# Steric effects on the rate of hydrolysis by palladium(II) complexes of the C-terminal amide bond in a series of methionine-containing dipeptides AcMet-Aa[*]

T. WADE JOHNSON[#] and NENAD M. KOSTIĆ[+]

*Department of Chemistry, Iowa State University, Ames, IA 50011, USA*
*(e-mail: nenad@iastate.edu.)*

*Abstract*: A series of N-acetylated, methionine-containing dipeptides designated AcMet-Aa containing various C-terminal amino acids designated Aa are hydrolyzed in aqueous solution at 50 ºC and $0.95 < pD < 1.10$ in the presence of three *cis*-$[Pd(L)(H_2O)_2]^{2+}$ complexes, in which L are bidentate ligands en, $Me_4$en, and 3-OH-dtco. The reactions were monitored by $^1$H-NMR spectroscopy. The rate constant for hydrolytic cleavage of the Met-Aa bond decreases as the steric bulk of the amino acid Aa increases. Correlations to Taft's $E_s$ values were made. The substituents on α-C and β-C atoms lower the rate constant most, those on the γ-C atom lower it less, and those on the δ-C have no detectable effect. Partial selectivity for leaving amino acid Aa is attributed to differences in the volume of the side chain and to discrimination between leaving groups of similar volume but different branching patterns.

*Keywords*: selective cleavage, kinetics, peptides, methionine, palladium(II).

## INTRODUCTION

Hydrolytic cleavage of peptides and proteins is an important biological process and a common procedure in chemistry and biochemistry. The half-life for the hydrolysis of the unactivated amide bond in neutral aqueous solution is around 500 years.[1] The exceptional inertness of the amide bond requires severe conditions for the hydrolytic cleavage. There is a great need for new and efficient reagents for selective cleavage of peptides and proteins.

Proteolytic enzymes are much used,[2] but are unsuitable in cases when uncommon selectivity is needed and when they would contaminate the proteinaceous digest. Only trpysin is truly regioselective,[3] but it tends to produce multiple, short fragments of the protein that is cleaved. Modern methods for protein sequencing prefer, and methods for protein semisynthesis require, relatively long peptides.

---

[*]   Dedicated to Professor Živorad Čeković on the occasion of his 70th birthday.

[#]   Present address: Susquehanna University, Selinsgrove, PA 17870, USA.

[+]   To whom correspondence should be addressed.

Chemical reagents, such as cyanogen bromide, *O*-iodosobenzoate, and hydroxylamine, are alternatives to enzymes.[2] Only cyanogen bromide is routinely used, but it is a volatile and toxic compound that requires harsh reaction conditions, and often produces incomplete cleavage.

Transition-metal complexes are emerging as new reagents for cleaving peptides and proteins.[4–10] Those of lanthanides,[4] cobalt(III),[5] copper(II),[6,7] copper(I),[8] and iron(II)[9,10] have been used by other researchers. Because cobalt(III) forms a substitutionally inert complex with the N-terminal amino acid in the peptide, only the N-terminal amide bond becomes cleaved. Biochemists, however, need to cleave amide bonds throughout the protein sequence. Iron chelates in the presence of some added chemicals are rapid cleavers, but these chelates must first be attached to the protein in a demanding synthetic procedure, which precludes catalytic turnover. Moreover, cleavage by iron chelates is not necessarily regioselective.

In this laboratory palladium(II) complexes have long been used for selective, hydrolytic cleavage of peptides and proteins in aqueous solutions, under relatively mild conditions.[11–24] These complexes spontaneously anchor to specific amino-acid side chains simply upon mixing with the substrate. Because the substrate, its fragments obtained by cleavage, and the cleaving reagents exist in an extended equilibrium, catalytic turnover is possible. Because these reagents are diamagnetic, cleavage reactions are easily followed by NMR spectroscopy. Previous studies in this laboratory have shown that palladium(II) complexes bind to the sulfur atom in methionine, as shown schematically in Eq. (1), and also to imidizole nitrogen in histidine.

After this anchoring to a side chain, the selectivity of subsequent clavage depends on the position of the anchoring amino-acid residue in the sequence and reaction conditions. If the methionine anchor occupies the position no. 3 or higher and the histidine anchor occupies position no. 4 or higher, the cleavage occurs at the second amide bond preceding the anchor (toward the amino terminus).[12] This selectivity is regularly observed with proteins and all but short peptides, that is, substrates in which the anchoring residue is several residues removed from the amino terminus. When, however, the substrates are short peptides lacking the second amide bond preceding the anchor, the anchored Pd(II) complex promotes se-



$$(1)$$

**external attack**  **internal delivery**

Scheme 1.

