of the barriers, MM2 (0.6 kcal/mol) + ab initio calculated steric and electronic or Fenske-Hall (1.8 or 1.5 kcal/mol), agree, perhaps fortuitously, with experiment (2.2 kcal/mol). The lack of precise crystallographic information on the H_2 location in the PCy_3 compounds does of course lead to some inherent inaccuracy in the comparison to the P-*i*-Pr_3 analogue. For example, the MM2 barrier for the PCy_3 compounds changes from 0.6 kcal/mol for a M-H distance of 1.89 Å (observed in W-P-*i*-Pr_3) to 0.9 kcal/mol for a separation of 1.79 Å, although increasing the distance to 1.99 Å (estimated for the Mo species from ab initio results) gives no change. There is also a small uncertainty in the barrier determined by INS for $Mo(CO)_3(PCy_3)_2(H_2)$ (Table VII) because it depends on the value of the reduced rotational constant B, which in turn depends on the H-H distance. Setting H-H to 0.82 Å, the experimental value for the W-P-*i*-Pr_3 complex, gives 1.7 kcal/mol while employing the 0.79 Å estimate from ab initio calculation gives 1.5 kcal/mol. The latter distance is probably more reasonable because of the known weaker binding of H_2 to Mo than W. Presumably the H-H separation in the W-PCy_3 complex is similar to that in the W-P-*i*-Pr_3 complex.

In summary, it must be noted that the agreement between the calculated and observed barriers to H_2 rotation is remarkably good, particularly in view of the small barrier heights involved and the limitations imposed by the lack of certain structural information. A meaningful detailed analysis of a dynamic process involving such low energies could not have been predicted. Most importantly, the data reveal that the direct electronic binding of the dihydrogen contributes significantly to this barrier, i.e., about half or more of the experimentally determined value. The rotational tunnel splitting is an extremely sensitive measure of this barrier as it depends exponentially[4b] on the value of the barrier. Thus, for example, the observation of a higher barrier (760 vs ~500 cm^{-1}, i.e., stronger M-H_2 binding) in the W complex compared with the Mo analogue is in agreement with a higher M-H_2 infrared stretch (ν_s = 953 cm^{-1} (M = W) versus 871 cm^{-1} (M = Mo)), but the effect on the rotational tunnel splitting is much more pronounced. It is therefore clear that rotational tunneling spectroscopy of η^2-bound H_2 by inelastic neutron scattering can be used as a probe of the electronic details of M-H_2 binding, since the directional properties of the electron wave functions, which help optimize the electron flow ($H_2 \rightarrow M$ σ donation, $M \rightarrow H_2$ σ^* back-donation) between H_2 and metal, also seem to be largely responsible for the barrier to rotation of the dihydrogen. Experimental evidence for metal to dihydrogen σ^* back-bonding is thus attainable as long as steric influences are minimal. The observation of rotational tunneling modes can also be expected to be an unambiguous diagnostic for H_2 binding, especially in complexes for which NMR or crystallographic evidence is tenuous.

Further INS experiments involving solution-state studies and dihydrogen complexes with different ligands and metals have been initiated to gain a more complete picture of the relationship of the rotational barrier to the bonding and ligand dynamics of these systems. Rotational tunneling peaks have been located by INS for iron complexes containing both H_2 and hydride ligands studied by other groups, and rotational barriers have been determined.[31] It is anticipated from preliminary experiments that quasi-elastic neutron-scattering studies will be useful in studying dynamic processes such as intramolecular exchange between H_2 and hydride ligands in these and other complexes containing both types of ligands.

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Copper-Mediated Oxidative C-Terminal N-Dealkylation of Peptide-Derived Ligands. A Possible Model for Enzymatic Generation of Desglycine Peptide Amides

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Abstract: A number of Cu(II) complexes of peptide derivatives that coordinate via N-deprotonation at the C-terminal amino acid residue have been characterized by titrimetry and the Cu(III)-Cu(II) electrochemical potentials. Reaction of these complexes with persulfate induces oxidative decarboxylation and hydrolysis of the resulting *N*-acylimines to carboxamide and either HCHO, CH_3CHO, or acetone depending on the identity of the C-terminal residue (Gly, Ala, or Aib, respectively). For complexes with Cu(III)-Cu(II) potentials of +1.4 V vs NHE or lower, reaction with $IrCl_6^{2-}$ results in C-N dehydrogenation at the C-terminus, giving (after hydrolysis) carboxamide and either glyoxylic or pyruvic acid for C-terminal Gly or Ala. In the case of C-terminal Aib, Ir(IV) oxidation results in a very slow production of acetone. Complexes with E_p above +1.5 V do not react with $IrCl_6^{2-}$, and sarcosine-terminal complexes, as well as those containing phenolate ligation, are inert to both persulfate and Ir(IV). The optimal complex studied here for investigating the mechanism of C-N dehydrogenation was picolinyl-Aib-Ala (E_p = +0.882 vs NHE), in which case the Ir(IV)-mediated cleavage to pyruvic acid and picolinyl-Aib-NH_2 proceeds via a Cu(III) intermediate. The same transformation could be effected electrochemically. The C-terminal oxidative N-dealkylation reaction, induced by Cu(III), has not been previously observed and may be a relevant model for the copper enzyme peptidyl α-amidating monooxygenase, which is responsible for conversion of glycine-extended peptide prohormones to the biologically active peptide carboxamides.

The majority of physiologic secretory polypeptide hormones, as well as many neuropeptides in the central nervous system, are COOH-terminal *amides*,[1] examples being gastrin, choleocystokinin, α-MSH, calcitonin, vasopressin, secretin, and certain

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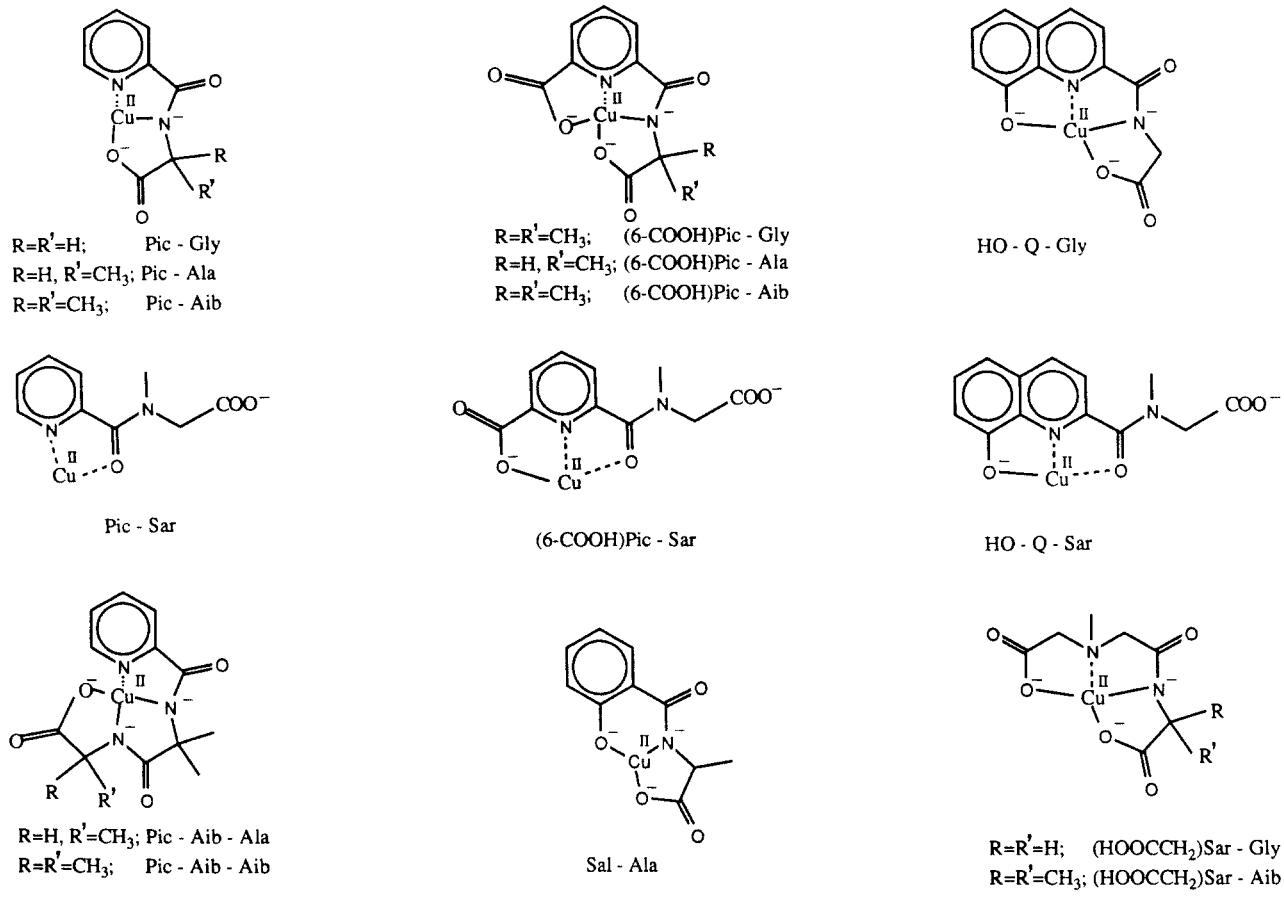
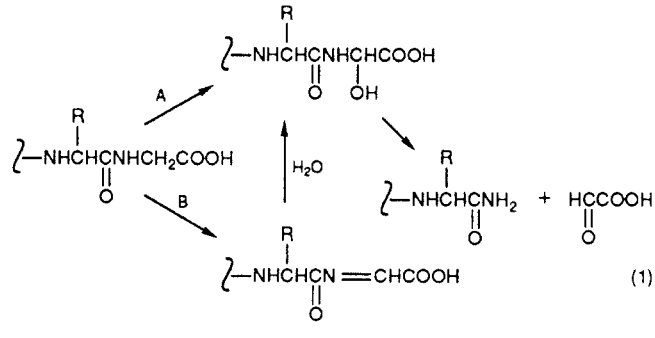


