Nickel Complexes of Cysteine- and Cystine-Containing Peptides: Spontaneous Formation of Disulfide-Bridged Dimers at Neutral pH

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A series of nine tripeptide ligands of the type XCH (X = glycine or lysine, C = cysteine, H = histidine) was prepared, and the coordination chemistry of these peptides with Ni(II) was investigated. The cysteine residues were incorporated as free thiols, as protected (*tert*-butyl) thiols, or as disulfide-bridged cystine dimers, and the histidine residues had either carboxylate (CO_2H) or carboxamide ($CONH_2$) C-termini. The Ni(II) complexes of the protected thiols exhibited no interaction of the side chain with the metal, giving UV and electrochemical data which were consistent with related tripeptide species. The Ni(II) complexes of the free thiol-containing ligands GCH−$CONH_2$, KCH−$CONH_2$, and GCH−CO_2H were found to dimerize rapidly via disulfide bond formation in the presence of air at pH 7. These processes were confirmed by independent synthesis of the dimeric (cystine) ligands and preparation of their Ni(II) complexes. The disulfide-bridged complex with a carboxylate terminus Ni_2(GCH−$CO_2H)_2$ showed no further reactivity with oxygen, which was unusual, since Ni(II) complexes of XXH−CO_2H peptides are known to spontaneously decarboxylate in air.

Introduction

Metal ion binding to peptides and proteins can be broadly divided into two classes. One of these involves ligation by side-chain residues, whereby the 3-dimensional structure of the protein plays an important role in the coordination geometry around the metal.[1] Secondly, peptide coordination has also been observed via binding of deprotonated amide (peptide) nitrogens, usually in conjunction with a terminal amino group and a histidine imidazole group at the third position of the peptide sequence.[2] This second coordination mode is utilized in nickel and copper binding by serum albumins, neuromedins C and K, human sperm protamine P2a, and histatins.[3]

There has been much interest in peptides containing the sequence XXH,[4] due in part to their ability to effect DNA damage and cross-linking in the presence of nickel(II). We previously reported DNA cleavage by the nickel complex of KGH−CO_2H in conjunction with O_2 or $KHSO_5$[5] and by the nickel complex of KGH−$CONH_2$ with either $KHSO_5$ or sulfite + dioxygen,[6] generally through formation of alkaline-labile sites. Long et al. have also shown that direct DNA strand cleavage can be effected by the nickel complex of KGH−$CONH_2$ with various oxidants under low salt conditions.[7] Kodadek and co-workers[8] have reported oxidative cross-linking of proteins by the nickel complex of GGH−CO_2H in the presence of oxidants such as $KHSO_5$ or magnesium monoperoxyphthalate (MMPP), whereas we recently observed oxidative cross-linking of DNA to the Ni(II) complex of KGH−CO_2H in the presence of dioxygen.[9] Nickel peptide complexes have also been attached to DNA recognition groups to generate affinity cleavage bioconjugates. These examples include the incorporation of the nickel GGH complex at the amino terminus of a DNA recognition unit such as a three-helix bundle from Hin recombinase,[10] a zinc-finger DNA binding domain from Sp1, a Jun/Fos hybrid, and a peptide−nucleic acid (PNA) oligomer.[11]

Further interest in this nickel peptide motif stems from an unusual autoxidative decarboxylation reaction that has been observed with nickel (and copper) complexes of XXH−CO_2H peptides (i.e., those with a carboxylate terminus).[12] The mechanism for this process (Figure 1) has been shown to involve a Ni(III) intermediate and is believed to proceed via a carbon-centered radical at the α-position of the histidine residue, and the hydroxylated product has been characterized crystallographically.[12a]

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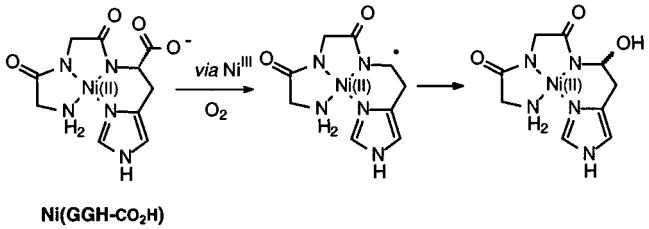

Figure 1. Autoxidative decarboxylation of (glycylglyclylhistidine)-nickel(II).

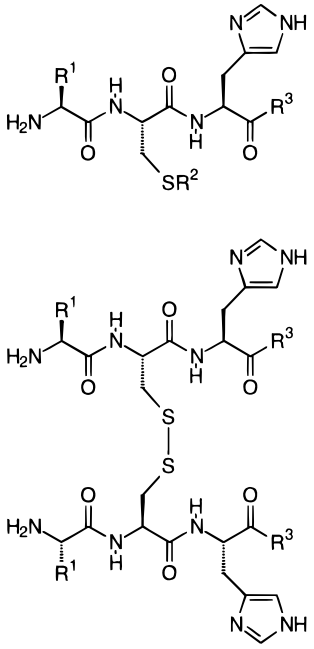

Figure 2. Monomeric and dimeric cysteine-containing tripeptide ligands (see Table 1 for substituents).

