# Regioselective Hydrolysis of Tryptophan-Containing Peptides Promoted by Palladium(II) Complexes

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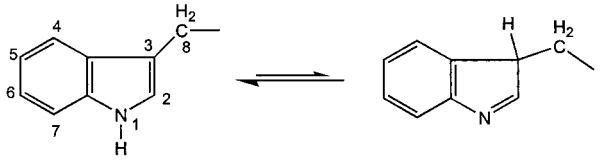
*Received November 23, 1998*
*Revised Manuscript Received July 5, 1999*

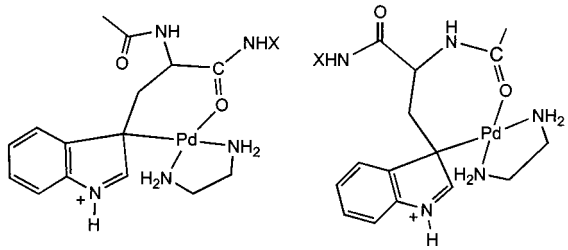
Selective cleavage of peptides and proteins is an important procedure in biochemistry and molecular biology. The half-life for the uncatalyzed hydrolysis of amide bonds is 350−500 years at room temperature and pH 4−8.[1] Clearly, efficient methods of cleavage are needed. Despite their great catalytic power and selectivity to sequence, proteinases have some disadvantages.[2] These enzymes cleave at numerous sites and yield relatively short peptides that are ill-suited for automatic sequencing.[2] Moreover, proteinases tolerate only a narrow range of reaction conditions. The only widely used chemical reagent for amide hydrolysis, cyanogen bromide, is toxic and volatile and requires harsh reaction conditions.[2] Transition-metal complexes are promising reagents for cleavage of amide bonds.[3] Peptides[4] and proteins[5] can be hydrolytically cleaved near histidine and methionine residues with several palladium(II) aqua complexes, often with catalytic turnover. Here, we report that palladium(II) complexes bind to *N*-acetylated tryptophan-containing peptides AcTrp-Ala, AcTrp-Val, and AcTrp-ValOMe in acetone solution and regioselectively cleave them upon addition of an equivalent of water.

**Binding of Tryptophan-Containing Peptides to Palladium-(II).** Coordination of indole (**1**) to metals in biological systems is unprecedented. Few abiological metal complexes with tryptophan are known.[6−8] Pyridine-type nitrogen in the tautomer 3H-indolenine (**2**) can coordinate to palladium(II).[9,10]




**1** **2**

Upon mixing of *N*-acetyl-L-tryptophanamide (AcTrp-$NH_2$) and *cis*-$[Pd(en)(sol)_2]^{2+}$ in acetone solution, complex **3** appears.[11,12] Downfield shift of the indole N(1)−H proton resonance by 0.32 ppm rules out both the deprotonation of **1** and its tautomerization to **2** upon coordination.[13] Downfield shift of the C(3) resonance by 41.6 ppm is characteristic of conversion of an aromatic atom (in **1**) to a tetrahedral atom (in **3**).[9,10,14] Downfield shifts of the proton resonances of $C(O)NH_2$ by 2.02 and 2.30 ppm and of α-CH by 0.40 ppm are diagnostic of coordination of the *C*-terminal amide oxygen (in **3**).[11,15,16] Without this auxiliary interaction indole alone would not bind to palladium(II). The blue shift of the palladium(II) d−d absorption bands upon tryptophan coordination is consistent with the relative strengths of the ligand fields of the carbanion at C(3), amide oxygen, and solvent (acetone and water, as sol) ligands.[17] Coordination of the *N*-terminal amide oxygen, as in **4**, was not observed, presumably because the seven-membered ring in **4** is less favorable than the six-membered ring in **3**. The ethyl ester AcTrp-OEt does not detectably react with *cis*-$[Pd(en)(sol)_2]^{2+}$ because the ester carbonyl oxygen is less nucleophilic than the amide oxygen. NMR spectra show that tryptophan-containing peptides AcTrp-NHX, for which $NH_2X$ would be alanine, valine, and the valine methyl ester, react with *cis*-$[Pd(en)(sol)_2]^{2+}$ to form complexes **3**.[11,18] The equilibrium