Figure 1. Proposed structures for Cu(II) complexes in solution.

enkephalin peptides.[2] These peptides are derived from large precursor proteins by a two-step posttranslational processing sequence that features the *C-terminal glycine-extended* sequence as the penultimate prohormone. Several investigators have confirmed the presence of specific enzymes in secretory granules that convert the glycine-extended peptides to the biologically active carboxamides.[3,4] Known at one time as "glycine-directed amidases", these enzymes have recently been shown to involve not an ammonia-for-glycine replacement but an oxidative N-dealkylation within the terminal glycine, retaining the glycine N and releasing the remaining C_2 unit as glyoxylate.[3] This enzyme class, now known as peptidyl α-amidating monooxygenase (PAM),[5] has been demonstrated to require copper, O_2, and ascorbate or similar ancillary two-electron reductant for reduction of the "second" oxygen atom to water,[3,4] reminiscent of dopamine β-monooxygenase (DBM).

The stoichiometry appears to be that shown in eq 1, but two very different mechanisms can be proposed: monoxygenation at the α-C to give a carbinolamide that subsequently dissociates to products (path A) or a dehydrogenation at the C–N position followed by hydrolysis (by way of the same carbinolamide) (path B). Path A corresponds to a typical mixed-function oxidase



(1)

stoichiometry characteristic of DBM and other monooxygenases. In contrast, path B corresponds to a tandem dehydrogenation of one substrate and one auxiliary reductant with reduction of O_2 to 2 mol of H_2O, a mechanism that has not been observed for any other redox metalloenzyme. Thus, although path A seems to be a more logical choice for the enzymatic mechanism, path B is not chemically unreasonable, since the facile oxidation of ascorbate concomitant with partial reduction of O_2 may yield a species with the high oxidation potential necessary to effect dehydrogenation of the substrate. Experimental distinction between the two possible mechanisms for the enzyme is difficult. An ^{18}O-labeling study to trace whether O_2 oxygen ends up in glyoxylic acid, as required for the path A mechanism, is precluded owing to the rapid ex-

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(5) Abbreviations used: *N*-(6-carboxypicolinyl)alanine, (6-COOH)Pic-Ala; *N*-(6-carboxypicolinyl)-α-aminoisobutyric acid, (6-COOH)Pic-Aib; *N*-(6-carboxypicolinyl)sarcosine, (6-COOH)Pic-Sar; 2,4-dinitrophenylhydrazine, 2,4-DNP; dopamine β-monooxygenase, DBM; *N*-(8-hydroxyquinolinyl-2-formyl)glycine, HO-Q-Gly; *N*-(8-hydroxyquinolinyl-2-formyl)-sarcosine, HO-Q-Sar; peptidyl α-amidating monooxygenase, PAM; *N*-picolinylalanine, Pic-Ala; *N*-picolinyl-α-aminoisobutyric acid, Pic-Aib; *N*-picolinylglycine, Pic-Gly; *N*-picolinylsarcosine, Pic-Sar; *N*-picolinyl-α-aminoisobutyrylalanine, Pic-Aib-Ala; *N*-picolinyl-α-aminoisobutyryl-α-aminoisobutryic acid, Pic-Aib-Aib; *N*-salicyloylalanine, Sal-Ala.

change of the aldehyde oxygen via the hydrate $HOOCCH(OH)_2$.

The well-known ability of Cu(II) to induce deprotonation of amides in chelate complexes[6] suggested to us that the PAM reaction may be initiated by coordination of the C-terminal amide position of peptide substrates to the active site copper. In this regard, chemical precedent exists for the path B mechanism in the work of Margerum[7,8] and others[9] who showed that the generation of Cu(III) in oligopeptide–Cu(II) complexes, which involve multiple amido coordination, results in dehydrogenation across one or more internal C_α–N bonds. Although these workers did not observe dehydrogenation at the C-terminal position, we considered that a selective C-terminal N-dealkylation could be enforced by the enzyme if the coordination of the peptide substrate to the copper center were restricted to the C-terminal amide position. It is not known whether substrate–copper coordination occurs in the PAM reaction, but if so, a Cu(III)-mediated C-terminal N-dealkylation mechanism could be relevant to the PAM enzyme.

Our strategy for developing model systems is to employ peptide-derived complexes that enforce C-terminal N-dealkylation either by confining amido ligation to the C-terminal residue or by blocking C–N cleavage at any "internal" amido positions with geminal dimethyl C_α-substitution. By limiting the reaction to a single cleavage site, these models permit a detailed look at the oxidation reaction mechanism. In some cases we employed a C-terminal glycine for relevance to the PAM enzyme, but more often we utilized a C-terminal alanine, where the expected product would be pyruvate rather than glyoxylate. Since Margerum has observed the ability of Cu(III) complexes to catalyze oxidative decarboxylation of glyoxylate (to CO_2),[10] our utilization of a C-terminal alanine seemed optimal for minimizing side reactions that could mask our ability to assess the C–N dehydrogenation reaction of interest.

For the 1:1 complexes of our model peptides with Cu(II), we measured the Cu(III)–Cu(II) potentials and determined the products arising from either treatment with external one-electron oxidants or, in some cases, electrochemical oxidation. Our results indicate that the desired oxidative N-dealkylation does occur but that a competing oxidative decarboxylation can also occur, depending on the substrate and choice of external oxidant.[11]

Results

The peptide-derived ligands used in this study were synthesized with standard peptide coupling and protection protocols. We made no attempt to obtain crystalline Cu(II) complexes of the various ligands, but we have characterized the complexation by potentiometric titration and voltammetry (Table I). The titrimetric results indicate that chelation-promoted amide N–H deprotonation by Cu(II) occurs at relatively low pH values, as expected. For some complexes, a high degree of cooperativity of multiple proton loss is observed, in which cases discrete pK^H values could not be discerned. This behavior is typical for systems of this type,[12] where the possibility for chelation arises only when two or more positions undergo deprotonation. In certain cases the free ligands were also titrated in order to assess directly the pK^H-lowering effect of Cu(II) coordination. In the case of Pic-Sar, which lacks a titratable N–H, Cu(II) had a negligible effect on the measured pK^H, as expected.

Table I. Titrimetric and Electrochemical Data

ligand or complex	pK^H (no. of H^+)[a]	E_p (NHE),[b] V
Pic-Gly–Cu(II)	3.08 (2)	1.63
Pic-Gly	3.85 (1)	
Pic-Ala–Cu(II)	2.94 (2)	1.59
Pic-Aib–Cu(II)	2.90 (2)	1.53
Pic-Aib	4.22 (1)	
Pic-Sar–Cu(II)	3.36 (1)	~1.84
Pic–Sar	3.30 (1)	1.63
Sal-Ala–Cu(II)	3.35 (1), 4.25 (1), 5.03 (1)[c]	1.04
HO-Q-Gly–Cu(II)	3.79 (1), 5.53 (1), 6.37 (1)	0.99
HO-Q-Sar–Cu(II)	3.83 (1), 6.76 (1)	1.06
(6-COOH)Pic-Gly–Cu(II)	3.20 (2), 5.48 (1)	1.40
(6-COOH)Pic-Ala–Cu(II)	3.25 (2), 5.61 (1)	1.36
(6-COOH)Pic-Aib–Cu(II)	3.14 (2), 5.34 (1)	1.33
(6-COOH)Pic-Sar–Cu(II)	3.12 (2)	>1.8
$(HOOCCH_2)$Sar-Gly–Cu(II)		1.36
Pic-Aib-Ala–Cu(II)	3.17 (1), 5.10 (1), 6.23 (1)[d]	0.88
Pic-Aib-Ala–Ni(II)	3.70 (1), 7.87 (2)	
Pic-Aib-Aib–Cu(II)	3.30 (1), 3.80 (1), 6.48 (1)[d]	0.82 (1.58)
Pic-Aib-NH_2–Cu(II)	3.50 (1), 7.37 (1)	0.88 (~1.79)

[a] $pK^H = pK_a$ in the case of the free ligands or the apparent pK_1, pK_2, etc., for stepwise deprotonation of the Cu(II) complexes. The conditions were 25 °C, with 1.6–3.3 mM solutions of the ligand in the absence or presence of an equimolar concentration of $Cu(NO_3)_2$. [b] From Osteryoung square-wave voltammetry at a glassy carbon electrode; ΔE_{sw} = 25 mV; ΔE_s = 4 mV; f = 15 Hz. [c] Potentiometric titration of Sal-Ala has been performed previously in the presence of up to only 0.2 equiv of Cu(II): Mukherjee, G. N.; Sarkar, S. *Indian J. Chem.* **1983**, 539. In this case, a bis complex is formed utilizing bidentate amido and carboxylate ligation and deprotonation of the phenolic hydroxyl is claimed to occur only at high pH (8–12). [d] The final deprotonation is accompanied by a color change from blue to pink.