**Chart 1**



cis -[Pd(en)(H$_2$O)$_2$]$^{2+}$    cis -[Pd(Me$_4$en)(H$_2$O)]$^{2+}$    cis-[Pd( 3-OH-dtco)(H$_2$O)]$^{2+}$

Scheme 2.

lective cleavage of the first amide bond following the anchor (toward the carboxy terminus).[16–22] In this study we used such shorter substrates, and cleavage occurred as in Eq. (1).

There are two general mechanisms for hydrolytic cleavage, shown in Scheme 1. In one, the anchored palladium(II) complex acts as Lewis acid, interacts with the carbonyl oxygen atom, and thus activates the scissile amide group towards nucleophilic attack by external water molecule. In the other mechanism, the palladium(II) complex internally delivers an aqua ligand to the proximate amide group. The two mechanisms give the same rate law, but indirect kinetic evidence favors external attack.[14] In either mechanism, cleavage requires a close approach of the anchored palladium(II) complex to the scissile group. Expecting that this approach will be hindered by steric bulk of the ancillary ligands on the Pd(II) atom and of the side chain R in the leaving amino acid, we set out to vary these ligands (see Scheme 2) and side chains, and to study steric effects on the kinetics of cleavage.

EXPERIMENTAL

*Chemicals*

Distilled water was demineralized and purified to a resistance greater than 18 MΩ cm. Deuterium-containing compounds D$_2$O and DClO$_4$ and also anhydrous AgClO$_4$ were obtained from Aldrich Chemical Co. Naturally occurring methionine-containing dipeptides were purchased from Sigma Chemical Co. Dipeptide Met-Ile was purchased from Bachem. Alpha-methylated amino acids α-Me-Val and α-Me-Leu were purchased from Bachem. Unnatural amino acids containing linear side chains (norleucine, norvaline, 2-aminobutyric acid, and 2-aminoisobutyric acid) were purchased from Aldrich.

The desalting column was packed with Bio-Rad analytical AG 11 A8 ion retardation resin. All common chemicals were of reagent grade. The salt $K_2[PdCl_4]$ more than 99.9 % pure was obtained from Aldrich or Pressure Chemical Company. The complexes cis-[Pd(3-OH-dtco)Cl₂], which contains 3-hydroxy-1,5-dithiacyclooctane, and cis-[Pd(en)Cl₂], which contains ethylenediamine, were prepared by published methods.[25,26] The complex cis-[Pd(Me₄en)Cl₂], which contains N,N,N',N'-tetramethyl ethylene-diamine, was purchased from Aldrich and converted into corresponding aqua complexes by treatment with 2 equivalents of AgClO₄ according to a published method.[25] Anhydrous tetrahydrofuran (THF) was distilled over sodium-benzophenone under a nitrogen atmosphere. Anhydrous methanol was dried over sodium and distilled under a nitrogen atmosphere. Anhydrous triethylamine was dried over calcium hydride and distilled under a nitrogen atmosphere.

*Measurements*

Proton NMR spectra were recorded at 300 MHz and 400 MHz by a Varian VXR 300 and Bruker DRX 400 NMR, respectively, The solvent, $D_2O$, was used as a lock, and DSS at known concentration was as an internal reference and concentration standard.

Two-dimensional COSY spectra were run at 400 MHz. The temperature was kept constant within ± 0.1 ºC by the NMR temperature controller in NMR experiments or within ± 1 ºC by an aluminum heating block in experiments for peptide cleavage. The pH was measured with a Fisher 925 pH instrument using an Aldrich Ag/AgCl combination NMR electrode. The values are not corrected for $D_2O$.

*Syntheses*

*Acetylation of dipeptides.*[17] The dipeptide was dissolved in glacial acetic acid. A slight molar excess of acetic anhydride was added. The solution was stirred overnight. The solvent was removed under reduced pressure with multiple dilutions of water. Acetone was added to assist in crystallization. To remove any residual acetic acid, the crystals were then dried under vacuum for 48 h.

*Esterification of 2-aminobutyric acid and 2-aminoisobutyric acid.* The synthesis of the methyl esters was based on a published procedure.[27] To a stirred suspension of 1.05 g (10 mmol) of the amino acid in 100 ml of 2,2-dimethoxypropane was added 5 ml of concentrated HCl, and the mixture was left overnight. The solvent was removed under reduced pressure, and a deep red oil was dissolved in 30 ml of methanol. Addition of 300 ml cold diethyl ether caused precipitation of the methyl ester. The precipitate was filtered, and analytically pure methyl ester was recovered, with yield of 100 %. Proton NMR spectrum of methyl 2-aminobutyrate: 1.04, t; 1.96, m; 3.84, s; 4.02, t; of methyl 2-aminoisobutyrate: 1.60, s; 3.84, s.