Table 1. Cysteine- and Cystine-Containing Tripeptide Ligands

peptide	R^1	R^2	R^3
GCH−$CONH_2$	H	H	NH_2
GC(*t*Bu)H−$CONH_2$	H	$-C(CH_3)_3$	NH_2
GCH−CO_2H	H	H	OH
GC(*t*Bu)H−CO_2H	H	$-C(CH_3)_3$	OH
KCH−$CONH_2$	$-CH_2(CH_2)_3NH_3^+$	H	NH_2
KC(*t*Bu)H−$CONH_2$	$-CH_2(CH_2)_3NH_3^+$	$-C(CH_3)_3$	NH_2
$(GCH-CONH_2)_2$	H	S−S dimer	NH_2
$(KCH-CONH_2)_2$	$-CH_2(CH_2)_3NH_3^+$	S−S dimer	NH_2
$(GCH-CO_2H)_2$	H	S−S dimer	OH

We were interested in studying the coordination chemistry of related peptide species which may be important in modes of metal binding to proteins and have relevance to toxicity of transition elements such as nickel.[13] As a first step, a cysteine residue was incorporated at the second position of the peptide backbone, giving the sequence XCH (see Table 1 and Figure 2), to study the effect of a cysteine residue upon transition metal binding and redox activity. Cysteine was chosen because oxidation chemistry leading to Ni^{III} could result in potential ligand oxidation, from which interesting DNA chemistry might arise. In addition, it was of interest to compare the two competing pathways possible for reaction with dioxygen—dimerization via disulfide bond formation vs oxidative decarboxylation.

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Experimental Section

Materials and Methods. Protected amino acids and resins were obtained from NovaBiochem. *N*,*N*-Dimethylformamide, acetonitrile, triphenylphosphine, NaCl, $NiCl_2$, and $Ni(OAc)_2$ were used as supplied by Fisher. Phenol, KOH, Na_2HPO_4, ethyl acetate, diethyl ether, and methanol were used as supplied by Mallinckrodt. Dicyclohexylcarbodiimide (DCC), diethylazodicarboxylate, hydroxybenzotriazole, thallium(III) trifluoroacetate, ethanedithiol, and triisopropylsilane were used as supplied by Aldrich. Piperidine and trifluoroacetic acid were used as supplied by Acros. D_2O was used as supplied by CIL. Tetrahydrofuran (THF, Fisher) was freshly distilled from Na/benzophenone. Ultrafiltered deionized water (Nanopure) was used for all reactions.

^1H NMR spectra were recorded on a Varian Unity-300 spectrometer and were referenced to internal solvent (HOD) at pH 7−8. FAB mass spectra were recorded on a Finnigan MAT 95 spectrometer, and ES mass spectra were recorded on a Micromass TRIO 2000. UV spectra were recorded on a Hewlett-Packard 8452a diode array spectrophotometer. Cyclic voltammetry was performed on a BAS CV-50W instrument using a glassy carbon working electrode and a Ag/AgCl reference electrode. Electrochemical measurements were carried out under buffered aqueous conditions (100 mM NaCl, 10 mM Na_2HPO_4, pH 7). All pH measurements were performed with a Corning general-purpose electrode calibrated between pH 4 and 7 on a Corning 250 ion analyzer. Peptide purification was carried out using a Waters automated gradient controller attached to a Lambda-Max 481 LC spectrophotometer (monitoring at 220 nm) using an Alltech alphabond C18 reverse-phase column (150 mm length, 22.5 mm i.d.) for preparative work and a Waters Bondapak C18 reverse-phase column for analytical work.

Peptide Synthesis. Solid-phase peptide syntheses were carried out on a 0.1−0.5 mmol scale according to established procedures.[14] Carboxamide C-terminal peptides were synthesized on Rink amide resin, employing triphenylmethyl (trityl) side-chain protection and fluorenylmethoxycarbonyl (Fmoc) terminal amine protection. Dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) coupling protocols were used. Side-chain deprotection and resin cleavage were carried out in a cocktail of 5% H_2O, 2% phenol, 2% ethanedithiol, and 2% triisopropylsilane in trifluoroacetic acid for 16 h. Carboxylate C-terminal peptides were synthesized on Wang resin, with the initial ester coupling being carried out under Mitsunobu conditions[15] with triphenylphosphine and diethylazodicarboxylate in THF. Side-chain deprotection and resin cleavage were carried out under the same conditions as with Rink resin with a reaction time of 4−6 h.

Reaction mixtures were then filtered, and the filtrates were concentrated under a stream of N_2 gas, after which the peptides were precipitated with diethyl ether and isolated by centrifugation. Crude peptides were purified by preparative HPLC, and their purity was confirmed by analytical HPLC. A concave gradient curve was employed using 100% solvent A (0.1% trifluoroacetic in deionized water) to 100% solvent B (60% acetonitrile, 0.04% trifluoroacetic acid in deionized water) over a period of 30−40 min, which gave retention times of the order 8−18 min. *S-tert*-Butyl-protected derivatives gave significantly longer retention times relative to those of peptides with free thiols. Peptide-containing fractions were then freeze-dried to give pure products in yields ranging from 70 to 95%.

Disulfide-bridged peptide dimers were prepared from the *S-tert*-butyl-protected thiol derivatives according to the method of Yajima.[16] The general procedure involved the reaction of 1 equiv of peptide with 1 equiv of thallium(III) trifluoroacetate in neat trifluoroacetic acid (5 mL) for 1 h at 0 °C. The reaction mixture was then concentrated under a stream of N_2 and washed with diethyl ether several times, and the

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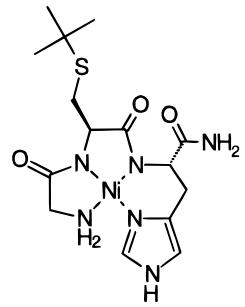

Figure 3. Ni{GC(*t*Bu)H−$CONH_2$}.

product was purified as described above. Peptide dimers gave shorter retention times during HPLC purification than did the *S*-*tert*-butyl derivatives from which they were prepared.

Chemical formulas and molecular weights are based upon all functional groups being neutral, although the overall charge on the peptides and complexes varied according to pH. Near pH 7, the lysine side chain is assumed to be protonated while the carboxylate terminus is deprotonated. Complexation to Ni(II) at pH 7−8 leads to deprotonation and coordination of the two peptide-bond nitrogens in conjunction with the amino terminus and one imidazole nitrogen of histidine (see Figure 3).[2] Cysteine thiols may also be deprotonated upon coordination to Ni(II) at pH 7−8.

