the respective rate constants being $k_a = (3.3 \pm 0.8) \times 10^3\ s^{-1}$ and $k_b = (3.6 \pm 0.4) \times 10^{-2}\ s^{-1}$. However, for II, A decayed by second-order kinetics to give another long-lived transient C which is absorbing relative to II at $\lambda > 425$ nm and underwent slow first-order decay to regenerate the initial spectrum.

These observations can be compared to those for III (L = PPh_3).[5] In benzene III was shown[5] to undergo CO photodissociation (eq 2) to give A, which undergoes second-order recombination with CO (eq 3) and competing dimerization to $[RhClL_2]_2$.

$$trans\text{-}RhCl(CO)L_2 \xrightarrow{h\nu} \underset{A}{RhClL_2} + CO \qquad (2)$$

$$A \xrightarrow{k_{CO}[CO]} trans\text{-}RhCl(CO)L_2 \qquad A \xrightarrow{+A} [RhClL_2]_2 \qquad (3)$$

The dimer displayed spectral properties and kinetics similar to those noted above for C. No bleached transient was observed except under H_2, in which case, the formation of the Rh(III) dihydride H_2RhClL_2 was concluded on the basis of spectral properties plus dihydrogen concentration and deuterium isotope effects on the reaction kinetics.

These observations and comparisons indicate that flash photolysis of I or II leads first to CO dissociation (eq 2). This view is reinforced by the observation that when I was flashed in benzene under 1% CO (the balance being argon), the disappearance of A was markedly accelerated ($k_{obsd} = (1.8 \pm 0.5) \times 10^4\ s^{-1}$),[8] but formation of B was about 35% that seen in the absence of CO. However, the decay rate of B back to I was unaffected. Similarly, for II in benzene, added CO accelerated the disappearance of A and suppressed formation of B. In contrast, there was little effect of adding $P(tolyl)_3$ (10^{-3} mol L^{-1}).

Formation of the bleached transient B is consistent with reaction of the highly unsaturated "tricoordinate" 14-electron species A[9] with the hydrocarbon solvent to form Rh(III) alkyl or aryl halide species (eq 4),[10] which are logical intermediates for photocatalytic

$$RhClL_2 + R\text{—}H \xrightarrow{k_a} \begin{matrix} R \\ \diagdown \\ H \end{matrix}\!\!>\!RhClL_2 \qquad (4)$$

functionalization of hydrocarbons above). For L = $P(tolyl)_3$, oxidative addition of benzene to the photogenerated intermediate A is slower and correspondingly B is less stable than for L = PMe_3, both observations consistent with the lower basicity and greater steric bulk of the triarylphosphine ligand. The same factors are consistent with the apparent failure of $RhCl(P(tolyl)_3)_2$ to react with cyclohexane. Remarkably, $P(tolyl)_3$ is enough more electron donating than PPh_3 that oxidative addition of solvent to A (eq 4) is the predominant decay pathway in benzene for L = $P(tolyl)_3$, while eq 3 is predominant for L = PPh_3 under analogous conditions. However, the differences may be rather subtle given that photocatalyzed carbonylation of benzene has also been observed to be a minor pathway in the photolysis of III.[2]

In summary, the flash photolysis of the Rh(I) complexes *trans*-$RhCl(CO)L_2$ (L = PMe_3, $P(tolyl)_3$, or PPh_3) leads to the following observations. (a) In each case, photodissociation of CO occurs to give the tricoordinate intermediate $RhClL_2$. (b) This species undergoes rapid recombination with CO, but, for L = PMe_3, it reacts with either solvent cyclohexane or solvent benzene via C–H activation at rates sufficient to compete with the second-order trapping by the low concentrations of CO generated in the flash.[11] (c) For L = $P(tolyl)_3$, similar, but slower, reaction with solvent is also the predominant decay pathway for A in benzene under these conditions; however, analogous reaction with cyclohexane was not seen. (d) The substituent effect of the *p*-Me group of $P(tolyl)_3$ is apparently sufficient to make C–H activation the predominant reaction of A in benzene. In contrast, the predominant reactions of this intermediate for the PPh_3 complex are second-order recombination with photogenerated CO plus competing dimerization. Continuing studies here regarding Rh(I) complexes are concerned with quantitatively characterizing ligand substituent effects on the photocatalyzed C–H activation of various substrates as well as examining the dynamics of the reactive intermediates formed by such reactions.