Solutions of equimolar mixtures of Cu(II) and the peptide were studied electrochemically at pH values above the final deprotonation pK^H. For the complexes of Pic-Aib-Ala and Pic-Aib-Aib, where the measured Cu(III)–Cu(II) potentials were below 1 V, the peak anodic and cathodic potentials were separated by 72 and 74 mV, respectively, at a scan rate of 100 mV/s, indicating close to electrochemical reversibility for a one-electron oxidation process. A quasi-reversible cyclic voltammogram was obtained for the complex of Pic-Aib-NH_2, but for the remaining complexes only irreversible waves were observed, even at fast sweep rates. The Cu(III)–Cu(II) peak potentials (E_p) obtained by Osteryoung square-wave voltammetry are listed in Table I. For two of the complexes, a second oxidation wave of unknown origin was observed at high potential (values listed in parentheses). The data for the the picolinyl and 6-carboxypicolinyl systems indicate a progressive decrease in E_p with increasing methyl-for-hydrogen replacement at C_α in the terminal peptide unit. Both the titrimetric and electrochemical trends are in keeping with the published work of Margerum.[8,13] The attribution of these potentials to the amido–Cu(II) coordination is further verified by the large increase in the measured potential that occurs upon replacement of the terminal amino acid with sarcosine. In fact, for Pic-Sar, the E_p is actually lower for the free ligand than it is in the presence of 1 equiv of Cu(II).

The reactions of the Cu(II) complexes with external oxidants at various selected pH conditions are listed in Table II. Although these reactions were generally conducted in open-air vessels, a number of reactions carried out under N_2 were found to give identical results. For the simple picolinyl derivatives of Gly, Ala, Aib, and Sar, binding to Cu(II) in a 1:1 stoichiometry was insufficient to prevent precipitation of copper hydroxides at higher

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Table II. Products of Reaction of the Cu(II) Complexes with External Oxidants

ligand (L)	reactant ratio Cu^{II}:L:oxidant	pH	oxidant	reaction time,[a] h	N–C cleavage products (% yield)[b]
Pic-Ala	1:1:1.5	10.5 (borate)	$K_2S_2O_8$	24	CH_3CHO
Pic-Ala	1:1:1.5	8–10.5 (NaOH)	$K_2S_2O_8$	24	CH_3CHO
Pic-Ala	1:1:1.5	7.0 (NaOH)	$K_2S_2O_8$	36	CH_3CHO (30)
Pic-Ala	1:1:1.5	10.5 (NH_4OH)	$K_2S_2O_8$	24	CH_3CHO
Pic-Ala	1:1:1.5	7.74 (NH_4OH)	$K_2S_2O_8$	72	CH_3CHO (38)
Pic-Ala	0:1:1.5	8.0 (NH_4OH)	$K_2S_2O_8$	48	no cleavage
Pic-Ala	1:1:10	11.0 (borate)	Na_2IrCl_6	24	no cleavage
Pic-Gly	1:1:1.5	6.4 (NaOH)	$K_2S_2O_8$	36	HCHO (25)
Pic-Gly	1:1:1.5	9.0 (NH_4OH)	$K_2S_2O_8$	24	HCHO
Pic-Sar	1:1:1.5	11.0 (NH_4OH)	$K_2S_2O_8$	24	no cleavage
Pic-Aib	1:1:1.5	11.9 (NH_4OH)	$K_2S_2O_8$	16	CH_3COCH_3 (46)
Pic-Aib	0:1:1.5	11.5 (NH_4OH)	$K_2S_2O_8$	24	no cleavage
Sal-Ala	1:1:1.5	8.0 (NH_4OH)	$K_2S_2O_8$	72	no cleavage
HO-Q-Gly	1:1:1.5	9.0 (NH_4OH)	$K_2S_2O_8$	72	no cleavage
HO-Q-Sar	1:1:1.5	9.0 (NH_4OH)	$K_2S_2O_8$	72	no cleavage
(6-COOH)Pic-Gly	1:1:1.5	9.0 (borate)	$K_2S_2O_8$	24	HCHO
(6-COOH)Pic-Gly	1:1:2	9.0 (borate)	Na_2IrCl_6	24	HCOCOOH
(6-COOH)Pic-Ala	1:1:1.5	8.25 (NaOH)	$K_2S_2O_8$	36	CH_3CHO (48)
(6-COOH)Pic-Ala	1:1:4	11.0 (borate)	Na_2IrCl_6	24	$CH_3COCOOH$
(6-COOH)Pic-Aib	1:1:1.5	11.0 (borate)	$K_2S_2O_8$	24	CH_3COCH_3
(6-COOH)Pic-Aib	1:1:2	11.0 (borate)	Na_2IrCl_6	24	CH_3COCH_3 (tr)
(6-COOH)Pic-Sar	1:1:1.5	8.0 (NaOH)	$K_2S_2O_8$	48	no cleavage
$(HOOCCH_2)$Sar-Gly	1:1:1.5	9.7 (NaOH)	$K_2S_2O_8$	36	HCHO (44%)
$(HOOCCH_2)$Sar-Gly	1:1:2	11.0 (borate)	Na_2IrCl_6	24	HCOCOOH, HCHO
Pic-Aib-Ala	1:1:2	8.5 (borate)	Na_2IrCl_6	24	$CH_3COCOOH$
Pic-Aib-Ala	1:1:2	10.5 (borate)	Na_2IrCl_6	24	$CH_3COCOOH$ (25%[c])
Pic-Aib-Ala	1:1:1.5	10.5 (borate)	$K_2S_2O_8$	24	CH_3CHO (10%)
Pic-Aib-Ala	1:1:3	10.5 (borate)	$K_2S_2O_8$	24	CH_3CHO (59%)
Pic-Aib-Ala	1:1:3	8.5 (borate)	aq H_2O_2	24	CH_3CHO (tr)
Pic-Aib-Ala	1:1:2	11.0 (borate)	$K_3Fe(CN)_6$	24	CH_3CHO (tr)
Pic-Aib-Aib	1:1:2	11.3 (borate)	Na_2IrCl_6	6	CH_3COCH_3 (tr)
Pic-Aib-Aib	1:1:1.5	11.5 (NaOH)	$K_2S_2O_8$	48	CH_3COCH_3 (28%)

[a] Carried out at 25 °C in air. Several reactions listed were also conducted under N_2 and gave identical results. [b] Products were qualitatively determined by TLC (with visualization by 2,4-DNP reagent) and by NMR of the isolated 2,4-dinitrophenylhydrazone. Quantification was carried out in some cases as indicated by the yields in parentheses; these values also indicate the determined yield of carboxamide product (which differed by less than 5%). The designation tr indicates that no carboxamide product could be detected and that the C_α cleavage product could be detected in trace amounts on TLC as the 2,4-dinitrophenylhydrazone but not by NMR. [c] This represents the yield of carboxamide product, which could be raised by increasing the amount of Ir(IV) used (e.g., to 4 equiv). The yield of pyruvic acid, as determined by the 2,4-DNP method, was never higher than 20% and decreased when excessive Ir(IV) was employed (see Results).

pH values desirable for ensuring complete N–H deprotonation. Initially, a variety of ancillary ligands were employed with the aim of achieving solubility at pH values up to 11.5. The ligands used were EDTA, nitrilotriacetic acid, tricarballylic acid, citric acid, tartaric acid, or oxalic acid, added in equimolar amounts, or pyridine, aniline, pyrazole, imidazole, or sodium acetate, added in equimolar or greater amounts. This approach was eventually abandoned because either (i) the ligand did not enhance solubility, (ii) the ligand was oxidized under the reaction conditions, or (iii) the ligand catalyzed the decarboxylation of glyoxylic or pyruvic acids to CH_2O or CH_3CHO, respectively. The latter decarboxylation was also observed in experiments where NH_4OH rather than aqueous NaOH was used to achieve homogeneous solution at high pH. In the NH_4OH case, although the *initial* fate of C_α in the oxidative cleavage (α-oxo acid or aldehyde) could thus not be evaluated, the efficiency of the reaction was nonetheless determinable by assessing the yield of the aldehyde produced and/or of the carboxamide product.

In other experiments listed in Table II with the simple picolinyl–amino acid complexes, NaOH or borate buffer was used directly, even though some precipitate was present, in order to assess the fate of C_α. These experiments permitted us to conclude the oxidative decarboxylation is the initial outcome in the case of persulfate. With the weaker oxidant $IrCl_6^{2-}$, no reaction could be effected under any condition.

For the remaining ligands in our study, involving more extended coordination to copper, no precipitation problems were encountered and lower Cu(II)–Cu(III) potentials were achieved. Again, only oxidative decarboxylation was observed when persulfate was used as external oxidant. Thus, acetone was obtained from Pic-Aib-Aib and (6-COOH)Pic-Aib; more importantly, CH_3CHO was obtained exclusively from Pic-Aib-Ala and (6-COOH)Pic-Ala and HCHO from (6-COOH)Pic-Gly. For the latter three systems, and in contrast to the simple picolinyl complexes discussed above, $IrCl_6^{2-}$ was capable of effecting oxidation. In these Ir(IV) reactions, the outcome was exclusively C–N dehydrogenation rather than oxidative decarboxylation, generating glyoxylate instead of HCHO and pyruvate instead of CH_3CHO.