**3** **4**

constants *K* for the binding of AcTrp-Ala to *cis*-$[Pd(en)(sol)_2]^{2+}$ in acetone-$d_6$ in the presence of 0.30 and 2.0 M $D_2O$ are 44 ± 10 and 5 ± 1 $M^{-1}$, respectively. Addition of 0.005 M NaOH to the latter solution does not much affect this binding ($K$ = 13 ± 7 $M^{-1}$). Water, itself a ligand, inhibits tryptophan coordination. In

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(11) (a) $^1$H NMR for AcTrp-$NH_2$: N(1)H 10.08 br s, C(2)H 7.15 s; C(4)H 7.64 d; C(5)H 7.00 t, C(6)H 7.08 t; C(7)H 7.35 d, C(8)H 3.18 m; amide NH 6.90 br, 6.35 br, and 7.12 br; α-CH 4.65 m; $CH_3$ 1.85 s. $^1$H NMR for **3**: N(1)H 10.40 br; C(2)H 7.57 s; C(4)H 7.77 d; C(5)H 7.18 t; C(6)H 7.12 t; C(7)H 7.54 d; C(8)H 3.70 m; amide NH 8.92 br, 8.65 br, and 7.3 br; α-CH 5.05 m; $CH_3$ 1.89 s. (b) $^{13}$C NMR for AcTrp-$NH_2$: C(2) 124.2; C(3) 111.6; C(4a) 128.8; C(4) 119.3; C(5) 119.3; C(6) 122.0; C(7) 112.0; C(7a) 137.2; C(8) 23.0; α-C 54.3; C(O) 174.0; $CH_3$ 29.0; $C(O)NH_2$ 170.0. $^{13}$C NMR for **3**: C(2) 119.0; C(3) 70.0; C(4a) 128.9; C(4) 119.5; C(5) 126.7; C(6) 122.2;, C(7) 113.0; C(7a) 137.3; C(8) 17.5; α-C 56.0; C(O) 175.0; $CH_3$ 31.5; $C(O)NH_2$ 187.0. $\lambda_{max}$ is 333 and 325 nm for *cis*-$[Pd(en)(sol)_2]^{2+}$ and **3**, respectively.

(12) Complex **3** is formed regardless of the mole ratio of Pd(II) to *N*-acetyl-L-tryptophanamide. Concentration of **3** never exceeded the lowest initial concentration of the substrates.

(13) 2D $^1$H−$^{15}$N HETCOR spectra ruled out the iminol species and deprotonation of the amide nitrogen.

(14) In *cis*-$[Pt(en)(C,O\text{-AcTrp-}NH_2)]^{2+}$ the $^{13}$C resonance of C(3) is at 70.0 ppm.

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(18) $^1$H NMR for AcTrp-Ala: N(1)H 10.10 br s; C(2)H 7.27 s; C(4)H 7.64 d; C(5)H 7.00 t; C(6)H 7.09 t; C(7)H 7.39 d; C(8)H 3.20 m; amide NH 8.12 br and 7.90 br; α-CH 4.75 q; α-CH 4.40 q; $CH_3$ 1.90 s; $CH_3$ (Ala) 1.32 d. For *cis*-$[Pd(en)(C,O\text{-AcTrp-Ala})]^{2+}$: N(1)H 10.50 br; C(2)H 7.60 s; C(4)H 7.70 d; C(5)H 7.17 t; C(6)H 7.11 t; C(7)H 7.68 d; C(8)H 3.60 m; amide NH 8.12 br and 8.21 br, α-CH 5.30 m; α-CH 4.75 m; $CH_3$ 1.90 s; $CH_3$ (Ala) 1.50 d. See also the Supporting Information.

