.[33−37]

Hydrolysis of the His−Gly Bond. When this reaction occurs, free glycine is easily detected in ^1H NMR spectra; see Figure 1. Upon addition of glycine to the reaction mixture its resonance is enhanced. Some of the liberated glycine reacts with the catalyst to form a small amount of the bis(bidentate) complex *cis*-$[Pd(en)(Gly\text{-}N,O)]^+$, easily detected by ^1H NMR spectroscopy; see Table 1. Indeed, this same complex is formed upon mixing of equimolar amounts of *cis*-$[Pd(en)(H_2O)_2]^{2+}$ and glycine. At the end of hydrolysis reaction palladium is completely removed as the insoluble complex $[Pd(ddtc)_2]$, and the ^1H NMR spectra show only the two fragments of the cleaved dipeptide. When hydrolysis occurs, it is a complete and "clean" reaction.

Coordination Modes and Reactivity. The mixture of complexes $\mathbf{B}_{Me}$ and $\mathbf{E}_{Me}$ did not show peptide hydrolysis except for the slight "background" cleavage upon prolonged heating. Clearly, neither of these complexes is reactive in hydrolysis. Palladium(II) atom bound to the N-1 atom is too distant from the His−Gly bond to cleave it by either of two mechanisms:

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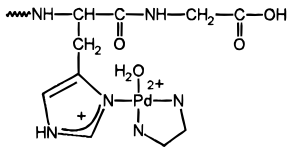
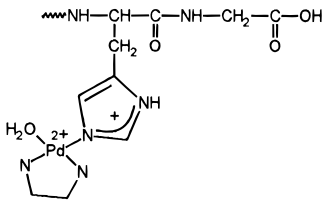
Chart 2

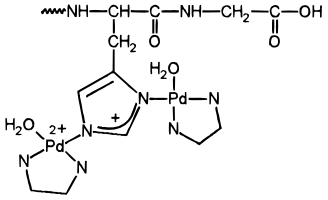
Complexes with AcHis−Gly



A



B



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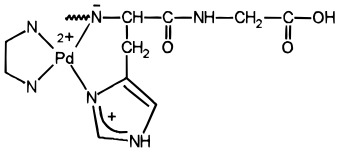

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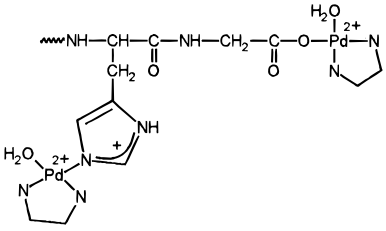
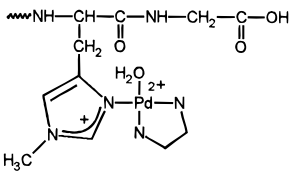
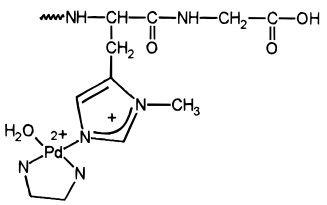


E

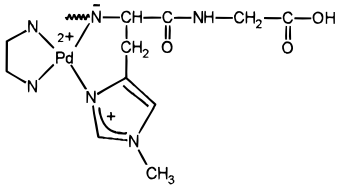
Complexes with 1-Me-AcHis−Gly and 3-Me-AcHis−Gly



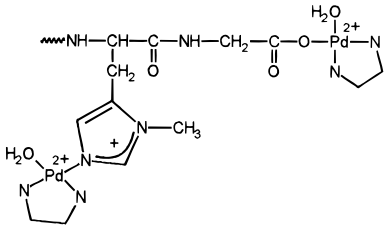
A_{Me}



B_{Me}



D_{Me}



E_{Me}

Table 1. Proton NMR Chemical Shifts of Compounds in D_2O Solutions, at $0.8 \leq pH \leq 5.0$

	chemical shifts, ppm		
compd or symbol[a]	H-2	H-5	α-CH_2
1-Me-AcHis−Gly	8.54	7.28	3.99
3-Me-AcHis−Gly	8.60	7.30	3.99
$\mathbf{A}_{Me}$	7.91	7.06	
$\mathbf{B}_{Me}$	7.81	6.87	
$\mathbf{D}_{Me}$	7.97	7.00	
$\mathbf{E}_{Me}$	7.68	6.77	
Gly			3.60−3.89[b]
cis-[Pd(en)(Gly-*N*,*O*)]$^+$			3.55