*Esterification of α-Me-Val, a-Me-Leu, norLeu, norVal, and t-Leu.* The synthesis of the methyl esters was based on a published procedure.[27] To 150 ml of anhydrous methanol, magnetically stirred under nitrogen at 0 ºC, in ice/water bath, was dropwise added 9 ml of thionyl chloride. The solution was stirred for 5 min, and 2.0 g of the amino acid was added to the solution. The ice bath was removed, and the solution was refluxed for 24 h. Excess thionyl chloride was quenched with 5 ml of water. The solution was reduced to an oil and pumped dry to yield 100 % of the crystalline product. Selected proton NMR peaks of α-Me-Val methyl ester: 1.04, d; 1.54, s; 3.84, s; of α-Me-Leu methyl ester: 0.99, d; 1.56, s; 3.84, s; of norLeu methyl ester: 0.98, t; 1.41, m; 3.85, s; 4.05, t; and of t-Leu methyl ester: 1.10, s; 3.79, s; 3.82, s.

*Coupling of the amino acid methyl ester to acetyl methionine.* The coupling of the AcMet to the amino acid is based on a published procedure.[27] Under a nitrogen atmosphere, a solution containing 956 mg (5.0 mmol) of N-acetyl-DL-methionine and 0.80 ml of anhydrous triethylamine in 150 ml of freshly distilled, dry THF was prepared at –15 ºC. The solution was stirred for 5 min before adding 470 µl (5.0 mmol) of ethyl chloroformate. A white precipitate formed in minutes. At –15 ºC to –20 ºC, the solution was stirred for one hour before adding 5.0 mmol of the desired amino acid methyl ester and 0.60 ml of anhydrous triethylamine. The solution was allowed to warm to room temperature over one hour and was stirred overnight. The precipitate was filtered off, and solvent was removed under reduced pressure. The remaining yellow oil was dissolved in *ca*. 30 ml of di-

chloromethane. The unspent AcMet was extracted with three 8-ml portions of 5 % ammonium carbonate, and the unspent amino acid methyl ester was extracted with three 8-ml portions of 5 % acetic acid. The remaining solution was washed with three 10-ml portions of water. The oil was dissolved in 50–150 ml water at 65 ºC. Over three hours, 2.5 equivalents of 1.0 M KOH was added dropwise. After the solution cooled to room temperature, it was acidified to pH < 2 with 6.0 M HCl or $HClO_4$. The solution was concentrated under reduced pressure to 2/3 of original volume and left at 4 ºC overnight. Any crystals formed were filtered off, and the solution was again concentrated to 1/3 of the volume and refrigerated. The second batch of crystals were filtered off and combined with the first batch. Dipeptides containing α-Me-Leu, norLeu, norVal, and α-Me-Val are not readily soluble in water and were washed with cold water to remove KCl. The final yield was 30 %. Dipeptides more soluble in water were dissolved in acetone or methanol. The precipitated salt was filtered off. The solution was concentrated under reduced pressure to an oil and dissolved in water. The aqueous solution was run through a desalting column sized 1 × 35 cm packed with a Bio-Rad AG11 A8 ion retardation resin. The eluate was concentrated and crystals obtained. The final yield was 30 %.

*Molecular modeling*

Desktop molecular modeler, version 1.2 by Oxford University Press, was used for determining the Cartesian coordinates of the amino acids. The various amino acids were constructed by template, and their geometry was optimized by molecular mechanics. The palladium(II) complexes were constructed, and their geometry was optimized with the following reasonable constraints: Pd–$OH_2$ = 2.20 Å, Pd–S = 2.295 Å, Pd–N = 2.037 Å, and square planar geometry at the palladium(II) atom.[28] These files were then converted to PDB format and imported by GRASP 2.0. The volumes and surface areas were calculated given the Van Der Waals dimensions for each atom, Table I.

TABLE I. Calculated volumes ($V$) of the amino acids (Aa) and differences ($\Delta V$) in volumes of their side chains and that of glycine, a H atom

| Aa | $V/\text{Å}^3$ | $\Delta V/\text{Å}^3$ |
| --- | --- | --- |
| Gly | 63 | 0 |
| Ala | 78 | 15 |
| Ser | 87 | 24 |
| AIB | 91 | 28 |
| ABA | 93 | 30 |
| norVal | 105 | 42 |
| Val | 105 | 42 |
| α-Me-Val | 119 | 56 |
| norLeu | 119 | 56 |
| Leu | 119 | 56 |
| *t*-Leu | 120 | 57 |
| Ile | 121 | 58 |
| α-Me-Leu | 133 | 70 |
| Lys-Ac | 166 | 103 |