In selected experiments we carried out quantitative analyses to relate the yield of carboxamide product (using NMR) to the yield of the C_α-derived cleavage product (using 2,4-DNP). Regardless of whether an oxidative decarboxylation or a dehydrogenation reaction was observed, the two yields were generally found to be comparable, as required, and the average values are listed in parentheses in Table II. However, even though the external oxidant was used in an amount theoretically sufficient to convert all the starting complex, the product yields were generally in the range of 25–50%. Apparently, the oxidatns are destroyed by disproportionation or other redox processes that become especially prominent at higher pH in the case of Ir(IV).[13a] Nonetheless, no side products derived from the peptide substrates were observed in our reactions, and thus the percent conversion to C–N cleavage products could be raised to some extent by increasing the amount of external oxidant employed. One limiting factor is the known oxidation of α-oxo acids by Ir(IV),[9a] such that the use of excess $IrCl_6^{2-}$ increased the yield of carboxamide product but did not significantly improve the yield of α-oxo acid product.

When $IrCl_6^{2-}$ was added to the Cu(II) complexes of Pic-Aib-Aib and (6-COOH)Pic-Aib, where C–N dehydrogenation is precluded, oxidative decarboxylation to acetone was observed as with persulfate, but in trace amounts that were detectable only by TLC as the 2,4-dinitrophenylhydrazone. This result indicates that the reaction leading to acetone in these cases is very slow and can barely compete with the nonproductive decomposition of the Ir(IV)

oxidant. We also attempted to effect oxidation of the Cu(II) complex of Pic-Aib-Ala with hydrogen peroxide[14] and $Fe(CN)_6^{3-}$; very little cleavage product was obtained, and this was identified as CH_3CHO rather than pyruvic acid.

For the ligand derived from (*N*-methylimino)diacetic acid, $(HOOCCH_2)$Sar-Gly, both HCHO and HCOCOOH were observed in the case of Ir(IV) oxidation, suggesting that oxidative decarboxylation was competing with C–N dehydrogenation in this case. However, we found that the related ligand $(HOOCCH_2)$Sar-Aib also gave HCHO upon reaction with $IrCl_6^{2-}$, clearly an unexpected result in view of the sluggish reactivity of the other Aib-terminal ligands with Ir(IV), and which gave acetone, not HCHO. Since we then found that the Cu(II) complex of (*N*-methylimino)diacetic acid itself also gives HCHO upon reaction with $IrCl_6^{2-}$ (results not shown), we concluded that the source of HCHO in the other experiments was the carboxymethyl arms attached to the tertiary amine nitrogen. In retrospect, these results are not surprising in view of the well-known ability of Cu(II) to catalyze oxidative decarboxylation of amino acids.[15] For this reason, (carboxymethyl)amino groups should not be used as ancillary ligand moieties in the construction of model systems aimed at observing the amido N-dealkylation of interest.

The phenolate-derived systems HO-Q-Gly and Sal-Ala exhibited no oxidative cleavage reactions, even with persulfate. In addition, bulk electrolysis of these Cu(II) complexes failed to reveal any alteration in the ligand. Since the redox potentials of these systems were lower than those for the 6-carboxypicolinyl system, where cleavage was observed, we suspected that the group undergoing oxidation was the Cu(II)-bound phenolate rather than the amido–Cu(II) site. This suspicion is supported by our finding that the Cu(II) complex of HO-Q-Sar, which lacks the crucial amide N–H, exhibits a potential that is essentially identical with those of HO-Q-Gly and Sal-Ala. These experiments indicate that phenolate donors are inappropriate in the design of desirable model systems for the amido N-dealkylation reaction.

The relevance of the reactions studied here to the PAM enzymatic process is postulated in terms of ligand degradation mediated by high-potential copper. Thus, it was essential to determine whether the reaction of the Cu(II)–peptide complexes with external oxidants involved (i) initial oxidation of the Cu(II) center to Cu(III) followed by a Cu(III)-mediated internal ligand oxidation or (ii) a direct oxidation of the Cu(II)-bound ligand by the external oxidant. We found that the free-ligand systems are not oxidized by $IrCl_6^{2-}$ or $S_2O_8^{2-}$, even at basic pH. Clearly, however, the uncoordinated ligands are not nearly as reducing as the N-deprotonated Cu(II)-bound ligands. We also found that the Ni(II) complex of Pic-Aib-Ala, which forms at somewhat higher pH than does the corresponding Cu(II) complex (Table I), undergoes oxidative decarboxylation to CH_3CHO upon treatment with persulfate but does not react with $IrCl_6^{2-}$. This suggests that the persulfate-induced oxidative decarboxylation is dependent only on metal ion coordination-induced N–H deprotonation at the amido C-terminus, but that the Ir(IV)-induced C–N dehydrogenation is selectively facilitated by Cu(II), consistent with a role of Cu(III) in the latter reaction.

We made no attempt to observe the intermediacy of Cu(III) for those complexes with high Cu(III)–Cu(II) potentials, where the lifetime of the Cu(III) state, if it were to form, would be very short prior to ligand oxidation. However, for the complexes with lower potentials, we expected to observe Cu(III), especially for the Aib-terminal ligands, where C–N dehydrogenation cannot occur. The generation of Cu(III) in the $IrCl_6^{2-}$ oxidations was confirmed for Pic-Aib-Aib and Pic-Aib-Ala. In the former case, the Cu(III) state (λ_{max} = 378 nm) was long-lived, with a $t_{1/2}$ of about 34 h at 10 °C, pH 8. The persistence of Cu(III) in the Pic-Aib-Aib complex is consistent with the inability of the ligand to undergo C–N dehydrogenation, and the Cu(III) state eventually decays through the apparently much slower oxidative decarboxylation pathway. Under the same conditions, the Cu(III) complex of Pic-Aib-Ala (λ_{max} = 378 nm) had $t_{1/2}$ = 3 min. The rapid disappearance of Cu(III) in this case is understood in terms of its ability to undergo a rapid C–N dehydrogenation. For the persulfate oxidations, we were unable to observe a Cu(III) absorbance even in the case of Pic-Aib-Aib. No other evidence implicating the generation of Cu(III) in the persulfate oxidations was obtained.

The most well-behaved system for observing the C–N dehydrogenation reaction of interest was Pic-Aib-Ala, and it was this Cu(II) complex for which we successfully achieved the same transformation through electrochemical means. Constant-potential electrolysis was carried out at 750 mV vs SCE with pH adjusted to 8.25, 8.5, and 10.7. In the former two cases, the current was low and no pyruvic acid was detected. However, at pH 10.7, the current decreased exponentially, and TLC run on aliquots of the reaction indicated the production of pyruvic acid. The electrolysis was stopped after 4 h, at which point the current ratio had decreased to 9.7% and the solution had turned from plum to a golden yellow. The calculated charge consumed accounted to 2.79 electrons, which is consistent with an overall two-electron oxidation of Pic-Aib-Ala to pyruvic acid and Pic-Aib-NH_2, coupled with oxidation of the Cu(II) complex of the latter to the corresponding Cu(III) complex at the electrolysis potential. The spectrum of the final complex (λ_{max} = 398 nm) was identical with that obtained independently upon bulk electrolysis of the Cu(II) complex of Pic-Aib-NH_2 at pH 9.0, which yielded a loss of 0.7 electron.

Discussion

The aim of the current study was to generate Cu(II)–peptide-like complexes that would undergo an oxidative N-dealkylation at the C-terminal amino acid when oxidized chemically or electrochemically. These systems were designed as models for a copper-containing enzyme of much current focus, peptidyl α-amidating monooxygenase (PAM), which is responsible for the production of peptide carboxamide hormones from the C-terminal glycine-extended precursors. Oxidative dealkylation of Cu(II)–peptide complexes was observed many years ago by Levitzki, Anbar, and Berger,[9] who showed that oxidation of tetra- and pentaalanine-bound Cu(II) to Cu(III) by $IrCl_6^{2-}$ was accompanied by oxidative cleavage of the C_α–N bond at the third amino acid from the N-terminus, producing Ala-Ala-NH_2 and a pyruvoyl peptide fragment (eq 2). A few years later, Margerum and

$$Cu^{II}(AlaAlaAlaAla) \xrightarrow{[O]} [Cu^{III}(AlaAlaAlaAla)] \longrightarrow AlaAla\text{-}NH_2 + O{=}C(CH_3)C(=O)Ala \quad (2)$$

$$Cu^{II}(Ala_5) \xrightarrow{[O]} [Cu^{III}(Ala_5)] \longrightarrow AlaAla\text{-}NH_2 + O{=}C(CH_3)C(=O)AlaAla$$

co-workers observed that certain Cu(II)–oligopeptide complexes reacted directly with O_2 in the dark, according to a complicated series of reactions that generated Cu(III) and organic radicals.[16] In this case, the observed N-dealkylation could have arisen at least in part from oxygenation at C_α. At the same time, however, these workers confirmed that the more general Ir(IV)-mediated oxidations of Cu(II)–oligopeptide complexes involved discrete Cu(III) intermediates and that the same reactions could be effected electrochemically.[7,8] Since the Ir(IV) and electrochemical oxidations occurred anaerobically, a mechanism for N-dealkylation involving C_α–N dehydrogenation followed by hydrolysis appears certain.

(14) H_2O_2 has been used to oxidize macrocyclic amido–Cu(II) complexes to the corresponding Cu(III) species: Hay, R. W.; Bembi, R.; Sommerville, W. *Inorg. Chim. Acta* **1982**, *59*, 147.

(15) Reddy, K. B.; Sethuram, B.; Rao, T. N. *Indian J. Chem.* **1978**, *16A*, 31. Reddy, M. G. R.; Sethuram, B.; Rao, T. N. *Indian J. Chem.* **1978**, *16A*, 591. Beileryan, N. M.; Chshmarityan, D. G. *Kinet. Katal.* **1973**, *15*, 1400.