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(8) Given the solubility of CO in benzene (6.9×10^{-3} mol L^{-1} atm^{-1}, ref 5a), this k_{obsd} allows the estimate of a k_{CO} value of about 2×10^8 L mol^{-1} s^{-1} for the tricoordinate species $RhCl(PMe_3)_2$. This value is consistent with those measured for other $MClL_2$ (M = Ir or Rh) (ref 5).

(9) In this paper we will follow the practice as referring to the reactive $RhClL_2$ intermediate as being tricoordinate, although it seems likely that the vacant coordination site may be occupied by a weakly coordinating solvent molecule.

(10) The spectral and kinetic behavior of the $RhClL_2$ intermediates (L = PMe_3 or $P(tolyl)_3$) would also be consistent with the formation of orthometalated phosphine ligand complexes. However, this appears to be an unlikely explanation given that the $P(tolyl)_3$ complex shows distinctly different behavior in benzene and in cyclohexane solutions. If orthometalation were the principal reaction of the tricoordinate complexes, the solvent would not be expected to have such an effect.

(11) (a) Notably, the intermediate generated by photodissociation of CO from *trans*-$RhCl(CO)(PMe_3)_2$ appears to be relatively unselective toward reaction with either cyclohexane or benzene. Earlier studies by Bergman and co-workers (ref 11b) suggested a similar low selectivity for the proposed intermediate $Ir(\eta^5\text{-}C_5Me_5)(PMe_3)$ but a significantly higher ratio of the reactivity toward a phenyl C–H bond vs a secondary C–H of an alkane has been suggested (ref 11c) for the proposed intermediate $Re(\eta^5\text{-}C_5H_5)(PPh_3)H_2$. (b) Buchanan, J. M.; Stryker, J. M.; Bergman, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 1537–1550. (c) Jones, W. D.; Maguire, J. A. *Organometallics* **1986**, *5*, 590–591.

Enzymatic Peptidyl α-Amidation Proceeds through Formation of an α-Hydroxyglycine Intermediate

Stanley D. Young* and Paul P. Tamburini

Department of Protein Chemistry
Unigene Laboratories, Inc.
Fairfield, New Jersey 07006

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The peptidyl α-amidating enzyme plays a critical role in the posttranslational bioactivation of many peptide hormones by catalyzing the oxidation of a carboxyl terminal glycine-extended precursor to yield both an α-amidated peptide and glyoxylic acid.[1] The mechanism of this N–C bond cleavage, which requires enzyme-bound copper ions, L-ascorbate, and molecular oxygen, is unknown; however, formation of a carbinolamine intermediate has been suggested.[2] Carbinolamine formation could occur either directly, as favored for dopamine-β-hydroxylase (DBM)[3] and cytochrome P450 catalyzed N-dealkylations,[4] or indirectly from hydrolysis of an imine-type intermediate.[1a] The capacity of a homogeneous α-amidating enzyme to convert either the model substrate *N*-dansyl-Tyr-Val-Gly (**1**) or its *N*- and α-hydroxyglycine analogues to *N*-dansyl-Tyr-Val-NH_2 (**2**) was examined in this

* Author to whom correspondence should be addressed.