[a] See Chart 2. [b] Dependent on pH.

polarization of the carbonyl group followed by external attack of solvent water or internal delivery of an aqua ligand to the scissile bond.[5,6,22]

The mixture of complexes $\mathbf{A}_{Me}$ and $\mathbf{D}_{Me}$ at relatively low pH values undergoes fast and complete hydrolysis of the peptide bond; see Figure 1. To determine whether hydrolysis occurs in one or both complexes, we treated *cis*-$[Pd(en)(H_2O)_2]^{2+}$ with an equimolar amount of His−Gly, the peptide in which the terminal amino group was not protected by acetylation. Upon mixing, at pH 2.6, the chelate complex shown below formed with the yield of 95%. This *N*,*N*-bidentate coordination of *N*-terminal histidyl residue is known.[38] The remaining 5% was its unidentate precursor, the complex of type **A** (or $\mathbf{A}_{Me}$). In two days, the time in which hydrolysis of the protected peptide AcHis−Gly is complete, only 10% of the unprotected peptide His−Gly was cleaved. This slight reactivity can be attributed to the minor, unidentate complex in the mixture. The bidentate

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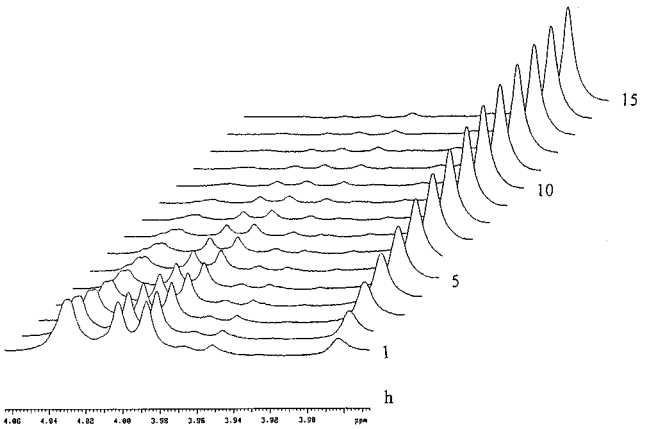
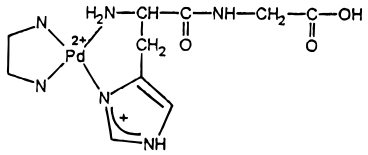


Figure 1. Proton NMR spectra at various times (in hours) of a solution in D_2O that was initially 29 mM in both 1-Me-AcHis−Gly and *cis*-$[Pd(en)(H_2O)_2]^{2+}$, at pH 1.2 and 60 ± 0.5 °C. Glycine CH_2 resonances of the peptide that is free and bound to the catalyst occur at 3.98−4.05 ppm, and this resonance of free glycine occurs at 3.89 ppm. As the peptide disappears, free glycine appears.

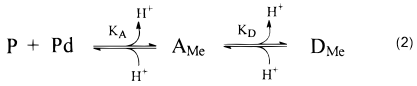


complex shown above, and therefore also its close analog $\mathbf{D}_{Me}$, is unreactive. The palladium(II) atom in it lacks aqua ligands and is held on the opposite side of the peptide bond. Neither the external attack nor the internal delivery of a water molecule is possible in this configuration.

The experiments with methylated peptides show that only the palladium(II) complex bound to the N-3 atom of imidazole can effect hydrolytic cleavage. Indeed, only this coordination mode permits the close approach of the metal atom and of its aqua ligand to the scissile peptide bond.

Effects of Acidity on the Concentrations of 1-Me-AcHis−Gly, Complex $\mathbf{A}_{Me}$, and Complex $\mathbf{D}_{Me}$. Formation of complex $\mathbf{D}_{Me}$ involves deprotonation of the N-3 atom in the imidazolium cation and subsequent coordination of the amide nitrogen atom originating from histidine. Naturally, both of these processes, shown in eq 2, depend on the concentration of hydrogen (actually, deuterium) ions in solution. The symbols **P** and **Pd** represent the free (uncoordinated) peptide and the complex *cis*-$[Pd(en)(H_2O)_2]^{2+}$. The data points in Figure 2 correspond to the following seven values of the hydrogen-ion concentration: 1.0×10^{-5}, 9.0×10^{-5}, 3.4×10^{-4}, 8.5×10^{-4}, 1.3×10^{-3}, 6.3×10^{-2}, and 1.6×10^{-1} M. Because of the linear scale on the horizontal axis, the first two or three points overlap.