(16) Burce, G. L. Paniago, E. B.; Margerum, D. W. *J. Chem. Soc., Chem. Commun.* **1975**, 261. Kurtz, J. L.; Burce, G. L.; Margerum, D. W. *Inorg. Chem.* **1978**, *17*, 2454.

In all these previous studies, the preferred C–N cleavage at the *third amino acid residue from the amino terminus*, regardless of oligopeptide length, appears to reflect amino-terminal anchoring of the peptide–copper chelate, though it was not clear what factors controlled the cleavage regiochemistry. In any case, the fact that no oxidative N-dealkylation was observed at the C-terminal residue suggests that cleavage at this latter position is energetically more difficult, probably on account of differing electronic effects between CO_2^- and CONHR. It should be noted that Margerum and co-workers did observe oxidation at the C-terminus in two cases: (1) photochemical degradation of Cu(III)–oligopeptide[7b,13b,17] and (2) thermal degradation of Ni(III)–oligopeptide complexes.[18] However, both of these reactions involved oxidative decarboxylation, not N-dealkylation (i.e., yielding CO_2 and HCHO rather than HCOCOOH for C-terminal glycine). Thus, the major advance of the current study over the previously published work is the unprecedented achievement of a *C-terminal oxidative N-dealkylation* that occurs upon chemical or electrochemical generation of C-terminal amido–Cu(III) complex. The key to this accomplishment was the elimination of the potential occurrence of oxidative N-dealkylation at "internal" sites.

The selective Ir(IV) and electrochemically mediated cleavage of Cu(II)–Pic-Aib-Ala to pyruvic acid and Pic-Aib-NH_2 reported in the current study proceeds via an observable Cu(III) intermediate. Replacement of the C-terminal Ala by Aib results in stabilizing the Cu(III) intermediate, and the only cleavage reaction that can now occur, oxidative decarboxylation, proceeds at a barely detectable level. In contrast, persulfate readily effects oxidative decarboxylation of the Cu(II) complexes of both Pic-Aib-Ala (giving CH_3CHO) and Pic-Aib-Aib (giving acetone), and in neither case is a Cu(III) intermediate spectroscopically observable. Similar chemistry occurs for the 6-carboxypicolinyl system. For this simple picolinyl–amino acid complexes, the Cu(III)–Cu(II) potentials are apparently out of reach of the Ir(IV) oxidant, but persulfate still manages to effect oxidative decarboxylation.

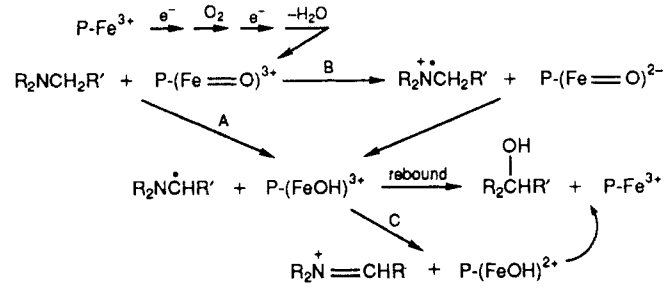
Persulfate oxidations were not observed in the absence of metal ions, and it is known that metal ion redox catalysis (e.g., by copper, eq 3) is an important initiating event for persulfate oxidations to proceed at reasonable rates.[19] The oxidative decarboxylations

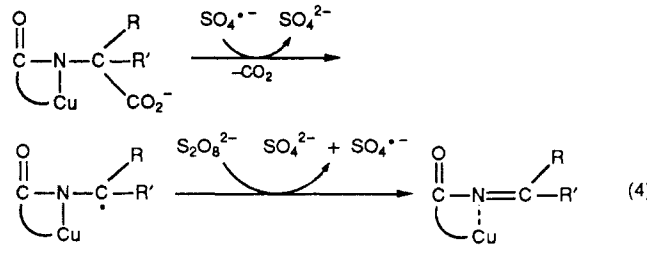
$$S_2O_8^{2-} + Cu(II) \rightarrow SO_4^{2-} + SO_4^{\bullet-} + Cu(III) \qquad (3)$$

induced by persulfate in the presence of Cu(II) cannot involve simple one-electron oxidation of the terminal carboxylate by the oxidant ($SO_4^{\bullet-}$ or Cu(III)), since the C-terminal Sar systems were found to be inert. Clearly, the oxidative decarboxylation is mediated by metal ion–amido coordination at the C-terminus. This requirement, for amido coordination coupled with the accepted generation of Cu(III) in persulfate oxidations,[20] would be consistent with the reaction proceeding via a Cu(III)–ligand intermediate,[21] which would have to be short-lived since it is not observed spectroscopically. However, it is difficult to envision how such an intermediate would differ from the Cu(III) intermediate proposed in the Ir(IV) oxidations, a crucial point, since the two reactions give distinct product outcomes.

Our best explanation at this time for the persulfate oxidations is a chain mechanism (eq 4) involving direct attack of $SO_4^{\bullet-}$ on the carboxylate of the Cu(II)-bound N-deprotonated ligand, the latter being particularly activated toward a concerted one-electron oxidative decarboxylation directly to a C_α-based radical stabilized by the adjacent *anionic* RCON$^-$ group.[22] The inertness of the Sar-terminal ligands would thereby arise because such radical would be flanked by an insufficiently stabilizing *neutral* RCON(CH$_3$) group. Although the oxidative decarboxylation process is of significant mechanistic interest, it has no relevance to the Cu(III)-mediated C–N dehydrogenation reaction proposed as a possible model for the PAM enzymatic mechanism.

Scheme I





(4)

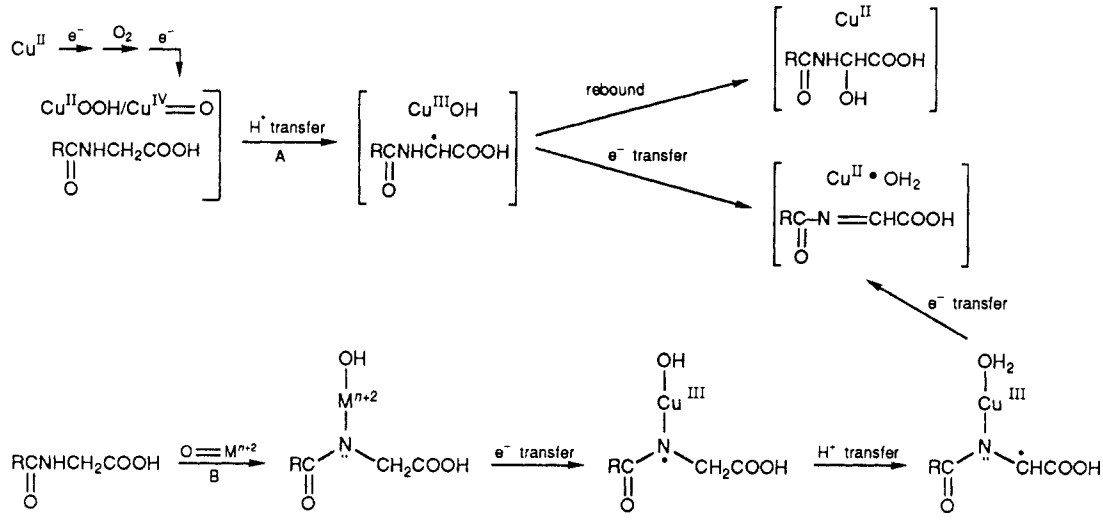
Very little is known about the active-site mechanism of the PAM reaction. The classification of this enzyme as a monooxygenase presumes that the enzyme functions as a mixed-function oxidase, incorporating 1 atom of O_2 into the substrate and reducing the other to water concomitant with oxidation of the auxiliary ascorbate substrate. The essential requirement for copper is ascribed to binding and activation of O_2,[3] as in the case of DBM.[23]

Enzymatic monooxygenation at aliphatic carbon is best characterized for cytochrome P-450.[24] The PAM reaction is closely analogous to the P-450-mediated dealkylation of amines, which generates intermediate carbinolamines.[25] This reaction is thought to involve, after two-electron reductive processing of O_2 to generate the active oxidant (Scheme I), either H atom abstraction from C_α (path A) or electron transfer[26] followed by proton transfer (path B), in both cases giving the same α-amino carbon radical. Although completion of the reaction by hydroxyl radical transfer from iron to the carbon radical (so-called "rebound"[27]) can be envisioned, the intermediate α-amino radical is so reducing that, in this case, simple one-electron oxidation to the iminium cation (path C) appears more likely,[25] in which case the reaction would be completed by hydroxide anion transfer.