Glycylcysteinylhistidinecarboxamide, GCH−$CONH_2$: $C_{11}H_{18}N_6O_3S$; MW = 314.36; ^{1}H NMR (D_2O) δ = 2.9 (2H, m, β-cys), 3.1−3.3 (2H, m, β-his), 3.93 (2H, s, gly), 4.6 (1H, m, α-his), 4.75 (1H, m, α-cys), 7.32 (1H, s, C_4H-his), 8.55 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 28.37, 29.40, 43.49 (α-positions), 55.50, 58.85 (β-positions), 120.49, 131.54, 136.67 (imidazole-his), 170.48, 174.89, 177.05 (amide C═O) ppm; FAB-MS *m*/*z* = 315 (MH^+).

Glycyl(*S*-*tert*-butylcysteinyl)histidinecarboxamide, GC(*t*Bu)H−$CONH_2$: $C_{15}H_{26}N_6O_3S$; MW = 370.47; ^{1}H NMR (D_2O) δ = 1.23 (9H, s, *S*-*tert*-butyl), 2.92 (2H, m, β-cys), 3.1−3.3 (2H, m, β-his), 3.89 (2H, s, gly), 4.6 (1H, m, α-his), 4.68 (1H, m, α-cys), 7.33 (1H, s, C_4H-his), 8.55 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 29.15 (α), 32.12 (α), 32.76 [*S*-*tert*-butyl C(CH_3)$_3$], 43.27 (α), 46.11 [*S*-*tert*-butyl $C(CH_3)_3$], 55.22, 56.64 (β-positions), 120.27, 131.50, 136.45 (imidazole-his), 170.06, 174.91, 179.99 (amide C═O) ppm; FAB-MS *m*/*z* = 371 (MH^+).

Glycylcysteinylhistidine, GCH−CO_2H: $C_{11}H_{17}N_5O_4S$; MW = 315.35; ^{1}H NMR (D_2O) δ = 2.94 (2H, m, β-cys), 3.1−3.3 (2H, m, β-his), 3.99 (2H, s, gly), 4.63 (1H, m, α-his), 4.74 (1H, m, α-cys), 7.32 (1H, s, C_4H-his), 8.70 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 28.54, 29.51, 43.51 (α-positions), 55.57, 58.46 (β-positions), 120.34, 131.52, 136.51 (imidazole-his), 171.03, 174.19 (amide C═O) ppm; FAB-MS *m*/*z* = 316 (MH^+).

Glycyl(*S*-*tert*-butylcysteinyl)histidine, GC(*t*Bu)H−CO_2H: $C_{15}H_{25}N_5O_4S$; MW = 371.45; ^{1}H NMR (D_2O) δ = 1.21 (9H, s, *S*-*tert*-butyl), 2.9−3.0 (2H, m, β-cys), 3.05−3.3 (2H, m, β-his), 3.88 (2H, s, gly), 4.61 (1H, m, α-his), 4.77 (1H, m, α-cys), 7.31 (1H, s, C_4H-his), 8.65 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 29.62 (α), 32.19 (α), 32.69 [S-*tert*-butyl C(CH_3)$_3$], 43.18 (α), 45.92 [*S*-*tert*-butyl $C(CH_3)_3$], 56.25, 56.59 (β-positions), 119.83, 131.84, 135.98 (imidazole-his), 170.11, 174.09 (amide C═O) ppm; FAB-MS *m*/*z* = 372 (MH^+).

Lysylcysteinylhistidinecarboxamide, KCH−$CONH_2$: $C_{15}H_{27}N_7O_3S$; MW = 385.48;^{1}H NMR (D_2O) δ = 1.38, 1.66 (2H + 2H, m + m, γ-lys + δ-lys), 1.87 (2H, m, β-lys), 2.87 (2H, d, β-cys), 2.95 (2H, dd, ϵ-lys), 3.15−3.35 (2H, m, β-his), 4.01 (1H, dd, α-lys), 4.45 (1H, dd, α-cys), 4.60 (1H, dd, α-his), 7.34 (1H, s, C_4H-his), 8.68 (1H, s, C_2H-his) ppm; FAB-MS *m*/*z* = 386 (MH^+).

Lysyl(*S*-*tert*-butylcysteinyl)histidinecarboxamide, KC(*t*Bu)H−$CONH_2$: $C_{19}H_{35}N_7O_3S$; MW = 441.59; ^{1}H NMR (D_2O) δ = 1.24 (9H, s, *S*-*tert*-butyl), 1.40, 1.68 (2H + 2H, m + m, γ-lys + δ-lys), 1.85 (2H, m, β-lys), 2.93 (2H, dd, ϵ-lys), 3.10−3.40 (4H, m, m, β-cys, β-his), 4.03 (1H, dd, α-lys), 4.44 (1H, dd, α-cys), 4.62 (1H, dd, α-his), 7.33 (1H, s, C_4H-his), 8.62 (1H, s, C_2H-his) ppm; FAB-MS *m*/*z* = 442 (MH^+).

Glycylcystinylhistidinecarboxamide dimer, (GCH−$CONH_2$)$_2$: $C_{22}H_{34}N_{12}O_6S_2$; MW = 626.71; ^{1}H NMR (D_2O) δ = 3.0−3.4 (4H, m, β-cys, β-his), 3.89 (2H, s, gly), 4.7 (2H, m, α-cys, α-his), 7.32 (1H, s, C_4H-his), 8.63 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 29.33, 41.25, 43.21 (α-positions), 55.25, 55.57 (β-positions), 120.11, 131.10, 136.32 (imidazole-his), 169.98, 174.27, 176.42 (amide C═O) ppm; FAB-MS *m*/*z* = 627 (MH^+).