10.1021/ja9840246 CCC: $18.00 
Published on Web 09/03/1999

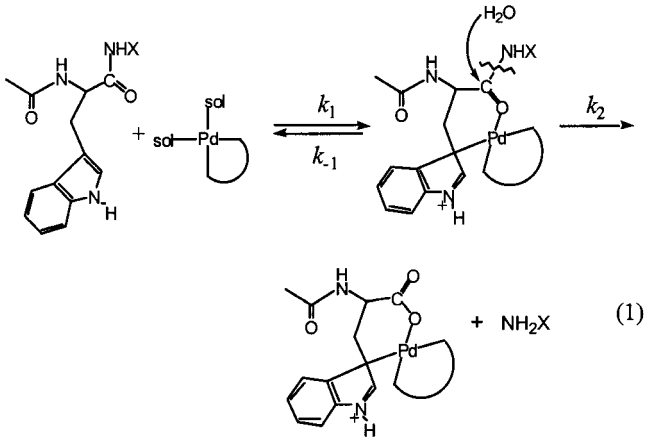
**Table 1.** Observed Rate Constants for the Hydrolysis of the Three Tryptophanyl Dipeptides Promoted by the Four Palladium(II) Complexes[a]

| | $10^3 k_{obs}$, $min^{-1}$ | | |
|---|---|---|---|
| complex[b] | AcTrp-Ala | AcTrp-Val | AcTrp-ValOMe |
| none | <0.0005 | <0.0005 | <0.0005 |
| *cis*-[Pd(en)(sol)$_2$]$^{2+}$ | 2.4 ± 0.2 | | 0.99 ± 0.1 |
| *cis*-[Pd(Me$_4$en)(sol)$_2$]$^{2+}$ | 0.21 ± 0.02 | | <0.01 ± 0.01 |
| *cis*-[Pd(dtcol)(sol)$_2$]$^{2+}$ | 2.7 ± 0.3 | 1.2 ± 0.1 | 0.95 ± 0.10 |
| [Pd(dien)(sol)]$^{2+}$ | <0.004 | | |

[a] At 323 K, in acetone-$d_6$ that was made 0.0010 M in $DClO_4$. Initial concentrations of the dipeptides, palladium(II) complexes, and $D_2O$ were 0.025, 0.025, and 0.300 M, respectively. [b] en is ethylenediamine, Me$_4$en is *N,N,N′,N′*-tetramethylethylenediamine, dtcol is 1,5-ditiocyclooctan-3-ol, and dien is diethylenetriamine.

aqueous solution this coordination is undetectable even in the presence of a 100-fold excess of *cis*-[Pd(en)(sol)$_2$]$^{2+}$.[19]

**Hydrolysis of Tryptophan-Containing Peptides.** As Table 1 shows, the "background" hydrolysis of the peptides is extremely slow. In the presence of the palladium(II) complexes and 1 equiv of water in an acetone-$d_6$ solution, however, these peptides are completely cleaved in less than a day. The active intermediates are complexes **3**, as Figure S1 in the Supporting Information shows. As eq 1 shows, the products are the *C*-terminal amino




(1)

acid and palladium(II) complex of *N*-acetyl-L-tryptophan. Acetic acid is not detected. In control experiments with AcAla-Trp, which lacks a *C*-terminal amide bond adjacent to the tryptophanyl residue, neither amide bond is cleaved. Evidently, palladium(II) regioselectively cleaves on the *C*-terminal side of tryptophan, not on the *N*-terminal side. This useful regioselectivity is presumably caused by the stereochemical preference for **3** over **4**. In aqueous solution coordination of the tryptophanyl residue to palladium(II) completely ceases, and the peptides do not hydrolyze.

The tridentate complex [Pd(dien)(sol)]$^{2+}$ has only one labile ligand and does not bind to the tryptophan-containing peptides because they can act only as bidentate ligands.[20] Consequently, there is no hydrolytic cleavage.

The peptide containing the smaller side chain (alanine) is cleaved more rapidly than the similar peptides containing the larger side chain (valine). Although the steric effect is small, it may be parlayed into sequence-selectivity (preferential cleavage next to a smaller leaving group).[4b,c] Similar rates for the valine and its ester indicate that the bulk of the peptide terminus does not affect the rate of cleavage.