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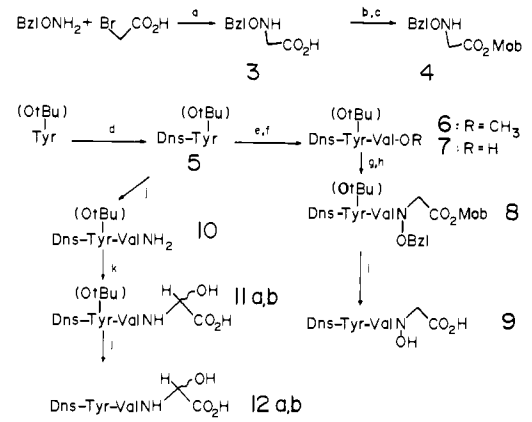
Scheme I[a]



[a] (a) MeOH (84%); (b) Cs_2CO_3 (0.5 equiv), MeOH; (c) $ClCH_2C_6H_4OCH_3$ (Cl-Mob), DMF (38%); (d) DnsCl, $NaHCO_3$, (2 equiv), acetone/water (2:1) (90%); (e) L-valine-OMe·HCl, $(i\text{-}PrN)_2C$, $(i\text{-}Pr)_2EtN$, HOBt, THF (100%); (f) Et_3N/water/MeOH (1:2:2), 50 °C (100%); (g) *i*-BuOCOCl, $(i\text{-}Pr)_2EtN$, CH_2Cl_2, 0 °C; (h) **4**, 0 °C to room temperature (70%); (i) $B(OTf)_3$ (7.7 equiv), TFA; (j) L-valine-NH_2·HCl, $(i\text{-}PrN)_2C$, $(i\text{-}Pr)_2EtN$, HOBt, THF (100%); (k) $OHCCO_2H\cdot H_2O$ (2.2 equiv), acetone, reflux (63%); (l) TFA (100%).

study by using a standard assay.[5] We provide strong evidence that enzymatic peptide α-amidation involves a stereoselective formation of an α-hydroxyglycine intermediate and that this intermediate is then enzymatically further converted to an α-amidated peptide product.

Scheme I details the synthesis of the *N*- and α-hydroxyglycine derivatives of **1**.[6] Bromoacetic acid was reacted with excess *O*-benzylhydroxylamine to obtain **3**.[7] The cesium salt[8] of **3** was then condensed with 4-methoxybenzyl chloride to form the trifluoroacetic acid labile ester **4**.[9] The low yield of **4** was due in part to the poor solubility of the cesium salt of **3** in DMF. *O-tert*-Butyl-L-tyrosine was N-labeled with 5-(dimethylamino)-1-naphthalenesulfonyl chloride (dansyl (Dns)) and then **5** was coupled to either L-valine methyl ester or L-valinamide to form **6** or **10**. The protected *N*-hydroxyglycine tripeptide **8** was formed by coupling **4** with the acid **7** using a mixed-anhydride procedure.[20] The tripeptide **8** was purified by using C18 reverse-phase high-performance liquid chromatography (RP-HPLC)[10] (retention time = 93 min) and then deprotected[7] to **9**. Product **9** was purified by RP-HPLC (retention time = 26 min).

The α-hydroxyglycine derivatives **12a,b** were obtained by condensing the amide **10** with glyoxylic acid[11] and then removing the *O-tert*-butyl protecting group from the tyrosine side chain in TFA.[12] The diastereomeric products **11a,b** formed during the nonstereospecific condensation of glyoxylic acid and **10** were purified as a single peak on RP-HPLC (retention time = 53 min). The diastereomers **12a,b** were subsequently separated by RP-HPLC by excluding a single mixed fraction (retention times: **12a** = 33.6 min, **12b** = 34.2 min). It has not yet been possible to make a stereochemical assignment for the diastereomers **12a** and **12b**. The hydroxyl peptides **9**, **12a**, and **12b** were completely stable for several hours at 25 °C in the pH range 4–10 and for several weeks at −20 °C in 0.1% TFA, 50% CH_3CN (v/v).