*Kinetics of hydrolysis*

Stock solutions of *cis*-[Pd(3-OH-dtco)$(D_2O)_2$]$^{2+}$, *cis*-[Pd(en)$(D_2O)_2$]$^{2+}$, and *cis*-[Pd(Me$_4$en)$(D_2O)_2$]$^{2+}$ were prepared fresh in $D_2O$. Each reaction mixture in $D_2O$ contained 50 µl of 100 mM DSS, a known

amount of 2.0 M DClO$_4$ to bring the pH to 1.0, and equimolar amounts of a dipeptide and a palladium(II) aqua complex. These ingredients were rapidly mixed in an NMR tube. The solution concentration was 2.5 mM to 25 mM, depending on solubility of the N-acetylated dipeptide. The total volume was between 675 μl and 715 μl. For rapid reactions, acquisition of the ^{1}H-NMR spectra began as soon as possible after the mixing, and 16 to 64 scans were taken each time. The temperature was kept at 50 ± 0.1 ºC. A typical kinetic plot consisted of at least 15 points taken over four to six half lives, as in Fig. 1. Concentrations of the peptide and hydrolysis products were determined from the known initial concentration, with an estimated error of 5 %.

In control experiments, peptide solutions without the palladium(II) complexes were kept at pH 1.05 and 50 ºC and occasionally examined by ^{1}H-NMR spectroscopy. The half-lifes of the so-called background cleavage were greater than 45 days and depended only slightly on the leaving amino acid.

TABLE II. Selected ^{1}H-NMR chemical shifts (δ, in ppm) of the acetylated dipeptide and the leaving amino acid, in D$_2$O solutions, at the (uncorrected) pH of 0.95 – 1.12

| Group | Hydrolysis substrate | Hydrolysis product |
|---|---|---|
|  | AcMet-Gly | Gly |
| Gly α-H | 4.00 s | 3.91 s |
|  | AcMet-Ala | Ala |
| Ala α-Me | 1.44 d | 1.58 d |
| Ala α-H | 4.39 q | 4.14 q |
|  | AcMet-Ser | Ser |
| Ser α-CH$_2$ | 3.97, 3.89 qd | 4.13, 4.03 qd |
| Ser α-H |  | 4.22 t |
|  | AcMet-AIB | AIB |
| AIB α-Me | 1.48 s | 1.61 s |
|  | AcMet-ABA | ABA |
| ABA β-Me | 0.95 t | 1.02 t |
| ABA α-H | 4.27 t | 4.06 t |
|  | AcMet-norVal | norVal |
| norVal γ-Me | 0.90 t | 0.95 t |
| norVal α-H | 4.35 t | 4.09 t |
|  | AcMet-Val | Val |
| Val β-Me | 0.96 d | 1.06 d |
| Val α-H | 4.28 d | 3.98 d |
|  | AcMet-α-Me-Val | α-Me-Val |
| α-Me-Val β-Me | 0.99 d | 1.03 d |
| α-Me-Val | 1.38 s | 1.55 s |
|  | AcMet-norLeu | norLeu |
| α-Me-norLeu α-H | 4.35 t | 4.08 t |
|  | AcMet-Leu | Leu |
| Leu γ-Me | 0.91 d | 0.97 d |
| Leu α-H | 4.43 t | 4.08 t |
|  | AcMet-Ile | Ile |
| Ile β-Me | 0.97 d | 1.06 d |
| Ile α-H | 4.34 d | 4.06 d |
|  | AcMet-t-Leu | t-Leu |
| t-Leu β-Me | 1.01 s | 1.11 s |

TABLE II. Continued

| Group | Hydrolysis substrate | Hydrolysis product |
| --- | --- | --- |
| t-Leu α-H | 4.24 s | 3.81 s |
| | AcMet-α-Me-Leu | α-Me-Leu |
| α-Me-Leu | 1.50 s | 1.57 s |
| α-Me | | |
| | AcMet-Lys | Lys |
| Lys α-H | | 4.09 t |
| | me$_4$en in | Free me$_4$en |
| | Pd(me$_4$en)(H$_2$O)$_2$$^{2+}$ | |
| Methyl | 2.68 s | 3.005 s |
| Methylene | 2.88 s | 3.68 s |

Free amino acids were identified by their $^1$H-NMR chemical shifts and by enhancement of their resonances upon spiking of the reaction mixtures with known samples, Table II. Free tetramethylethylenediamine was identified by recording a solution of the free compound at pH 1.05.

## RESULTS AND DISCUSSION

*Volume determination*. There are several methods for describing the steric bulk of the amino acids. We first used average volumes of amino-acid pieces as defined by a data base of protein structures,[29] but the cumulative error margins in piecing together large side chains became too high. Newer values of whole side-chain volumes inadequately describe the unnatural amino acids.[30] Since all amino-acid residues have the same backbone, we consider the side chains only. Differences in $\Delta V$ between the amino acids in question and glycine are shown in Table I.