Lysylcystinylhistidinecarboxamide dimer, (KCH−$CONH_2$)$_2$: $C_{30}H_{52}N_{14}O_6S_2$; MW = 768.95; ^{1}H NMR (D_2O) δ = 1.40, 1.72 (2H +2H, m + m, γ-lys + δ-lys), 1.90 (2H, m, β-lys), 2.98 (2H, dd, ϵ-lys), 3.1−3.4 (4H, m, β-cys + β-his), 4.04 (1H, dd, α-lys), 4.68 (2H, m, α-cys + α-his), 7.35 (1H, s, C_4H-his), 8.61 (1H, s, C_2H-his) ppm; FAB-MS *m*/*z* = 769 (MH^+).

Glycylcysteinylhistidine dimer, (GCH−CO_2H)$_2$: $C_{22}H_{32}N_{10}O_8S_2$; MW = 628.68; ^{1}H NMR (D_2O) δ = 2.9−3.3 (4H, m, β-cys, β-his), 3.82 (2H, s, gly), 4.6−4.7 (2H, m, m, α-cys, α-his), 7.25 (1H, s, C_4H-his), 8.59 (1H, s, C_2H-his) ppm; FAB-MS *m*/*z* = 629 (MH^+).

Complex Formation. Nickel(II) complexes were prepared by adding 1.05 equiv of $NiCl_2$ or $Ni(OCOCH_3)_2$ to 1 equiv of peptide in deionized water. The pH of the solution was then adjusted to between 7.0 and 8.0 using 1 M NaOH (or neat Et_3N), and the mixture was freeze-dried. The residue was taken up in water (D_2O for NMR measurements, phosphate buffer for electrochemical measurements), and the mixture was filtered through a syringe filter to remove small amounts of NiOH, which usually formed. All yields of nickel(II) complexes were essentially quantitative; no evidence for free ligand or other species was detected by NMR, and the extinction coefficients are all in accord with the well-characterized parent complexes, Ni(GGH−CO_2H) and Ni(GGH−$CONH_2$).[2] The chemical formulas of the nickel complexes presented below are based upon the deprotonation of the amide nitrogens of the cysteine and histidine residues.

(Glycylcysteinylhistidinecarboxamide)nickel(II), Ni(GCH−$CONH_2$): $C_{11}H_{16}N_6O_3SNi$; MW = 371.04; λ_{max} (upon initial formation, pH 6.4) = 324 nm (438 dm^3 mol^{-1} cm^{-1}), 464 nm (74 dm^3 mol^{-1} cm^{-1}); λ max (upon standing in air) = 420 nm (80 dm^3 mol^{-1} cm^{-1}); ^{1}H NMR paramagnetically broadened initially, upon standing overnight in air: δ = 2.91 (2H, m, gly), 3.28 (1H, dd, β-his), 3.35 (2H, dd, cys), 3.76 (1H, dd, β-his), 4.16 (1H, m, α-cys), 4.25 (1H, dd, α-his), 6.95 (1H, s, C_4H-his), 7.53 (1H, s, C_2H-his) ppm; ES-MS *m*/*z* = 368.8.

[Glycyl(*S*-*tert*-butylcysteinyl)histidinecarboxamide]nickel(II), Ni{GC(*t*Bu)H−$CONH_2$}: $C_{15}H_{24}N_6O_3SNi$; MW = 427.14; λ_{max} (pH 6.8) = 424 nm (102 dm^3 mol^{-1} cm^{-1}); ^{1}H NMR (D_2O) δ = 1.38 (s, 9H, *S*-*tert*-butyl), 2.80−2.92 (2H, m, gly), 3.11 (1H, s, β-his), 3.22 (2H, s, cys), 3.38 (1H, dd, β-his), 4.0 (1H, m, α-cys), 4.06 (1H, dd, α-his), 6.92 (1H, s, C_4H-his), 8.39 (1H, s, C_2H-his) ppm; ^{13}C NMR (D_2O) δ = 32.37 (α), 32.56 (α), 33.23 [*S*-*tert*-butyl C(CH_3)$_3$], 45.21 [*S*-*tert*-butyl $C(CH_3)_3$], 50.38 (α), 54.92, 61.64 (β-positions), 117.89, 136.28, 139.26 (imidazole-his), 182.34, 184.76, 186.63 (amide C═O) ppm; FAB-MS *m*/*z* = 427; ES-MS *m*/*z* = 427.3.

(Glycylcysteinylhistidine)nickel(II), Ni(GCH−CO_2H): $C_{11}H_{15}N_5O_4SNi$; MW = 372.02; λ_{max} (upon initial formation, pH 8.0) = 322 nm (507 dm^3 mol^{-1} cm^{-1}), 446 nm (149 dm^3 mol^{-1} cm^{-1}); λ_{max} (upon standing for 24 h) = 420 nm (73 dm^3 mol^{-1} cm^{-1}). This solution was then acidified (to pH 3.0) with trifluoroacetic acid, and the ligand was recovered by preparative HPLC (major fraction R_t = 13.7 min). The product was confirmed as the dimer, (GCH−CO_2H)$_2$: $C_{22}H_{32}N_{10}O_8S_2$; MW = 628.68; ^{1}H NMR (D_2O) δ = 2.9−3.3 (4H, m, β-cys, β-his), 3.82 (2H, s, gly), 4.6−4.7 (2H, m, α-cys, α-his), 7.25 (1H, s, C_4H-his), 8.59 (1H, s, C_2H-his) ppm; FAB-MS *m*/*z* = 629 (MH^+).