The capacity of a homogeneous α-amidating enzyme[13] to convert **1** or its *N*- or α-hydroxyglycine analogues (**9** or **12a,b**) to **2** was compared.[14,15] α-Amidation of **1** required L-ascorbate and exhibited an apparent K_m of 8.2 μM and an apparent V_m of 309 min^{-1} (r = 0.98). Under identical conditions, neither **9** nor **12a** was metabolized. In contrast, **12b** was rapidly converted to **2**. The reaction was independent of L-ascorbate, exhibiting an apparent K_m of 15.6 μM and an apparent V_m of 2930 min^{-1} (r = 0.99) in the presence of 3 mM ascorbate, and an apparent K_m of 12.9 μM and an apparent V_m of 2860 min^{-1} (r = 0.98) in the absence of ascorbate. Although the apparent pH optima for formation of **2** from either **1** or **12b** differed (pH 5.0 and pH 6–6.5, respectively), the conversion of **12b** was significantly faster than the conversion of **1** throughout the pH range 4–9.[17]

The α-amidation enzyme is inhibited by certain metal ion chelators such as 1,10-phenanthroline,[1b] presumably due to interference with molecular oxygen binding and activation. The effects of 1,10-phenanthroline on enzymatic α-amidation of **1** and **12b** were compared (n = 4).[18] The 1,10-phenanthroline completely inhibited the metabolism of **1** (v = 61 ± 10 min^{-1} in the absence of phenanthroline) whereas conversion of **12b** to **2** was essentially unaffected (v = 1050 ± 30 and 795 ± 130 min^{-1}, respectively, in the absence and presence of phenanthroline).

The peptide **12b** fulfills certain important criteria expected for a stable intermediate in the pathway of enzymatic α-amidation of **1**. First, only the diastereomer **12b** was enzymatically converted to **2**. This is consistent with the known stereoselectivity of the α-amidating enzyme catalyzed peptide α-amidation by the oxidation of a C-terminal D-, but not L-, alanine.[19] Second, conversion of **12b** to **2** occurred at rates substantially greater than those observed for the conversion of **1**. While this further implicates **12b** as an intermediate, it does not prove that a rate-limiting step precedes **12b** formation. Since **12b** reversibly binds the enzyme, dissociation of a portion of **12b** formed from **1** would artificially reduce the net rate of formation of **2**. The size of this effect, however, cannot be calculated without an accurate knowledge of the relevant microscopic rate constants, and therefore the positioning of **12b** relative to the rate-limiting enzymatic step is tentative. Third, formation of **2** from **12b** was independent of L-ascorbic acid and unaffected by 1,10-phenanthroline. This shows that the catalytic steps involving the copper center(s), including reduction by L-ascorbate and molecular oxygen binding and activation, precede those steps involved in enzymatic conversion of **12b** to **2**. This is consistent with the catalytic mechanisms operative in other copper-dependent monooxygenases.[3] Studies are in progress to assign the absolute configuration of **12b**.

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(15) Reactions were initiated at 37 °C by addition of α-amidating enzyme preparation to achieve the following component concentrations in 50 mM sodium acetate, pH 5.0: α-amidating enzyme, 0.05–0.25 μg/mL; L-ascorbate, 0.0 or 3.0 mM; *N*-dansyl tripeptide, 0.4–20 μM. At 2.6, 5.3, and 10 min following enzyme addition, aliquots of the reaction mixture were removed and adjusted to 1% TFA (v/v). Apparent kinetic parameters were obtained by using Hanes–Woolf plots[16] of the initial velocities; the correlation coefficients (r) of these plots are given in parentheses.

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(17) Reactions were initiated at 37 °C by enzyme addition to achieve 0.05 μg/mL (for **12b**) or 0.25 μg/mL (for **1**) α-amidating enzyme, 3 mM L-ascorbate, and 30 μM dansyl tripeptide in a buffer comprising 50 mM in each of Tris, acetate, and Bis-tris adjusted to unitary or half-unitary pH values from **4** to 9.

(18) The α-amidating enzyme (1 μg/mL) was preincubated with or without 2 mM 1,10-phenanthroline for 15 min prior to initiating reactions by dilution (25-fold) into a standard assay system containing dansyl tripeptide (10 μM). Reactions were terminated after 10 min. All other conditions were as described.[15]

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