Integration of characteristic ^1H NMR resonances of the species shown in eq 2 allowed determination of the formation constants: $K_A = 3.4$, and $K_D = 5 \times 10^{-3}$ M. The latter value



(2)

corresponds to $pK_D = 2.3$ and agrees with a reported value of *ca* 2.0.[35] Indeed, palladium(II) anchored to a side chain is highly effective in inducing deprotonation of the amide nitrogen atom.[35,39,40]

Because the peptide and the catalyst are equimolar and form 1:1 complexes $\mathbf{A}_{Me}$ and $\mathbf{D}_{Me}$, concentrations [**P**] and [**Pd**] are

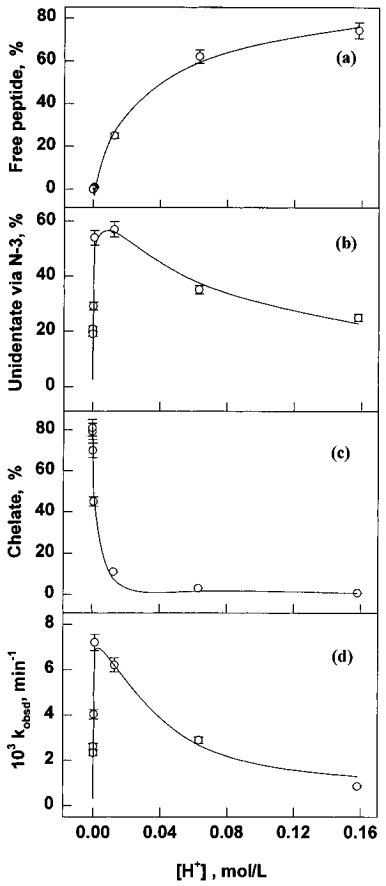


Figure 2. Effects of acid on a solution in D_2O that was initially 29 mM in both 1-Me-AcHis−Gly and *cis*-$[Pd(en)(H_2O)_2]^{2+}$, at 60 ± 0.5 °C. Values of $[H^+]$, uncorrected for the deuterium effect, are 1.0×10^{-5}, 9.0×10^{-5}, 3.4×10^{-4}, 8.5×10^{-4}, 1.3×10^{-3}, 6.3×10^{-2}, and 1.6×10^{-1} M. Data points corresponding to the first two or three of these values overlap. When error bars are not seen, they are smaller than the circles. (a) Fraction of the peptide bound to palladium(II). (b) Fraction of the complex designated $\mathbf{A}_{Me}$, in which the peptide coordinates to palladium(II) as a unidentate ligand, via the N-3 atom of imidazole. (c) Fraction of the complex designated $\mathbf{D}_{Me}$, in which the peptide coordinates to palladium(II) as a bidentate ligand, via the N-3 atom of imidazole and the deprotonated nitrogen atom of the amide bond on the amino side of the histidyl residue. (d) The observed rate constant for hydrolytic cleavage of the His−Gly peptide bond.

equal. They are related to the total peptide concentration, $[\mathbf{P}]_{tot}$, according to eq 3. In these experiments $[\mathbf{P}]_{tot}$ was 29 mM. In

$$[\mathbf{P}] = \frac{[\mathbf{P}]_{tot}}{1 + \frac{K_A[\mathbf{Pd}]}{[H^+]} + \frac{K_A K_D[\mathbf{Pd}]}{[H^+]^2}} \qquad (3)$$

order to make the results more general, only the concentration of hydrogen ion is given in absolute units, as molarity. Concentrations of the free peptide, of the free catalyst, and of the complexes $\mathbf{A}_{Me}$ and $\mathbf{D}_{Me}$ are given relative to $[\mathbf{P}]_{tot}$, hence the percentages on the vertical axes in Figures 2a, 2b, and 2c.[41] Because the peptide 1-Me-AcHis−Gly and the product of its cleavage 1-Me-AcHis form similar complexes with the catalyst and are involved in analogous equilibria, the concentration of the free catalyst, [**Pd**], is constant during the reaction. This constancy makes fitting to eq 3 possible.