[Glycyl(*S*-*tert*-butylcysteinyl)histidine]nickel(II), Ni{GC(*t*Bu)H−CO_2H}: $C_{15}H_{23}N_5O_4SNi$; MW = 428.13; λ_{max} (upon initial formation, pH 7.0) = 422 nm (102 dm^3 mol^{-1} cm^{-1}); λ_{max} (upon exposure to air) = growth of new bands at 321 and 383 nm ($\sim 10^3$−10^4 dm^3 mol^{-1} cm^{-1}).

(Lysylcysteinylhistidinecarboxamide)nickel(II), Ni(KCH−$CONH_2$): $C_{15}H_{25}N_7O_3SNi$; MW = 442.16; λ_{max} (upon initial formation, pH 7.2) = 328 nm (480 dm^3 mol^{-1} cm^{-1}), 462 nm (140 dm^3 mol^{-1} cm^{-1}); λ_{max} (upon standing in air) = 420 nm (76 dm^3 mol^{-1} cm^{-1}); ^{1}H NMR paramagnetically broadened.

(Glycylcystinylhistidinecarboxamide dimer)dinickel(II), Ni$_2$-(GCH−$CONH_2$)$_2$: $C_{22}H_{30}N_{12}O_6S_2Ni_2$; MW = 740.06; λ_{max} = 420 nm (82 dm^3 mol^{-1} cm^{-1} per nickel); ^{1}H NMR (D_2O) δ = 2.91 (2H, m, gly), 3.28 (1H, dd, β-his), 3.35 (2H, dd, β-cys), 3.76 (1H, dd, β-his),

4.16 (1H, m, α-cys), 4.25 (1H, dd, α-his), 6.95 (1H, s, C_4H-his), 7.53 (1H, s, C_2H-his) ppm; FAB-MS no discernible molecular ion; ES-MS no discernible molecular ion.

(Lysylcystinylhistidinecarboxamide dimer)dinickel (II), Ni_2-$(KCH-CONH_2)_2$: $C_{30}H_{48}N_{14}O_6S_2Ni_2$; MW = 882.30; λ_{max} = 422 nm (76 dm^3 mol^{-1} cm^{-1} per Ni); ^{1}H NMR (D_2O) δ = 1.4−1.8 (6H, m, β,γ,δ-lys), 2.88 (1H, dd, α-lys), 3.03 (2H, t, ϵ-lys), 3.20 (1H, dd, β-his), 3.27 (2H, m, β-cys), 3.65 (1H, dd, β-his), 3.92 (1H, m, α-cys), 4.07 (1H, t, α-his), 6.89 (1H, s, C_4H-his), 7.24 (1H, s, C_2H-his) ppm; FAB-MS m/z = 883 (MH^+); ES-MS m/z = 902.9 (MNa^+).

(Glycylcystinylhistidine dimer)dinickel(II), $Ni_2(GCH-CO_2H)_2$: $C_{22}H_{28}N_{10}O_8S_2Ni_2$; MW = 742.03; λ_{max} = 424 nm (66 dm^3 mol^{-1} cm^{-1} per nickel).

Results

Synthesis and Characterization of C-Terminal Carboxamide Complexes. A series of tripeptides was prepared by manual solid-phase synthesis using N-terminal Fmoc protection and acid-labile, side-chain protection according to established procedures.[14] All peptides contained either glycine or lysine at the first position, cysteine at the second position, and histidine at the third position, with either a carboxylate ($-CO_2H$) or carboxamide ($-CONH_2$) C-terminus. Three alternative forms of the cysteine residue were synthesized. These included a free thiol, an *S-tert*-butyl-protected thiol, or a disulfide-linkage to afford a dimeric peptide. The disulfide-bridged peptide dimers were prepared by oxidative cleavage of the corresponding *S-tert*-butyl-protected peptides using thallium(III) trifluoroacetate.[16] Dimeric species (hexapeptides) were readily identified by significant changes in the position and multiplicity of the β-cysteine ^{1}H NMR signal, which was shifted from a narrow doublet around 2.9 ppm for the monomer to a broad multiplet around 3.2 ppm for the dimer. Further confirmation of dimer formation was obtained by FAB-MS analysis.

The syntheses resulted in a total of nine novel metal-binding units for study, as illustrated in Table 1 and Figure 2 (with designated abbreviations), six of which were carboxamide-terminal peptides. The purity and identity of these species were confirmed by ^{1}H NMR spectroscopy,[17] analytical HPLC, and FAB mass spectrometry.

Nickel(II) complexes of these ligands were prepared by mixing molar equivalents of nickel chloride or nickel acetate with the appropriate ligand in deionized water and adjusting the pH of the solution to a value between 7.0 and 8.0 to ensure deprotonation of the peptide amide nitrogens for coordination. The thiol-protected ligand with a carboxamide C-terminus, GC(*t*Bu)H−$CONH_2$, was studied initially, since this complex was expected to be stable in the presence of dioxygen. Upon reaction with nickel(II), this peptide gave rise to a yellow species with a single UV maximum at 420 nm (102 dm^3 mol^{-1} cm^{-1}) which could be isolated and characterized by ^{1}H NMR and FAB mass spectrometry, confirming the formation of a simple diamagnetic, square-planar 1:1 Ni{GC(*t*Bu)H−$CONH_2$} complex (Figure 3). The cyclic voltammogram of this species at pH 6.3 exhibited a quasi-reversible oxidation at +0.77 V (vs Ag/AgCl, scan rate = 0.1 Vs^{-1}), which is appropriate for nickel tripeptide complexes of the XXH type.[2]