*Hydrolysis rate as a function of leaving group and the palladium(II) complex*. The rate constant for hydrolysis of the AcMet-Aa dipeptides generally decreases as the leaving group Aa and the ligands attached to palladium(II) atom increase in volume, as shown in Tables III and IV and Fig. 2. Interestingly, the rate constants differ for amino acids of the same or similar volumes, such as isomers of valine and leucine, because their side chains differ in shape. Addition of a methyl group to the α-C atom in Ala, Val and Leu lowers the rate constant to varying extents, depending upon the palladium(II) complex and the side chain. The effectiveness of the promoters decreases in the order $[Pd(en)(H_2O)_2]^{2+}$, $[Pd(Me_4en)(H_2O)_2]^{2+}$, and $Pd(3\text{-}OH\text{-}dtco)(H_2O)_2^{2+}$. The respective half-lives for the hydrolysis promoted by the three complexes range from 13 min to 1800 min. The respective slopes of the plots in Fig. 2 are very similar: –0.023, –0.017, and –0.020. Evidently, the volumes of the leaving group Aa and of the bidentate ligand act independently in inhibiting the cleavage reaction.

$E_s$ *values*. The rate of acid hydrolysis of esters depends on the steric bulk of the substituent on the acid group.[31–38] As this substituent increases in size, the rate of hydrolysis decreases. Taft's[39] $E_s$ values, or steric constants, are defined in Eq. (2)[37] with methyl group as standard $k^0$ ($E_s = 0$). Substituents with greater steric require-

Fig. 1. A typical set of raw experimental results: Progress in time of the hydrolysis of Acmet-aib promoted by $[Pd(3\text{-}OH\text{-}dtco)(H_2O)_2]^{2+}$. The initial concentrations of Acmet-aib, $[Pd(3\text{-}OH\text{-}dtco)(H_2O)_2]^{2+}$, and $DClO_4$, were 25, 25, and 100 mM, respectively. The solvent was $D_2O$, and the temperature was 323 K. The mixing time is taken as zero.

ments will have a negative $E_s$ value. We applied this treatment to hydrolysis of the amide bond in depetides using the H substituent (*i.e.*, glycine as Aa) as standard. Hence the constant difference of 1.24 between the previous and our parameters, the corresponding entries in the last two columns in Table IV. Side chains for which $E_s$ constants have not been published before are excluded also from our list.

$$E_s = \log\left(\frac{k}{k^0}\right) \qquad (2)$$

*Shape of the side chain*. Not only the volume of the leaving group, but also the shape and the location of the side chain relative to the scissile amide bond affect the hydrolysis rate. Table V shows a lesser inhibition when the branching is farther from the backbone, at $\gamma$-C atom in Leu, than when branching is nearer to the backbone, $\beta$-C in Val. Comparison of norleucine and isoleucine shows that the shape of the leaving group also affects the inhibition.

Linear alkyl side chains show the same trend in the established and our $E_s$ values. Both become more negative with increasing volume and show that $\alpha$-C and $\beta$-C substituents and branching pattern are more important than $\gamma$-C and $\delta$-C substituents.

The data in Tables III and IV for the following five series of leaving groups Aa show the kinetic effects of side-chain substitution: (1) Gly / Ala / AIB; (2) Ala / ABA /Val / *t*-Leu; (3) ABA / norVal / Leu; (4) Val / Ile / *t*-Leu; and (5) *t*-Leu / Ile / Leu /

Fig. 2. Dependence of the hydrolysis rate constant on the steric bulk of the leaving amino acid Aa. The cleavage promoters are $Pd(en)(H_2O)_2^{2+}$ (▲), $Pd(me_4en)(H_2O)_2^{2+}$ (●), and $Pd(3\text{-OH-dtco})(H_2O)^{2+}$ (◆). The slopes of the plots are $-0.023$, $-0.017$, and $-0.020$, respectively.

norLeu. In the first series, two methyl groups are successively introduced on the α-C atom of Aa, the leaving group. In the second series, successive methylation of the β-C atom creates a branched chain. In the third series, branching occurs at the γ-C atom. In the fourth series, isoleucine ant *t*-leucine side chains have the same calculated volume, but the latter one reacts more slowly, presumably because steric bulk is closer to the scissile bond. Methylation of the γ-C atom, in "conversion" of valine to isoleucine, has a marginal effect, but methylation of the β-C atom, in "conversion" of valine to *t*- leucine, nearly halves the rate constant for hydrolysis by the bulkiest reagent, *cis*-[Pd(3-OH-dtco)(H_2O)_2]^{2+}. In the fifth series, as the side chains in the isomers of leucine progress from tertiary, to secondary, to branched primary, to linear primary butyl group, the hydrolysis rate constant increases. As the bulk of the side chain becomes farther removed from the backbone and thus from the scissile bond, the cleavage reaction becomes faster.