As Figure 2a shows, the experimental data are fitted well. When $[H^+]$ is small, so that protons do not effectively compete

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(40) Kasselauri, S.; Garoufis, A.; Hadjiliadis, M.; Hadjiliadis, N. *Coord. Chem. Rev.* **1990**, *104*, 1.

(41) It can easily be shown that when concentrations of the compounds are expressed in percentage of $[P]_{tot}$ and not in molarity the value of K_A has to be multiplied by 2.9×10^{-4}, whereas K_D needs no correction.

Table 2. Effects of Hydrogen Ion Concentration on Hydrolysis of the His−Gly Bond in 1-Me-AcHis−Gly, Promoted by *cis*-$[Pd(en)(H_2O)_2]^{2+}$

$10^5[H^+]$, mol/L	10^3k_{obsd}, min^{-1}	$10^5[H^+]$, mol/L	10^3k_{obsd}, min^{-1}
15800	0.9	34	4.3
6300	2.9	9	2.6
126	6.2	1	2.3
85	7.2		

with palladium(II) for the peptide, the third term in the denominator in eq 3 becomes very large, and [**P**] practically vanishes. Indeed, the first three points in Figure 2a almost overlap because peptide coordination is essentially complete. When $[H^+]$ becomes relatively large, so that these ions displace palladium(II) from the peptide, the third term in the denominator becomes negligibly small, and eq 3 reduces to eq 4. Indeed, the curve in Figure 2a is a hyperbola, showing partial coordination in weakly-acidic solutions.

$$[\mathbf{P}] = \frac{[\mathbf{P}]_{tot}[H^+]}{K_A[\mathbf{Pd}] + [H^+]} \tag{4}$$

Data in Figure 2b were successfully fitted to eq 5. Decrease in acidity favors imidazole coordination, and the unidentate complex $\mathbf{A}_{Me}$ reaches maximum concentration at $[H^+] = 1.3 \times 10^{-2}$ M. Further decrease in acidity suddenly allows deprotonation of the amide group and conversion of complex $\mathbf{A}_{Me}$ into the chelate complex $\mathbf{D}_{Me}$. Figures 2b and 2c show sharp, concomitant disappearance of the former and appearance of the latter. At very low acidity the third term in the denominator becomes much larger than the other two, and eq 5 reduces to eq 6. In this region dependence of $[\mathbf{A}_{Me}]$ on $[H^+]$ is practically vertical; indeed, the calculated slope is 2×10^4.

$$[\mathbf{A}_{Me}] = \frac{[\mathbf{P}]_{tot}}{1 + \frac{[H^+]}{K_A[\mathbf{Pd}]} + \frac{K_D}{[H^+]}} \tag{5}$$

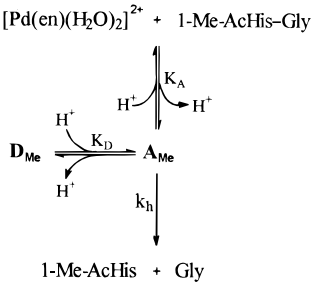
$$[\mathbf{A}_{Me}] = \frac{[\mathbf{P}]_{tot}}{K_D}[H^+] \tag{6}$$

Data in Figure 2c were nicely fitted to eq 7. Complex $\mathbf{D}_{Me}$ begins to form at $[H^+] \approx 0.030$ M and quickly becomes dominant as the acidity decreases further and amide deprotonation becomes possible. The curve in Figure 2c can be viewed as a sum of two straight lines intersecting at 5×10^{-3} M, exactly the value of K_D. If the horizontal scale were logarithmic (in pH units) rather than linear (in concentrations), the plot would be a familiar titration curve with the pK_D of 2.3, characteristic of palladium(II)-induced deprotonation of the amide nitrogen atom.

$$[\mathbf{D}_{Me}] = \frac{[\mathbf{P}]_{tot}}{1 + \frac{[H^+]}{K_D} + \frac{[H^+]}{K_A[\mathbf{Pd}]}} \tag{7}$$