Reaction of $NiCl_2$ with the free thiol-containing ligand GCH−$CONH_2$ at pH 7 under argon gave rise to a red solution which exhibited UV maxima at 324 (ϵ = 438 dm^3 mol^{-1} cm^{-1}) and 464 (ϵ = 93 dm^3 mol^{-1} cm^{-1}) initially. The ^{1}H NMR of this material was paramagnetically broadened, and the cyclic voltammogram did not show any appreciable features. A solution

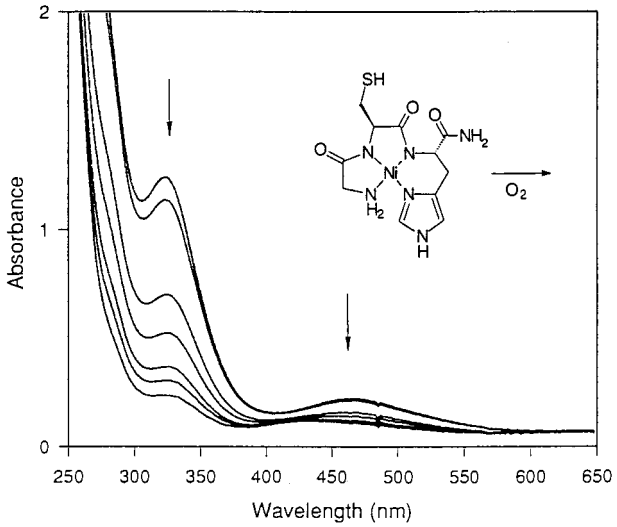

Figure 4. UV−vis spectra illustrating oxidation of Ni(GCH−$CONH_2$) over time: 0, 1, 10, 20, 60, 120, and 360 min (in descending intensity, respectively).

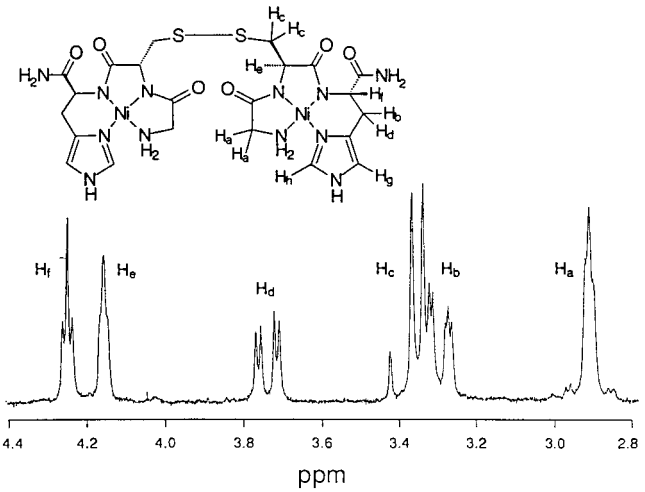

Figure 5. ^{1}H NMR spectrum (D_2O) of α- and β-proton signals for disulfide-bridged $Ni_2(GCH-CONH_2)_2$.

of this species was found to be very sensitive to dioxygen and, upon exposure to air, rapidly turned yellow and exhibited a single UV maximum at 420 nm (ϵ = 80 dm^3 mol^{-1} cm^{-1}). The UV spectra illustrating these changes are presented in Figure 4. The oxidized solution was freeze-dried, and the residue gave a well-defined ^{1}H NMR spectrum. Variation in pH did not effect any change back to the red material. The yellow product also exhibited a quasi-reversible oxidation at +0.82 V (vs Ag/AgCl, scan rate = 0.1 V s^{-1}). These observations suggested that some form of oxidation process had taken place, potentially via disulfide bond or sulfenate formation.

To distinguish between these two possibilities—oxidative dimerization vs oxygen atom transfer to form a sulfenate (R(S=O)H)—the dinuclear nickel(II) complex of the independently synthesized cystine dimer was prepared. Thus, reaction of $NiCl_2$ with the disulfide-bonded dimeric ligand $(GCH-CONH_2)_2$ gave rise to a yellow solution with a single UV maximum at 420 nm (ϵ = 82 dm^3 mol^{-1} cm^{-1}). The ^{1}H NMR spectrum of this material was not paramagnetically broadened and was *identical* to the spectrum obtained from the $NiCl_2$ + GCH−$CONH_2$ reaction mixture upon standing overnight. Figure 5 illustrates the region of the ^{1}H NMR containing the α and β signals for the dimeric complex. The distinctive doublet of doublets

(17) Bal, W.; Lukszo, J.; Jezowska-Bojczuk, M.; Kasprzak, K. S. *Chem. Res. Toxicol.* **1995**, *8*, 683.

centered at 3.35 ppm is due to the β-cysteine methylene protons adjacent to the disulfide bond.

For further confirmation of this chemistry, the reactions of the lysine-containing peptide ligands KC(*t*Bu)H−$CONH_2$, KCH−$CONH_2$, and $(KCH-CONH_2)_2$ with $NiCl_2$ were examined and found to be essentially the same as those of the glycine-containing ligands GCH−$CONH_2$ and its cystine dimer $(GCH-CONH_2)_2$. Accordingly, the nickel(II) complex of KCH−$CONH_2$ exhibits UV maxima at 328 ($\epsilon = 480$ dm^3 mol^{-1} cm^{-1}) and 462 nm ($\epsilon = 140$ dm^3 mol^{-1} cm^{-1}) initially, although these bands collapse to give one maximum at 420 nm ($\epsilon = 76$ dm^3 mol^{-1} cm^{-1}) upon formation of $[Ni_2(KCH-CONH_2)_2]$. The lysine-containing peptides and their complexes exhibited higher intensity molecular ions (MH^+) in the FAB mass spectra, and no evidence for sulfenate formation could be discerned.