**Mechanism of Hydrolysis.** Absence of aqua ligands in the hydrolytically active complexes **3** rules out the internal attack of Pd(II)-bound nucleophile at the amide carbon.[4] Coordination of the amide oxygen renders the amide carbon more electrophilic and susceptible to external attack of solvent water, which causes the hydrolytic cleavage on the *C*-terminal side of tryptophan (eq 1). There is no cleavage on the *N*-terminal side, presumably because that amide bond is not activated by the palladium(II) atom. Fitting the data in Figure S1 to the equations in the Supporting Information yielded the rate constant for hydrolysis $k_{obs} = (k_1k_2[cis\text{-Pd(en)(sol)}_2^{2+}])/(k_1[cis\text{-Pd(en)(sol)}_2^{2+}] + k_{-1})$ of $(2.4 \pm 1.0) \times 10^{-3}$ $min^{-1}$ and the equilibrium constant $K = k_1/k_{-1}$ of 27 ± 6 that agrees with the result of binding studies. The microscopic rate constants $k_2$ in the presence of 0.30 and 2.0 M $D_2O$ in acetone-$d_6$ solution are $(6.3 \pm 1.0) \times 10^{-4}$ and $(4.6 \pm 1.0) \times 10^{-3}$ $min^{-1}$, respectively.[21] That $k_2$ increases 7.3-fold as the concentration increases 6.7-fold is consistent with the external attack of water at the coordinated AcTrp-Ala, as in eq 1. Initial rate for alanine formation is a composite quantity: $v_0 = k_2[\mathbf{3}]$; because $k_2$ increases and [**3**] decreases as the water concentration increases, the overall effect on $v_0$ is small.[22]

To our knowledge, this is the first report that coordination of the tryptophan side chain is followed by hydrolysis of a nearby peptide bond. Unlike methionine and histidine, which coordinate to palladium(II) in aqueous as well as acetone solutions,[4] tryptophan coordinates only in acetone solution. Tryptophan has lower affinity to palladium(II) than methionine and histidine do. Rate constants for palladium(II)-promoted hydrolysis next to these residues show the same trend.[23] Therefore, the overall selectivity of cleavage, can be altered simply by changing the solvent or the concentration of the palladium(II) promoter. Cleavage will occur near histidine and methionine in aqueous solution, and near histidine, methionine, and tryptophan in acetone solution, if the promoter is present in excess. The fine selectivity, preference for the *C*-terminal over the *N*-terminal amide bond of tryptophan, is due to the stereochemistry of chelate rings. A prospect for cleaving hydrophobic or membrane-bound proteins in nonaqueous solutions, by palladium(II) complexes, has emerged.

**Acknowledgment.** We thank NSF for Grant No. CHE-816522.

**Supporting Information Available:** Syntheses of the palladium(II) promoters and of the substrates; kinetic and other experimental procedures; structures of the promoters; $^{13}C$ NMR data for the complex *cis*-[Pd(dtcol)(*C,O*-AcTrp-NH$_2$)]$^{2+}$; $^1H$ NMR data for the two dipeptides and their palladium(II) complexes; Figure S1 showing the progress of hydrolysis; and kinetic equations used to fit the data in Figure S1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The *K* values are independent of the initial concentrations of AcTrp-Ala and *cis*-[Pd(en)(sol)$_2$]$^{2+}$. We report average results of several experiments.

(20) Upon addition of [Pd(dien)(sol)]$^{2+}$ to AcTrp-NHX the $^1H$ NMR spectra remain unchanged.

(21) Calculated from the initial rate of alanine formation and the known initial concentration of **3**.

(22) The initial rates in acetone-$d_6$ that is 2.0 M in $D_2O$ and 0.0050 M in NaOH, 2.0 M in $D_2O$, and 0.30 M in $D_2O$ are $(1.0 \pm 0.5) \times 10^{-4}$, $(1.1 \pm 0.2) \times 10^{-4}$, and $(6.3 \pm 1.0) \times 10^{-6}$ M $min^{-1}$, respectively, at 323 K. Initial concentrations of the dipeptides and palladium(II) complexes were 0.10 M each.

(23) In acetone solution in which there is an excess of *cis*-[Pd(en)(sol)$_2$]$^{2+}$ over a mixture of Met-, His-, and Trp-containing peptides, upon addition of water only the Trp-containing peptide is cleaved.