In the case of PAM, whether the active oxidant generated upon two-electron processing of O_2 /is $Cu^{II}OOH$ or a high-potential copper–oxo species can only be surmised, but a reasonable mechanistic framework is depicted in Scheme II. Amides have a relatively high redox potential, and thus an initial H atom

(17) Hamburg, A.; Margerum, D. W. *Inorg. Chem.* **1983**, *22*, 3884.
(18) Bossu, F. P.; Paniago, E. B.; Margerum, D. W.; Kirksey, S. T., Jr.; Kurtz, J. L. *Inorg. Chem.* **1978**, *17*, 1034.
(19) Sosnovsky, G.; Rawlinson, D. J. In *Organic Peroxides*; Swern, D., Ed.; Wiley: New York, 1971; Vol. II, p 269.
(20) Cu(II)–$S_2O_8^{2-}$ reactions appear to involve Cu(III) in many cases: Anbar, M. *Adv. Chem. Ser.* **1965**, *49*, 126. Bacon, R. G. R.; Hill, H. A. O. *Q. Rev. Chem. Soc.* **1965**, *19*, 95. However, many Cu(II)–$S_2O_8^{2-}$ reactions appear to involve direct le oxidation of substrate by $SO_4^{\bullet-}$: Walling, C.; Zhao, C.; El-Taliawi, G. M. *J. Org. Chem.* **1983**, *48*, 4910. Walling, C.; El-Taliawi, G. M.; Zhao, C. *Ibid.* 4914. Walling, C.; El-Taliawi, G. M.; Amarnath, K. *J. Am. Chem. Soc.* **1984**, *106*, 7573.
(21) Oxidation of macrocyclic amido–Cu(II) complexes to the corresponding Cu(III) species has been reported: Fabbrizzi, L.; Kaden, T. A.; Perotti, A.; Seghi, B.; Siegfried, L. *Inorg. Chem.* **1986**, *25*, 321.
(22) A concerted electron-transfer–fragmentation mechanism has been found to operate for the persulfate-induced oxidative decarboxylation of aliphatic carboxylic acids: Tanner, D. D.; Osman, S. A. A. *J. Org. Chem.* **1987**, *52*, 4689.
(23) Brenner, M. C.; Klinman, J. P. *Biochemistry* **1989**, *28*, 4664, and references cited therein.
(24) Ortiz de Montellano, P. R., Ed. *Cytochrome P-450. Structure, Mechanism, and Biochemistry*; Plenum Press: New York, 1986.
(25) Lindsay Smith, J. R.; Mortimer, D. N. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1743. Burka, L. T.; Guengerich, F. P.; Willard, R. J.; Macdonald, T. L. *J. Am. Chem. Soc.* **1985**, *107*, 2549.
(26) Macdonald, T. L.; Gutheim, W. G.; Martin, R. B.; Guengerich, F. P. *Biochemistry* **1989**, *28*, 2071. Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9. Hollenberg, P. F.; Miwa, G. T.; Walsh, J. S.; Dwyer, L. A.; Rickert, D. E.; Kedderis, G. L. *Drug Metab. Dispos.* **1985**, *13*, 272.
(27) Groves, J. T.; McClusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859. See also: McMurry, T. J.; Groves, J. T. in reference 24, p 1.

Scheme II



abstraction step (path A) makes sense. However, N-deprotonation of the amide moiety by coordination of the active-site copper[6] would probably switch the mechanism over to the alternative electron-transfer/proton-transfer sequence (path B).[28] Regardless of how the carbon radical intermediate is formed, the α-nitrogen should make it easily oxidizable by the copper–O_2-derived oxidant (conceivably a Cu(III) species at this stage). Subsequently, delivery of the copper-bound hydroxide to the initial acyl imine product would generate the same carbinolamide product that would arise from a hydroxyl radical transfer "rebound" step.

Conclusion

The model studies presented here confirm that the generation of a strongly oxidizing copper species coordinated to amido nitrogen of *N*-acylglycine (or alanine) is capable of effecting the C–N dehydrogenation depicted in Scheme II, path B, and the mechanism shown in terms of the organic moiety is a reasonable one (though additional studies are needed to confirm this). If the PAM enzyme enforces direct coordination of the active-site copper to the C-terminus of peptide substrates, our studies here constitute a reasonable model. Of the complexes studied that undergo this transformation, Pic-Aib-Ala possesses the lowest Cu(II)–Cu(III) potential, +0.88 V vs NHE. This is not a prohibitively high value for a physiologic enzyme to be able to achieve in the reductive processing of O_2. Nonetheless, we are currently working on additional models in an effort to establish how low the Cu(II-I)–Cu(II) potential can be brought down with preservation of the capability of effecting the *C-terminal* C–N dehydrogenation reaction of interest. Our preliminary results suggest that the required potential may not be far from the +0.5 V vs NHE value observed by Margerum[7,8] to successfully effect the more energetically facile *internal* C–N dehydrogenation.

If the PAM enzyme does not involve copper–substrate coordination, the proposal of an initial electron-transfer step is less likely, and a H atom abstraction pathway becomes more reasonable (Scheme II, path A). However, even in this case, there is still a good possibility that the radical intermediate will undergo one-electron oxidation rather than hydroxyl radical rebound, giving the C–N dehydrogenation product as the precursor to the carbinolamide. If so, then the PAM reaction would be better described as an oxidase than as a monooxygenase, since the carbinolamide product would be arising from a hydrolysis step. No experiments have been described to date that offer direct evidence for one or the other hypothetical PAM reaction. Even if an oxygen-labeling experiment could be performed, it may not solve this mechanistic ambiguity since, as stated above, the enzyme could deliver an O_2-derived water molecule specifically to the acylimine product prior to its release from the active site. In fact, the findings of a recent study indicate that the carbinolamide may not even be the product released from the active site, since the enzyme appears capable of facilitating carbinolamide dissociation to peptide amide and glyoxylate.[29]

In light of the difficulty of obtaining direct mechanistic information for the PAM reaction, model studies such as those presented here should be valuable in ascertaining the reasonability of various proposed mechanisms, as has been the case for cytochrome P-450. Of special utility would be the development of suicide substrates designed on the basis of a particular assumed mechanism, and model studies would be helpful to "screen" candidate suicide moieties. Suicide inactivators have played a crucial role in the development of mechanistic concepts for other redox metalloenzymes (e.g., DBM[30] and cytochrome P-450[31]) and will undoubtedly become instrumental in the near future in efforts to establish the mechanism of action of PAM.

Experimental Section

General Procedures. UV–vis spectra were recorded with a Perkin-Elmer Lambda 3 instrument. ^{1}H NMR spectra were obtained with a Varian XL-200 spectrometer, and chemical shift data are reported (ppm) downfield from Me_4Si internal standard, except in the case of D_2O, where sodium 3-(trimethylsilyl)-1-propanesulfonate was employed. Mass spectra were obtained on a Kratos MS-25 instrument. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. All solvents, reagents, and organic fine chemicals were the most pure available from commercial sources. *N*-Salicyloylalanine was prepared according to ref 32. The water used for titrimetry and electrochemical measurements was doubly distilled.

Picolinyl Amino Acids. (A) DCC Method. An equimolar solution of picolinic acid, the amino acid ethyl or benzyl ester salt, and Et_3N in CH_3CN–THF (2:1) was treated with 1.05 equiv of DCC. After the solution was stirred for 2–3 days, DCU was removed by filtration, the filtrate was concentrated in vacuo, and the residue was partitioned between half-saturated aqueous $NaHCO_3$ and $CHCl_3$. The crude esters obtained after evaporation of the organic layer were purified by silica gel flash chromatography (EtOAc eluant). For the benzyl esters, hydrogenolysis with 10% Pd/C (5% w/w) in THF–H_2O (1:1), filtration of the catalyst, and evaporation of the filtrate gave the crude products, which were recrystallized from H_2O (Ala and Gly) or $CHCl_3$–hexane (Sar and Aib). For the ethyl esters, hydrolysis was achieved by dropwise addition of 1 equiv of 1 M aqueous NaOH over 1 h to a suspension of ester in H_2O

(28) A mechanism involving initial single electron transfer has recently been documented for DBM in addition to the more traditional H atom abstraction pathway: Wimalasena, K.; May, S. W. *J. Am. Chem. Soc.* **1989**, *111*, 2729; *Ibid.* **1987**, *109*, 4036, and references cited.

(29) Young, S. D.; Tamburini, P. P. *J. Am. Chem. Soc.* **1989**, *111*, 1933.

(30) For a recent review, see: Fitzpatrick, P. F.; Villafranca, J. J. *Arch. Biochem. Biophys.* **1987**, *257*, 231.

(31) (a) Bondon, A.; Macdonald, T. L.; Harris, T. M.; Guengerich, F. P. *J. Biol. Chem.* **1989**, *264*, 1988, and references cited therein. (b) Lee, J. S.; Jacobsen, N. E.; Ortiz de Montellano, P. R. *Biochemistry* **1988**, *27*, 7703, and references cited therein. (c) Ortiz de Montellano, P. R.; Reich, N. O. in reference 24, p 273.

(32) Van Brussel, W.; Van Sumere, C. F. *Bull. Soc. Chim. Belg.* **1978**, *8*, 791.

(1 mL/mmol) heated at 80 °C. After the solution was cooled, the product acids crystallized upon addition of 1 equiv of concentrated HCl. Overall yields averaged 90%.

(B) Mixed-Anhydride Method. A solution of $(CF_3CO)_2O$ (2.53 g, 12 mmol) in 30 mL of dry CH_3CN was added slowly to picolinic acid (1.23 g, 10 mmol) in 50 mL of CH_3CN at −10 °C. After this solution was allowed to warm to room temperature, the amino acid benzyl ester HOTs salt (Ala, Sar, or Aib) was added followed by Et_3N (3.53 g, 35 mmol), and the resulting solution was stirred overnight. The solvent was removed in vacuo, and the oily residue was partitioned between half-saturated aqueous $NaHCO_3$ and $CHCl_3$. Evaporation of $CHCl_3$ and silica gel flash chromatography (EtOAc eluant) yielded the pure benzyl esters, which were deprotected as in (A).