Synthesis and Characterization of C-Terminal Carboxylate Peptide Complexes. Attention was next focused on the protected-thiol ligand with a *carboxylate* terminus, GC(*t*Bu)H−CO_2H. The nickel complex of this peptide exhibited a single UV maximum at 422 nm ($\epsilon = 102$ dm^3 mol^{-1} cm^{-1}) initially; however, when the complex was allowed to stand in air, two new bands appeared at 321 and 383 nm, with extinction maxima of ca. 10^3-10^4 dm^3 mol^{-1} cm^{-1}. This behavior is characteristic of the autoxidative decarboxylation process which has been reported previously[12] and was not investigated further. These data clearly demonstrate that the presence of the *S-tert*-butyl-protected thiol group did not significantly affect the complexation and autoxidation properties of the peptide derivatives in the present study.

Reaction of $NiCl_2$ with the tripeptide GCH−CO_2H was of particular interest because this ligand has both a free cysteine thiol and a carboxylate terminus. As shown above, either one of these moieties could undergo spontaneous reaction in the presence of air after complexation of nickel(II). In the event, reaction of $NiCl_2$ with GCH−CO_2H afforded an initially red solution with UV maxima at 322 ($\epsilon = 507$ dm^3 mol^{-1} cm^{-1}) and 446 nm ($\epsilon = 149$ dm^3 mol^{-1} cm^{-1}). The color of the aqueous solution changed to yellow upon exposure to air, with a concomitant collapse of the bands in the UV spectrum to give one band at 420 nm ($\epsilon = 73$ dm^3 mol^{-1} cm^{-1}). Interestingly, there was also a transient UV band at 360 nm during this process ($\epsilon \sim 400$ dm^3 mol^{-1} cm^{-1}), which may be indicative of a Ni(III) intermediate. Upon acidification (to pH 3.0) with trifluoroacetic acid and HPLC purification, it was possible to recover the ligand from this reaction in ca. 90% yield. ^{1}H NMR spectroscopy and FAB mass spectrometry confirmed the isolation of the disulfide-bridged $(GCH-CO_2H)_2$ ligand, which was also synthesized independently. There was no evidence of any decarboxylation reaction having taken place. After the initial dimerization of the Ni(GCH−CO_2H) complex to afford the Ni_2-$(GCH-CO_2H)_2$ complex (Figure 6), there appeared to be no further reactivity with O_2. This was confirmed by preparing the nickel(II) complex of the dimerized ligand $(GCH-CO_2H)_2$, which also exhibited no reaction with O_2.

Discussion

The following observations are significant in the reactions described above. *Carboxamide*-terminal tripeptide ligands containing an *S-tert*-butyl-protected cysteine residue behave similarly to the well-studied Ni^{II}(GGH−$CONH_2$)-type complexes,[2,3] providing air-stable, diamagnetic, square-planar nickel(II) complexes with N_4 ligation as shown in Figure 3. The analogous *carboxylate*-terminal tripeptides with a side-chain-protected cysteine residue behave similarly to other non-cysteine-containing peptides in undergoing spontaneous autoxidative decarboxylation after complexation to nickel(II).

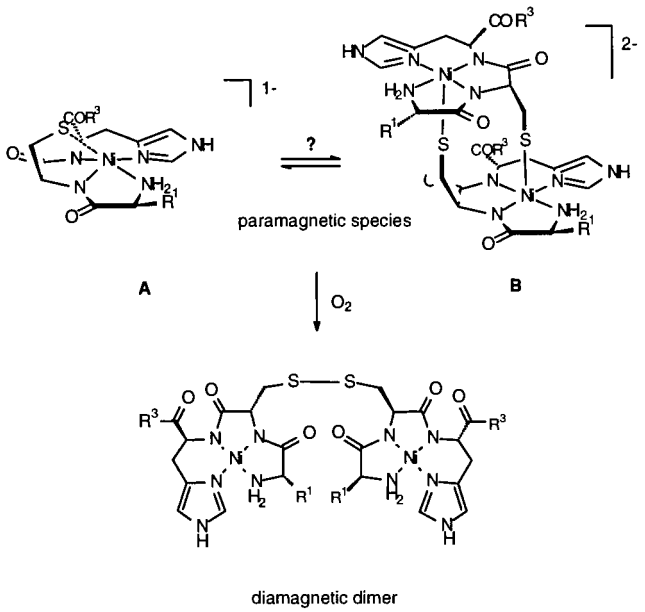

Figure 6. Proposed mechanism of oxidative dimerization of Ni^{II}XCH-type complexes with dioxygen. The initially formed species, in the absence of O_2, are paramagnetic when a free cysteine thiol group is present. Sulfur coordination to the nickel center, either in an intramolecular fashion (**A**) or via formation of a dimeric complex (**B**), might then lead, on exposure to O_2, to the characterized disulfide dimer, a diamagnetic species with square-planar coordination.

Table 2. . Reactions of Nickel(II) Complexes with Dioxygen

ligand	oxidative decarboxylation	S−S dimerization
GC(tBu)H−$CONH_2$	n.a.[a]	no
GCH−$CONH_2$	n.a.[a]	yes
KC(*t*Bu)H−$CONH_2$	n.a.[a]	no
KCH−$CONH_2$	n.a.[a]	yes
GC(*t*Bu)H−CO_2H	yes	no
GCH−CO_2H	no	yes
$(GCH-CO_2H)_2$	no	n.a.[a]

[a] Not applicable.