Effect of Acidity on Hydrolysis Rate. Peptide coordination in palladium(II) complexes, discussed above, is significant in its own right, but its real importance lies in its relevance to the new hydrolysis reaction. Because the pK_a value of the aqua ligand in *cis*-$[Pd(en)(H_2O)_2]^{2+}$ is 5.6,[31] this ligand is not deprotonated to a significant extent in the pH interval used. As Table 2 and Figure 2d show, the rate constant depends on acidity in an interesting way. Similarity between Figures 2b and 2d corroborates our conclusion that the hydrolytically-active complex is the unidentate one designated $\mathbf{A}_{Me}$. Dissimilarily between Figures 2c and 2d likewise corroborates our conclusion that the bidentate complex designated $\mathbf{D}_{Me}$ is unreactive. Since the rate constant is directly proportional to the concentration of the complex designated $\mathbf{A}_{Me}$, acidity of the solution should similarly affect the concentration of $\mathbf{A}_{Me}$ and the rate constant. Indeed, an increase in $[H^+]$ from 1.0×10^{-5} to 8.5×10^{-4} M causes a sharp increase in both of these quantities, and a further increase in $[H^+]$ to 1.6×10^{-1} M causes a gradual decrease in both. The kinetic data in Figure 2d are nicely fitted to eq 8, derived from Scheme 1.

Scheme 1. Kinetic Mechanism for Hydrolysis of 1-Me-AcHis−Gly



$$k_{obsd} = \frac{k_h}{1 + \frac{[H^+]}{K_A[\mathbf{Pd}]} + \frac{K_D}{[H^+]}} \tag{8}$$

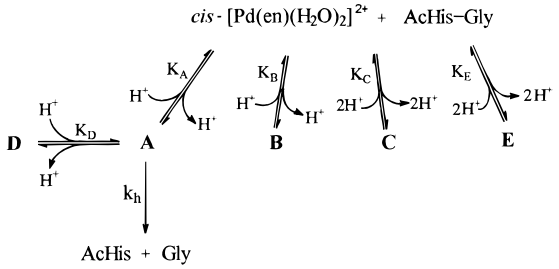
Parallelism between the plots in Figures 2b and 2d proves that acid concentration influences the hydrolysis rate only by controlling the concentration of the hydrolytically-active complex, not by catalyzing the reaction itself. Indeed, the rate constant decreases as the acid concentration increases over almost the entire range of hydrogen-ion concentrations; the only exception is the sharp rise in the rate constant at the beginning of this range, as explained above. The cleavage of the peptide bond is catalyzed by the palladium(II) complex anchored to the side chain, not by the hydrogen ion. Acidic solution is needed only to suppress ologomerization of the catalytic palladium(II) aqua complex, shown in eq 1.

Because the thio ether group is not a Brønsted acid or base under the usual conditions, our thorough studies of hydrolytic cleavage of methionyl peptides[21−25,27] offered only partial evidence concerning the need for acidic solution in hydrolysis. Control experiments in the absence of metal complexes repeatedly showed slight or negligible cleavage. In this study, because imidazole and amide groups undergo (de)protonation under conditions compatible with peptides and metal complexes, we are finally able to prove that the crucial cleavage step is not catalyzed by hydrogen ions.

Kinetic Mechanism for Hydrolysis of 1-Me-AcHis−Gly. The effects of acidity on concentrations of complexes and on the rate of hydrolysis support the mechanism in Scheme 1, according to which $\mathbf{A}_{Me}$ is reactive and $\mathbf{D}_{Me}$ is unreactive. The rate constant for hydrolysis, k_h, can be calculated from eq 9 because $[\mathbf{P}]_{tot}$ is known and all the other quantities in eq 9 are experimentally determined. The value $k_h = 5.8 \times 10^{-3}$ min^{-1} at $[H^+] = 6.3 \times 10^{-2}$ M corresponds to the half-life of 2.0 h.

$$\frac{d[Gly]}{dt} = \frac{k_h[\mathbf{P}]_{tot}}{1 + \frac{[H^+]}{K_A[\mathbf{Pd}]} + \frac{K_D}{[H^+]}} \tag{9}$$

Scheme 2. Kinetic Mechanism for Hydrolysis of AcHis−Gly



Kinetic Mechanism for Hydrolysis of AcHis−Gly. In a previous study[26] we showed that AcHis−Gly is completely hydrolyzed into *N*-acetylhistidine and glycine in the presence of *cis*-$[Pd(en)(H_2O)_2]^{2+}$, but we did not examine the mechanism of this new reaction. Now we do so. Scheme 2 is more complex than Scheme 1 because the unmethylated peptide forms all five complexes designated **A** through **D**, which exist in an extended equilibrium with the peptide, the catalyst, and one another. Effects and noneffets of imidazole methylation show that complex **A** is active in hydrolysis and that complexes **B**, **D**, and **E** are inactive. Since methylation prevents formation of complex **C**, we have to rely on other evidence concerning it. Fortunately, this complex is readily monitored by ^{1}H NMR spectroscopy.