(C) Schotten–Baumann Method. Picolinyl chloride hydrochloride was prepared by refluxing picolinic acid with a slight excess of distilled $SOCl_2$ in benzene, evaporation to dryness, and crystallization from hexane, giving pale yellow needles. An excess of this material was added portionwise as the solid to a vigorously stirred solution of amino acid dissolved in 1 equiv of 1 M aqueous NaOH, which was maintained at pH 10.5 by addition of 4 M aqueous NaOH. The acid chloride addition was continued until TLC indicated complete acylation of the amino acid (~2 equiv was required). The pH was adjusted to pH 4.5, and the solvent was removed in vacuo. Repeated crystallization of the residue permitted separation of the desired picolinyl amino acid from picolinic acid.

Picolinylglycine: ^1H NMR (D_2O) δ 3.94 (s, 2 H), 7.48 (m, 1 H), 7.88 (m, 2 H), 8.47 (d, 1 H, J = 4.8 Hz, C_6–H). This compound has been reported previously.[33]

Picolinylalanine: ^1H NMR (D_2O) δ 1.57 (d, 3 H, J = 7.3 Hz), 4.63 (q, 1 H, J = 7.3 Hz), 7.73 (m, 1 H), 8.08 (m, 2 H), 8.66 (m, 1 H); HRMS (15 eV, of precursor benzyl ester) m/z calcd 284.1161 (M^+), obsd 284.1164; mp 144 °C.

Picolinylsarcosine: ^1H NMR (D_2O) δ 3.07 and 3.17 (2s, 3 H, NCH_3), 4.15 and 4.33 (2s, 2 H, CH_2), 7.64–7.87 (m, 2 H), 8.08–8.25 (m, 1 H), 8.67 (m, 1 H); HRMS (15 eV, of benzyl ester precursor) m/z calcd 284.1161 (M^+), obsd 284.1164; mp 88 °C.

Picolinyl-α-aminoisobutyric acid: ^1H NMR (D_2O) δ 1.41 and 1.42 (2 s, 6 H), 7.45 (m, 1 H), 7.83 (m, 2 H), 8.43 (m, 1 H); mp 103 °C.

General Method for the Preparation of Picolinyl-α-aminoisobutyryl Amino Acid Derivatives (Pic-Aib-Amino Acid). An equimolar mixture of Pic-Aib and the appropriate amino acid benzyl ester HOTs salt in CH_3CN–THF (2:1) was treated with a 1.05 equiv of DCC, 1.0 equiv of Et_3N, and 0.20 equiv of HOBt, and the resulting mixture was stirred at room temperature for 2–3 days. The DCU was removed by filtration, the filtrate was mixed with 10–15 mL of H_2O and stirred for 2–3 h, and a second crop of DCU was removed by filtration. The final filtrate was evaporated in vacuo, and the residue was partitioned between half-saturated aqueous $NaHCO_3$ and $CHCl_3$. The organic layer was evaporated, and the crude products were purified by silica gel flash chromatography (EtOAc eluant), giving 80–90% isolated yields as oils.

A solution of the esters in THF–H_2O (1:1) containing 5% by weight of 10% Pd/C was exposed to 1 atm of H_2 for 5 h. After filtration of the catalyst, the final products crystallized as neutral acids from THF–H_2O.

Pic-Aib-Ala: ^1H NMR (D_2O) δ 1.22 (d, 3 H, J = 7.3 Hz, Ala CH_3), 1.45 and 1.48 (2s, 3 H each, Aib CH_3), 4.23 (q, 1 H, J = 7.3 Hz, Ala CH), 7.52 (m, 1 H), 7.89 (m, 2 H), 8.49 (d, 1 H, J = 4.6 Hz, C_6–H); HRMS (15 eV, of benzyl ester precursor) m/z calcd 370.1767 (MH^+), obsd 370.1776; mp 75–77 °C.

Pic-Aib-Aib: ^1H NMR (DMSO-d_6) δ 1.37 and 1.60 (2 s, 6 H each), 7.60 (m, 1 H, C_5–H), 7.99 (m, 3 H, C_3–H, C_4–H, and NH), 8.64 (m, 1 H, C_6–H), 9.15 (s, 1 H, NH); HRMS (15 eV, of benzyl ester precursor) m/z calcd 384.1923 (MH^+), obsd 384.1931; mp 185 °C.

Preparation of Pic-Aib-NH_2. A solution of Pic-Aib (1.04 g, 5 mmol) in dry CH_3CN (10 mL) was cooled to 0 °C, and $(CF_3CO)_2O$ (1.05 g, 5 mmol) in 10 mL of CH_3CN was added over 1 h. The resulting solution was added over 5 min to concentrated NH_4OH (50 mL) at 0 °C. After the solution was warmed to room temperature overnight, removal of solvent in vacuo, partitioning of the residue between half-saturated aqueous $NaHCO_3$ and $CHCl_3$, and evaporation of the organic layer, 0.85 g (85%) of a white solid was obtained: ^1H NMR ($CDCl_3$) δ 1.71 (s, 6 H), 5.80 and 6.83 (2 br s, 1 H each, NH), 7.45 (m, 1 H), 7.86 (dt, 1 H, J = 1.7 and 7.8 Hz), 8.16 (d, 1 H, J = 7.8 Hz), 8.56 (m, 1 H), 8.51 (s, 1 H, NH); HRMS (15 eV) m/z calcd 208.1086 (MH^+), obsd 208.1091; mp 108 °C.

6-Carboxypicolinyl Amino Acids (Mixed-Anhydride Method). To a solution of 2,6-pyridinedicarboxylic acid (3.34 g, 20 mmol) and Et_3N (2.02 g, 20 mmol) in 50 mL of dry CH_3CN, cooled to −10 °C with an ice–salt bath, was added a solution of $(CF_3CO)_2O$ (5.04 g, 24 mmol) in 30 mL of CH_3CN over 1 h. The resulting white suspension was allowed to return to room temperature, and the amino acid benzyl ester HOTs salt (20 mmol) was added followed by Et_3N (4.04 g, 40 mmol). Vigorous stirring of the resulting mixture resulted in clearing in either 2 h (Gly, Ala, Sar) or 10 h (Aib). After 1 day, the solvent was removed in vacuo, and the residue was treated with 1000 mL of half-saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (3 × 50 mL) to remove the diester byproduct (~20%). The aqueous layer was acidified with HCl to pH 2.5–3.5 at which time the desired monoesters were obtained as crystalline white solids (Gly, 2.1 g, 33%; Sar, 2.4 g, 52%; Aib, 2.1 g, 44%) or as an oil (Ala, 2.6 g, 57%). Hydrogenolysis of the benzyl esters as described above yielded the final products that crystallized as white needles from boiling H_2O.

(6-Carboxypicolinyl)glycine: ^1H NMR (DMSO-d_6) δ 4.08 (d, 2 H, J = 5.4 Hz, CH_2), 8.26 (br s, 3 H, Ar–H), 9.44 (br t, 1 H, NH); HRMS (15 eV, of benzyl ester precursor) m/z calcd 315.0981 (MH^+), obsd 315.0979; mp 198 °C.

(6-Carboxypicolinyl)alanine: ^1H NMR (DMSO-d_6) δ 1.46 (d, 3 H, J = 7.3 Hz), 4.54 (p, 1 H, J_{app} = 7.5 Hz), 8.26 (m, 3 H, Ar–H), 9.32 (d, 1 H, NH, J = 7.7 Hz); HRMS (15 eV, of benzyl ester precursor) m/z calcd 329.1137 (MH^+), obsd 329.1136; mp 232 °C.

(6-Carboxypicolinyl)sarcosine: ^1H NMR (D_2O) δ 2.89 and 3.02 (2s, 3 H, NCH_3), 4.05 and 4.20 (2 s, 2 H, CH_2), 7.72 (m, 1 H), 8.00–8.11 (m, 2 H); HRMS (15 eV, of benzyl ester precursor) m/z calcd 328.1059 (M^+), obsd 328.1050; mp 164 °C.

(6-Carboxypicolinyl)-α-aminoisobutyric acid: ^1H NMR (D_2O) δ 1.46 (s, 6 H), 7.9–8.2 (m, 3 H); HRMS (15 eV, of benzyl ester precursor) m/z calcd 343.1294 (MH^+), obsd 343.1296; mp 254 °C.

Preparation of 2,6-Pyridinedicarboxylic Acid, Monobenzyl Ester. A mixture of 2,6-pyridinedicarboxylic acid (16.7 g, 0.1 mol), water (40 mL), benzyl alcohol (115 mL), and concentrated H_2SO_4 (5.5 mL) was refluxed for 2 h and allowed to stir at room temperature overnight. The mixture was neutralized with 1 L of saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ to remove the diester (6.9 g, 20%). The aqueous layer was acidified to pH 3.8, at which point the monoester crystallized as white needles. Additional monoester was obtained by extracting the resulting mother liquor with $CHCl_3$, to give a combined yield of 7.7 g (30%): ^1H NMR ($CDCl_3$) δ 5.48 (s, 2 H), 7.48 (m, 5 H, Ph), 8.12 (t, 1 H, J = 7.6 Hz), 8.40 (t, 2 H, J = 7.6 Hz).

Preparation of 6-Carboxypicolinamide. A solution of 1.0 g of the above monobenzyl ester in 40 mL of concentrated NH_4OH was heated in a sealed pressure bottle at 90 °C (oil bath) for 6 h. The product was obtained in quantitative yield upon evaporation of the solvent: ^1H NMR (D_2O) δ 7.8 (br s); HRMS (15 eV) m/z calcd 166.0378 (M^+), obsd 166.0372.