TABLE III. Observed rate constants for the hydrolysis of methionine-containing dipeptides AcMet-Aa promoted by equimolar amounts of each of three palladium(II) complexes at 323 K, in a 0.1 M solution of DClO$_4$ in D$_2$O

| Aa | $\Delta V$/Å3 | Pd(en)(H$_2$O)$_2^{2+}$ $k_{obs} \times 10^4$/min$^{-1}$ | Pd(Me$_4$en)(H$_2$O)$_2^{2+}$ $k_{obs} \times 10^4$/min$^{-1}$ | Pd(3-OH-dtco)(H$_2$O)$_2^{2+}$ $k_{obs} \times 10^4$/min$^{-1}$ |
|---|---|---|---|---|
| Gly | 0 | 370 | 82 | 3.7 |
| Ala | 15 | 180 | 65 | 28 |
| Ser | 24 | 120 | 54 | 15 |
| AIB | 28 | 140 | 63 | 18 |
| ABA | 30 | 140 | 53 | 19 |
| norVal | 42 | 110 | 37 | 15 |
| Val | 42 | 85 | 24 | 7.2 |
| α-Me-Val | 56 | 65 | 25 | 6.4 |
| norLeu | 56 | 110 | 38 | 14 |
| Leu | 56 | 69 | 25 | 9.9 |
| $t$-Leu | 57 | 85 | 18 | 3.8 |
| Ile | 58 | 94 | 23 | 8.8 |
| α-Me-Leu | 70 | 56 | 19 | 7.1 |
| Lys-Ac | 103 | 23 | 19 | 6.1 |

*Second methylation of the α-carbon atom.* To probe the kinetic effect of steric bulk close to the scissile amide bond, we replaced the α-hydrogen atom with a methyl group. The comparisons in Table III of Ala and AIB and of Leu and α-Me-Leu shows average decreases of 25 % and 36 %, respectively, in rate for the three palladium(II) complexes, respectively. The comparison of Val and α-Me-Val, however, shows no significant change.

The effect of second methylation on the hydrolysis rate constant depends upon the original side chain. In the Ala/AIB pair, the steric bulk at the α-C is doubled, but in the Val/α-Me-Val pair the addition of 14 Å$^3$ to an existing volume of 42 Å$^3$ already positioned near the backbone did not produce a detectable steric effect. In the Leu/α-Me-Leu pair, however, addition of 14 Å$^3$ to 56 Å$^3$ produced detectable inhibition because the existing volume, although larger, was located farther away from the backbone.

*Effect of the chelating ligand on the kinetics of hydrolysis.* As Table V shows, steric bulk of the palladium(II) complex depends on the size of the bidentate ligand. As the horizontal trends in Table III show, the rate constant for cleavage of a given dipeptide decreases as the size of this ligand increases. The vertical trends in Table III are surprising. The larger the bidentate ligand, the lesser the apparent dependence of the rate constant on the size of the leaving group.

TABLE IV. Calculated $E_s$ values for the side chains of the leaving groups Aa in dipeptides AcMet-Aa whose hydrolytic cleavage is promoted by three palladium(II) complexes containing different bidentate ligands L

| Aa | $\Delta V$ | $Pd(L)(H_2O)_2^{2+}$ | | | Average of three Ls | $E_s$ published[a] $CH_3 = O$ | This work $H = O$ |
|----|----|----|----|----|----|----|----|
| | | L is en | L is Me$_4$en | L is 3-OH-dtco | | | |
| Gly | 0 | 0 | 0 | 0 | 0 | 1.24 | 0 |
| Ala | 15 | $-0.31$ | $-0.11$ | $-0.12$ | $-0.18$ | 0 | $-1.24$ |
| Ser | 24 | $-0.49$ | $-0.18$ | $-0.40$ | $-0.36$ | $+0.03$ | $-1.21$ |
| AIB | 28 | $-0.42$ | $-0.11$ | $-0.31$ | $-0.28$ | $-$ | $-$ |
| ABA | 30 | $-0.42$ | $-0.19$ | $-0.29$ | $-0.30$ | $-0.07$ | $-1.31$ |
| norVal | 42 | $-0.53$ | $-0.35$ | $-0.39$ | $-0.42$ | $-0.36$ | $-1.60$ |
| Val | 42 | $-0.64$ | $-0.53$ | $-0.71$ | $-0.63$ | $-0.47$ | $-1.71$ |
| $\alpha$-Me-Val | 56 | $-0.94$ | $-0.52$ | $-0.76$ | $-0.74$ | $-$ | $-$ |
| norLeu | 56 | $-0.53$ | $-0.33$ | $-0.42$ | $-0.42$ | $-0.39$ | $-1.63$ |
| Leu | 56 | $-0.73$ | $-0.51$ | $-0.57$ | $-0.60$ | $-0.93$ | $-2.21$ |
| $t$-Leu | 57 | $-0.63$ | $-0.66$ | $-0.99$ | $-0.76$ | $-1.54$ | $-2.78$ |
| Ile | 58 | $-0.60$ | $-0.55$ | $-0.62$ | $-0.59$ | $-1.13$ | $-2.37$ |
| $\alpha$-Me-Leu | 70 | $-0.82$ | $-0.64$ | $-0.71$ | $-0.72$ | $-$ | $-$ |
| Lys-Ac | 103 | $-1.02$ | $-0.64$ | $-0.78$ | $-0.87$ | $-$ | $-$ |