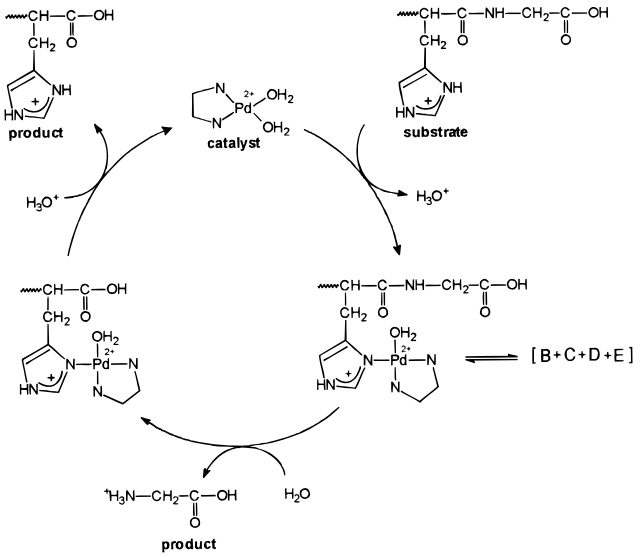
With decreasing acidity of the solution the rate constant mostly decreases, as explained above, but the concentration of complex **C** increases. Clearly, it is unreactive in hydrolysis. We can conclude that the only reactive complex is the one designated **A**. The rate constant k_h was calculated from the rate law in eq 10, in which $[\mathbf{P}]_{tot}$ is known and all other quantities are experimentally determined. The value of 6.4×10^{-3} min^{-1} at $[H^+] = 7.9 \times 10^{-2}$ M is virtually equal to the value of k_h in Scheme 1, given above. Evidently, a methyl group at the uncoordinated nitrogen atom of imidazole does not affect the

$$\frac{d[Gly]}{dt} = \frac{k_h[\mathbf{P}]_{tot}}{1 + \frac{K_B}{K_A} + \frac{[H^+]}{K_A[\mathbf{Pd}]} + \frac{1}{[H^+]}\left(K_D + \frac{K_C + K_E}{K_A}\right)} \quad (10)$$

hydrolytic reaction. This finding justifies our use of methylated peptides in this mechanistic study. Hydrolytic cleavage depends only on the interaction of the scissile peptide bond with the palladium(II) aqua complex anchored at the N-3 atom in the imidazole side chain.

The Catalytic Cycle. Even though *cis*-$[Pd(en)(H_2O)_2]^{2+}$ binds incompletely to AcHis−Gly and to 1-Me-AcHis−Gly in equimolar mixtures under acidic conditions, each peptide becomes completely cleaved. Clearly, the catalyst turns over these substrates. Indeed, 1 equiv of the catalyst can completely cleave as many as 4 equiv of the peptide. This is a modest turnover, but it proves the principle of catalysis. As Scheme 3 shows, the reactive complex in the catalytic cycle exists in an extended equilibrium with the unreactive ones. Because only a fraction of the catalyst forms the reactive complex **A**, actual turnover is greater than the catalyst:substrate molar ratio of 1:4 indicates.

Scheme 3. Catalytic Cycle for Hydrolysis of AcHis−Gly



Two factors luckily combine to make possible this catalytic turnover. Because complexes of palladium(II) are relatively labile, the catalyst can detach from the histidyl fragment of the cleaved peptide molecule and attach to an intact peptide molecule. Because the anchoring imidazole is a Brønsted base, this process is assisted by hydrogen ions in solution.

Conclusion and Prospects

This study is the main achievement of our laboratory to date in our quest for artificial metallopeptidases. It reveals the mechanism of hydrolysis to an extent unattainable in previous studies with methionine as the anchoring group or with platinum(II) complexes as the anchored cleaving agents. It is now clear that hydrolysis of the peptide bond is catalyzed by the proximate palladium(II) aqua complex and not by the acid in solution. Turnover is achieved and will be made greater in future studies. Cleavage is now possible in mildly acidic solutions (pH 5.0), which do not perturb the conformation of most proteins. This study points the way for further applications of our palladium(II) complexes in biochemistry and structural biology.

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