In contrast, all complexes containing unprotected cysteine thiols were reactive with O_2 to yield disulfide dimers (Table 2). Thus, coordination of nickel(II) to a free thiol-containing tripeptide XCH−$CONH_2$ in the absence of O_2 initially gives rise to a paramagnetic species which is likely a 5-coordinate or potentially a severely distorted 4-coordinate complex. Simple ball-and-stick models suggest that the cysteine thiol moiety is incapable of strong binding to the same metal ion as the other donors in the same peptide without perturbing the square-planar N_4 ligation of the peptide (Figure 6, **A**). An attractive explanation is formation of a dimeric intermediate (Figure 6, **B**), in which two thiolates ligated to redox-active nickel centers are brought into close proximity. Oxidation of a coordination dimer of this type by dioxygen, potentially via a Ni(III) intermediate, would be expected to be facilitated by the intramolecular nature of the dimerization. The final product is a disulfide-bridged dinuclear nickel complex as illustrated in Figure 5. The structure of the final product has been confirmed by independent preparation of the disulfide-bridged ligand, followed by metal insertion, which leads to identical NMR, UV, and MS spectral data. Most importantly, this process is spontaneous in the presence of air at neutral pH. Under these conditions, the rate of formation of intermolecular disulfide

bonds for simple thiols is normally very slow, requiring hours to days for completion.[18] Transition metals are known to catalyze the oxidation of thiols,[19] with a maximum rate usually being attained between pH 8 and 9. In fact, the rapid and total conversion of XCH tripeptides to the corresponding dimers in the presence of nickel(II) and dioxygen without formation of other oxidized side products represents an effective means of generating this class of dimeric peptides. The alternative synthesis of the disulfide-bridged dimers via thallium(III)-mediated oxidation required further HPLC purification.

Maroney and co-workers have reported a related ligand oxidation reaction in a nickel complex with bridging thiolate ligands between metal centers.[20] However, this product (which exhibited a coordinated disulfide) was only formed upon chemical or electrochemical oxidation. In other work, Darensbourg et al. have described the oxidation of coordinated thiols by O_2 to afford metallosulfoxides and metallosulfones.[21] Such species have not been observed in the cysteine-containing peptide complexes studied here. Rather, rapid disulfide bond formation always resulted from aerobic oxidation of nickel(II) peptides containing unprotected cysteine thiols. The general observations are summarized in Figure 7.

The autoxidation reaction of the complex containing both an unprotected cysteine thiol and a carboxylate terminus was particularly interesting. Exposure of Ni(GCH−CO_2H) to air resulted in disulfide bond formation rather than decarboxylation. This observation was initially surprising, since this was the first carboxylate-terminal XXH-type tripeptide prepared that did not appear to undergo a typical decarboxylation reaction with nickel(II). Apparently, the dimerization reaction of this complex via disulfide bond formation is more rapid than a potential decarboxylation, and after dimerization, the decarboxylation is inhibited in some fashion. This may be because O_2 binding (believed to be important in the decarboxylation) does not take place in the same manner in the dimer as in monomers. Alternatively, if O_2 does bind, the $O_2^{\bullet-}$ that forms may short circuit the decarboxylation by acting as a reductant for the S−S bond,[22] creating a rapid cycle of oxidation and reduction of the thiols that does not allow the slow, oxidative decarboxylation to compete. Whatever the mechanism, it should be possible to observe autoxidative decarboxylation if dimerization is somehow inhibited, either by steric effects or through association with other species (e.g., DNA).

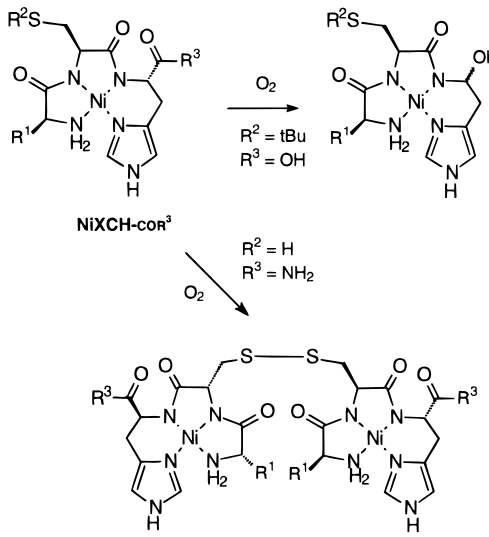

Figure 7. Nickel(II) complexes of the XCH motif reacting with dioxygen via two pathways: (top) oxidative decarboxylation when the C-terminus is a carboxylate group and the cysteine thiol is protected; (bottom) oxidative disulfide bond formation to generate the cystine dimer when a free thiol is present.

Our motives for incorporating the positively charged lysine group were three-fold: to aid in detection via mass spectrometry, to potentially facilitate crystallization, and to increase affinity toward anionic DNA. Double deprotonation of peptide ligands through coordination to nickel(II) generates neutral complexes, and it was found that these neutral species did not "fly" well in FAB or electrospray mass spectrometry experiments, especially when a disulfide bond was present. Furthermore, single crystals of these complexes (for X-ray diffraction purposes) were difficult to obtain. The positively charged, lysine-containing peptide dimer $(KCH-CONH_2)_2^{2+}$ did, in fact, give a much more intense FAB molecular ion peak than the neutral $(GCH-CONH_2)_2$ dimer. It was also possible to observe the positively charged $[Ni_2(KCH-CONH_2)_2]^{2+}$ complex by both FAB and electrospray mass spectrometry, while the neutral species $Ni_2(GCH-CONH_2)_2$ was not observed. Attempts to crystallize either the monomeric or dimeric complexes have so far proven unsuccessful. Current studies focus on the reactivity of these complexes with nucleic acids, where the positively charged lysine residue should increase the affinity of these species for the anionic phosphoribose backbone of DNA and RNA. One might anticipate that association of a nickel tripeptide complex with a nucleic acid would inhibit the cysteine dimerization reaction, potentially allowing the nucleic acid to intercept reactive intermediates formed upon oxidation with O_2.

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