[a]Ref. 34

TABLE V. Calculated volumes of the complexes

| Complex | Volume/Å$^3$ |
|----|----|
| $Pd(H_2O)_4^{2+}$ | 85 |
| $Pd(en)(H_2O)_2^{2+}$ | 106 |
| $Pd(Me_4en)(H_2O)_2^{2+}$ | 163 |
| $Pd(3\text{-}OH\text{-}dtco)(H_2O)_2^{2+}$ | 180 |

The volumes in Table V must be taken skeptically because of possible displacement of chelating ligand by water. It has previously been shown that en is a relatively labile ligand, which rapidly dissociates from the palladium(II) in acid solution.[19] This leaves the likely hydrolysis promoter as an aqua or mixed aqua-hydroxo complex (depending on the p$K_a$ value of the aqua ligand). The ligand Me$_4$en was chosen for its increase in both volume and basicity relative to en. This ligand on Pd(II) atom is relatively inert under our reaction conditions for several days, longer than the duration of our experiments. Upon binding of the Pd(Me$_4$en)(H$_2$O)$_2^{2+}$ to methionine, however, the labilizing trans effect of the

TABLE VI. Comparative kinetics for the displacement of Me$_4$en ligand from the palladium(II) atom and dipeptide hydrolysis promoted by an equimolar amount of the palladium(II) complex. Temperature was 323 K, and the solvent was a 0.10 M solution of DClO$_4$ in D$_2$O

| $k_{obs} \times 10^3$ min$^{-1}$ for loss of Me$_4$en | $k_{obs} \times 10^3$ min$^{-1}$ for dipeptide hydrolysis | Dipeptide |
|---|---|---|
| 10.0 | – | Ac-Met |
| 17.3 | 8.2 | Ac-met-Gly |
| 9.4 | 6.0 | Ac-Met-AIB |
| 6.9 | 1.8 | Ac-Met-$t$-Leu |
| 3.1 | 1.9 | Ac-Met-Lys |
| 7.4 | 3.9 | Ac-Met-Ile |
| 11.0 | 3.7 | Ac-Met-norVal |

thioether ligand causes displacement (aquation), and the free Me$_4$en ligand is easily detected by NMR spectroscopy. Again, the actual species that promotes the hydrolysis is probably a complex containing mixed ligands. As Table VI shows, the rate constants for the loss of the Me$_4$en ligand and the peptide hydrolysis are of similar magnitudes. Because 3-hydroxy-1,5-dithiacyclooctane is a very weak Bronsted base (with respect to the H$^+$ ion), this ligand remains attached to the palladium(II) atom throughout the reaction. This stability is a desirable property in metal complexes used for hydrolytic cleavage of peptides.

ИЗВОД

## СТЕРНИ ЕФЕКТИ НА БРЗИНУ ХИДРОЛИЗЕ КОМПЛЕКСИМА ПАЛАДИЈУМА(II) С-ТЕРМИНАЛНЕ АМИДНЕ ВЕЗЕ У НИЗУ МЕТИОНИНСКИХ ДИПЕПТИДА AcMet-Aa

Т. ВЕЈД ЏОНСОН и НЕНАД М. КОСТИЋ

*Хемијски одсек, Државни универзитет Ајове, Ејмс, Ајова 50011, С.А.Д.*

Низ N-ацетилованих дипептида AcMet-Aa, који садрже метионин и разне аминокиселине Aa, хидролизују у воденом раствору на 50 ºC при 0,95 < pD < 1,10 у присуству три *cis*-[Pd(L)(H$_2$O)$_2$]$^{2+}$ комплекса у којима је L бидентатни лиганд en, Me$_4$en, или 3-OH-dtco. Реакције су праћене $^1$H-NMR спектроскопијом. Константа брзине за хидролитичко кидање Met-Aa везе опада како запремина аминокиселине Aa расте. Ти подаци корелисани су са Тафтовим $E_s$ параметром. Супституенти на α-C и β-C атомима највише снижавају константу брзине, они на γ-C атому снижавају је мање, а они на δ-C атому немају мерљиви утицај. Делимична селективност у односу на одлазећу аминокиселину Aa приписана је разликама у запремини бочног низа и дискриминацији међу одлазећим групама сличним по запремини али различитим по гранању супституената.