Preparation of *N*-(8-Hydroxyquinolinyl-2-formyl)glycine (HO-Q-Gly). The acid chloride of 8-hydroxyquinolinic acid[34] (2.44 g, 10 mmol), glycine benzyl ester *p*-tosylate (3.37 g, 10 mmol), and Et_3N (3.13 g, 31 mmol) were mixed in dry benzene (60 mL), and the resultant mixture was stirred at room temperature for 20 h. After evaporation of benzene, 100 mL of water was added, and the aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ layer was dried (anhydrous Na_2SO_4) and evaporated, and the resulting brown oil was purified by silica gel flash chromatography (EtOAc eluant) to yield 3.0 g (89%) of the ester intermediate. Hydrogenolysis of the benzyl ester in 95% aqueous EtOH over Raney Ni (1 atm of H_2, 55 °C, 4.5 h), filtration of the catalyst, and evaporation afforded near stoichiometric conversion to the crude product acid, which was recrystallized from acetone–ether: yield 2.3 g (93% overall); ^1H NMR (DMSO-d_6) δ 4.09 (d, 2 H, J = 5.9 Hz), 7.18 (d, 1 H, J = 6.6 Hz), 7.50 (m, 2 H), 8.13 and 8.51 (2 d, 1 H each, J = 8.4 Hz, C_3- and C_4-H), 9.97 (br s, 1 H, NH), 10.13 (s, 1 H, OH); HRMS (15 eV, of precursor benzyl ester) m/z calcd 336.1110 (M^+), obsd 336.1111 (100%); mp 292 °C. An attempt to prepare this compound by the Schotten–Baumann method resulted in only a 40% yield. In addition, when the acid chloride was coupled to glycine ethyl ester, saponification of the coupled ester was accompanied by partial amide hydrolysis.

Preparation of *N*-(8-Hydroxyquinolinyl-2-formyl)sarcosine (HO-Q-Sar). This was accomplished as above except that debenzylation was effected with 5% Pd/C. The use of 10% Pd/C catalyst resulted in partial hydrogenation of the quinoline moiety. In one preparation, the product was dissolved in water adjusted to pH 1.5 with HCl, evaporation of which and recrystallization from acetone–ether gave 2.5 g (84%) of the HCl salt: ^1H NMR (HCl salt, DMSO-d_6) δ 3.09 (s, 3 H), 4.22 and 4.49 (2 s, 2 H), 7.17 (d, 1 H, J = 6.5 Hz), 7.45 (m, 2 H), 7.64 (m, 1 H), 8.42 (m, 1 H); HRMS (15 eV, of benzyl ester precursor) m/z calcd 350.1266 (M^+), obsd 350.1259; mp 184 °C (HCl salt), 110 °C (free base).

Preparation of *N*-(Carboxymethyl)sarcosyl Amino Acids. A mixture of the anhydride of (*N*-methylimino)diacetic acid[35] (1.29 g, 10 mmol),

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10 mmol of amino acid (Gly or Aib), and Et_3N (1.1 g, 11 mmol) in DMF–THF (1:1, 50 mL) was refluxed for 2 h. After the solution was cooled to room temperature, some solid residue was filtered, and the filtrate was evaporated. The residue was dissolved in H_2O, and CH_3CN was added until cloudiness appeared; cooling yielded the crystalline product (1.6 g, 78% for Gly; 1.9 g, 82% for Aib).

$(HOOCCH_2)$Sar-Gly: ^1H NMR (DMSO-d_6) δ 2.35 (s, 3 H, NCH_3), 3.18 (s, 2 H), 3.31 (s, 2 H), 3.77 (d, 2 H, J = 4.7 Hz, Gly CH_2), 8.05 (br s, NH); mp 168 °C.

$(HOOCCH_2)$Sar-Aib: ^1H NMR (DMSO-d_6) δ 1.38 (s, 6 H), 2.34 (s, 3 H, NCH_3), 3.11 (s, 2 H), 3.28 (s, 2 H), 7.95 (s, 1 H); mp 243 °C.

Chemical Oxidation of the Cu(II)–Peptide Complexes. (A) Oxidation with Na_2IrCl_6. A solution of 1 mmol of peptide and 1 mmol of $Cu(NO_3)_2 \cdot 2.5H_2O$ in 20 mL of 0.1 M aqueous borate buffer (initially at pH 8.5) was adjusted to pH 8.5–11.0 (see Table II) with aqueous NaOH. To the vigorously stirred solution at room temperature was added solid Na_2IrCl_6 (2 mmol). The resulting dark brown solution gradually faded to light brown over the course of the experiment (5 or 24 h), during which time the pH was maintained by addition of aqueous NaOH (otherwise the pH dropped 1–2 pH units). Aliquots were periodically removed and quenched with 3 M aqueous HCl to pH 1.0 and a few drops of EtOH-free 2,4-DNP reagent for the qualitative analysis of HCHO, CH_3CHO, CH_3COCH_3, HC(O)COOH, and $CH_3C(O)COOH$ by TLC (eluant EtOAc–CH_3OH, 2:1). Various modifications of the experimental conditions are given in Table II.

(B) Oxidation with $K_2S_2O_8$. A solution of 1 mmol each of the peptide and $Cu(NO_3)_2 \cdot 2.5H_2O$ in 20 mL of 0.1 M aqueous borate buffer adjusted to pH 10.5 with aqueous NaOH was prepared as above. Solid $K_2S_2O_8$ (1.5 mmol) was added, and the pH was maintained at 10.5 for 24 h by addition of aqueous NaOH. TLC analysis for products was carried out as in (A).

(C) Oxidation with H_2O_2. To a solution of 1 mmol each of the peptide and $Cu(NO_3)_2 \cdot 2.5H_2O$ in 20 mL of 0.1 M aqueous borate buffer adjusted to pH 8.5 with aqueous NaOH was added 0.34 mL (3 mmol) of 30% H_2O_2 solution.

Quantitative Determination of Products of Oxidation of Cu(II)–Peptide Complexes. Completed reactions conducted as above were quenched with 3 M aqueous HCl to pH 1.0 and divided into two equal portions. One portion was treated with a slight excess of EtOH-free 2,4-DNP reagent, and the resulting derivative was removed by filtration, dried, and weighed for determination of the yield of carbonyl product. When the product was pyruvic acid, the acidified half-reaction mixture was added to 20 mL of 12 N HCl containing excess 2,4-DNP, and the derivative was extracted with five portions of EtOAc and quantified upon evaporation of solvent. The second reaction portion was used for determination of the yield of picolinamide, 6-carboxypicolinamide, or Pic-Aib-NH_2. The Cu(II) was removed by bubbling H_2S through the solution and filtering off the precipitated CuS; the filtrate was evaporated in vacuo, and the residue was partitioned between half-saturated aqueous $NaHCO_3$ and $CHCl_3$ to remove unreacted substrate acids. A weighed amount of hexamethylbenzene (HMB) was added to the organic layer, which was then evaporated in vacuo and analyzed by ^1H NMR. The yield of amide product was determined by integration relative to the HMB singlet, and its identity and purity were confirmed by TLC.

UV–Vis Studies To Monitor Cu(III). A solution of $Cu(ClO_4)_2 \cdot 6H_2O$ and the peptide ligand in 3% excess was prepared in 0.1 M borate buffer, pH 8–11, to a concentration of 0.42 mM and purged with N_2 for 30 min. A 3-mL sample in a cuvette was equilibrated in the sample holder at 10 °C, and the spectrum was recorded. To this was added 50 μL of a 50 mM solution of Na_2IrCl_6 with vigorous shaking, and the spectrum was recorded immediately and over a period of time sufficient to reach >90% decay of the Cu(III) absorbance.

Electrochemistry. Cyclic voltammetry (CV) and Osteryoung square-wave voltammetry (OSWV) were performed with a Bioanalytical Systems Model 100 electrochemical analyzer, with the standard three-electrode cell arrangement with a saturated calomel electrode (SCE) as the reference and Pt wire as the auxiliary electrode. Two different working electrodes were used: a glassy carbon (GC) disk electrode and wax-impregnated graphite (WIG) electrode. The electrochemical runs were carried out on N_2-degassed solutions containing 1 mM ligand, 1 mM $Cu(ClO_4)_2$, and 0.08 M $NaNO_3$, with pH adjusted through addition of aqueous NaOH. The E_p values reported in Table I were obtained by OSWV at a freshly cleaned GC electrode. Scans were initiated at the rest potential and were typically recorded to 1.8 V vs SCE. Since these scans proceeded to potentials corresponding to the anodic limit of the GC electrode, OSWV runs were made on blank solutions containing only supporting electrolyte and adjusted to the same pH as the corresponding solution of Cu(II) complex. The background current in these blank solutions begins to rise at ~1.0 V and smoothly increases to ~30 μA at 1.8 V vs SCE. The redox processes, which are given as >1.25 V vs NHE in Table I, appear as sharply defined, prominent peaks on the rising background current. Nearly identical values of E_p were obtained with the WIG electrode.

For bulk electrolysis, the auxiliary electrode (Pt foil) was kept separate from the working and reference (SCE) electrodes through use of a two-compartment cell containing a fritted glass partition; 1–2 mM solutions of the Cu(II) complex were employed. The working electrode used in the electrolyses was a bundle of three WIG rods, each with an exposed surface of 3-mm diameter and ~25-mm length. During the electrolysis, the solution in the main compartment was vigorously stirred with a magnetic stirrer. Aliquots were periodically removed to monitor the appearance of Cu(III) by UV–vis spectroscopy. Bulk electrolyses were run until the current ratio was less than 10% of the initial current. The background current was then estimated, and a correction was made to yield the number of electrons transferred for 100% electrolysis according to the Faraday relation.

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