## REFERENCES

1. A. Radzicka, R. Wolfenden, *J. Am. Chem. Soc.* **118** (1996) 6105
2. *Methods in Enzymology, Volume 182, Guide to Protein Purification*, M. Deutscher, (Ed.), Academic Press, New York, 1990
3. L. R. Croft, *Handbook of Protein Sequence Analysis*, 2nd ed.; Wiley, Chichester, 1980
4. M. Komiyama, *Metal Ions Biol. Syst.* **38** (2001) 25
5. D. A. Buckingham, C. R. Clark, *Metal Ions Biol. Syst.* **38** (2001) 43
6. G. M. Polzin, J. N. Burstyn, *Metal Ions Biol. Syst.* **38** (2001) 103
7. G. Allen, *Metal Ions Biol. Syst.* **38** (2001) 197
8. M. Kito, M. Urade, *Metal Ions Biol. Syst.* **38** (2001) 187
9. S. A. Datwyler, C. F. Meares, *Metal Ions Biol. Syst.* **38** (2001) 213
10. T. Heyduk, N. Baichoo, E. Heyduk, *Metal Ions Biol. Syst.* **38** (2001) 255
11. N. M. Milović, N. M. Kostić, *Metal Ions Biol. Syst.* **38** (2001) 145
12. N. M. Milović, N. M. Kostić, *J. Am. Chem. Soc.* **124** (2002) 4759
13. N. M. Milović, N. M. Kostić, *Inorg. Chem.* **41** (2002) 7053
14. N. M. Milović, N. M. Kostić, *J. Am. Chem. Soc.* **125** (2003) 781
15. N. M. Milović, L. M. Dutca, N. M. Kostić, *Inorg. Chem.* **42** (2003) 4036
16. I. E. Burgeson, N. M. Kostić, *Inorg. Chem.* **30** (1991) 4299
17. L. Zhu, N. M. Kostić, *Inorg. Chem.* **31** (1992) 3994
18. L. Zhu, N. M. Kostić, *J. Am. Chem. Soc.* **115** (1993) 4566
19. L. Zhu, N. M. Kostić, *Inorg. Chim. Acta* **217** (1994) 21
20. E. N. Korneeva, M. V. Ovchinnikov, N. M. Kostić, *Inorg. Chim. Acta* **243** (1996) 9
21. T. N. Parac, N. M. Kostić, *J. Am. Chem. Soc.* **118** (1996) 51
22. T. N. Parac, N. M. Kostić, *J. Am. Chem. Soc.* **118** (1996) 5946
23. L. Zhu, N. M. Kostić, *Inorg. Chim. Acta* **339** (2002) 104
24. L. Zhu, L. Qin, T. N. Parac, N. M. Kostić, *J. Am. Chem. Soc.* **116** (1994) 5218
25. H. Hohmann, R. Van Eldik, *Inorg. Chim. Acta* **174** (1990) 87
26. A. K. Covington, M. Paabo, R. A. Robinson, R. G. Bates, *Anal. Chem.* **40** (1968) 700
27. A. Bodansky, M. Bodansky, *The Practice of Peptide Synthesis*, Springer-Verlag, New York, 1984
28. *International Tables for Crystallography*, A. J. C. Wilson, (Ed.), Kluwer Academic Publishing, Boston, 1992
29. F. W. Richards, *J. Mol. Biol.* **82** (1974) 1
30. J. Singh, J. M. Thornton, *Atlas of Protein Side-Chain Interactions* Vol. I + II, Oxford Press, Oxford, 1992
31. R. W. Taft, *Steric Effects in Organic Chemistry*, M. S. Newman, (Ed.). Wiley, New York, 1956
32. R. W. Taft, *Progress in Physical Organic Chemistry*, Vol. 12, Wiley, New York, 1976, and references within
33. P. W. Atkins, J. S. E. Holker, A. K. Holliday, *Correlation Analysis in Chemistry*, Claredon Press, Oxford, 1973
34. N. B. Chapman, J. Shorter, *Correlation Analysis in Chemistry*, Plenum Press, New York, 1978
35. O. Exner, *Correlation Analysis of Chemical Data*, Plenum Press, New York, 1988
36. T. Fujita, C. Takayama, M. Nakajima, *J. Org. Chem.* **38** (1973) 1623
37. D. F. DeTar, *J. Org. Chem.* **45** (1980) 5166
38. M. Charton, *J. Am. Chem. Soc.* **97** (1975) 1552
39. R. W. Taft, *J. Am. Chem. Soc.* **74** (1952) 3120.