Dioxygen-induced Decarboxylation and Hydroxylation of [Ni^{II}(Glycyl-Glycyl-L-Histidine)] Occurs *via* Ni^{III}: X-Ray Crystal Structure of [Ni^{II}(Glycyl-Glycyl-α-hydroxy-D,L-Histamine)]·3H_2O

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Electrochemical and EPR studies show that the dioxygen-induced decarboxylation and hydroxylation of [Ni^{II}(GGH-H_{-2})]$^-$, where GGH is glycyl-glycyl-L-histidine (HL), in aqueous solution occurs *via* a Ni^{III} intermediate; the product [Ni^{II}(Gly-Gly-α-hydroxy-D,L-histamine-H_2)]·3H_2O is shown by X-ray crystallography to contain square-planar Ni^{II} coordinated to the terminal amino group [Ni–N, 1.932(3) Å], two deprotonated amide N's [1.884(3) and 1.831(3) Å] and imidazole δN [1.908(3) Å].

There is much current interest in the ability of Ni^{II} and Cu^{II} complexes of the tripeptide Gly–Gly–L–His (GGH) to catalyse the oxidation and cleavage of DNA.[1,2] These metal ions are thought to bind with square-planar coordination geometry *via* the terminal amino N, two deprotonated amide N's and the Nδ of histidine, and to mimic the *N*-terminal Ni^{II} and Cu^{II} binding site of serum albumin. The X-ray structure of [Cu^{II}(GGH-N-methyl-amide-H_{-2})] has confirmed this mode of binding,[3] but few crystal structures of Ni^{II} peptide complexes have been determined.[4,5] Recent potentiometric studies have shown that [Ni^{II}(GGH-H_{-2})]$^-$ **1** is the major species present in millimolar aqueous solutions of Ni^{II} and GGH at pH values > *ca.* 6.4.[6] Complex **1** is known to be highly sensitive to O_2 in aqueous solution.[7,8] We show here by electrochemical studies and electronic absorption and electron paramagnetic resonance (EPR) spectroscopy that the reaction involves a Ni^{III} intermediate, and establish by X-ray crystallography that the Ni^{II} peptide product is decarboxylated and hydroxylated at His Cα.

Yellow solutions containing Ni^{II} and GGH (Sigma) in a 1 : 1 mol ratio (5 mmol dm^{-3}) in 0.1 mol dm^{-3} phosphate buffer pH 6.5 to 8 gave rise to yellowish-brown crystals on standing for *ca.* 48 h at ambient temperature in capped tubes. The X-ray crystal structure† of this product **2** shows that it is a square-planar Ni^{II} complex [N(1)–Ni–N(4) 95.09(13)°, N(2)–Ni–N(1) 96.12(13)°, N(3)–Ni–N(2) 84.30(13)°, N(3)–Ni–N(4) 84.49(13)°] with four N ligands provided by the terminal NH_2 [Ni–N(4) 1.932(3) Å], two strong bonds to deprotonated amide N's [Ni–N(2) 1.884(3), Ni–N(3) 1.831(3) Å], and Nδ of His [1.932(3) Å], giving two five-membered and one six-membered chelate rings, Fig. 1. The patterns of bond lengths and bond angles are closely comparable with those reported for Na_2[Ni^{II}(GGGG-H_{-3})]·8H_2O[5] and [Cu^{II}(GGH-N-methyl amide-H_{-2})]·H_2O,[3] however, in contrast to the latter structure, there are no short axial Ni–O contacts. The Ni–N(peptide) bond lengths are shorter than in the bischelated octahedral Ni^{II} complexes Na_2[Ni^{II}(GG-H_{-1})$_2$]·8H_2O and 9H_2O (1.99–2.02 Å),[4] but comparable with those in the square-planar complexes [Ni^{II}(L-Pro-amide-H_{-1})$_2$]·2H_2O[9] and Na_2[Ni^{II}(GGGG-H_{-3})]·8H_2O (1.83–1.87 Å).[5]

It is clear that autoxidation of **1** leads to the decarboxylation and hydroxylation at Cα of coordinated His, and also to racemization since the X-ray structure of **2** is centrosymmetric, in agreement with the suggestions of Sakurai and Nakahara.[8] Complex **2** has a characteristic His CαH peak at 5.14 ppm, and ^{1}H NMR studies showed that in dilute solutions of **1** (0.6 mmol dm^{-3}) autoxidation was almost complete (90%) after 32 h, whereas in more concentrated solutions it was only partial (15% after 24 h), consistent with the O_2 content of the solution being a limiting factor in the reaction. The presence of HCO_3^- (25 mmol dm^{-3}) also hindered the autoxidation. The UV–VIS spectrum of **1** is characterized by a low intensity d-d band at *ca.* 425 nm (ε 160 dm^3 mol^{-1} cm^{-1}), and the autoxidized product by an additional intense absorption band at 305 nm (ε 5930 dm^3 mol^{-1} cm^{-1}). The intensity of this peak is dependent on the buffer, the oxygen concentration and the pH, and reaches maximum intensity *ca.* 23 h after exposure to air. No CD band was associated with this peak showing that **2** is optically inactive, in agreement with the X-ray structure of **2**.

Electrolysis of [Ni^{II}(GGH-H_{-2})]$^-$ **1** results in the development of a new band at 305 nm which on standing converts to the same spectral product as that from autoxidation (*i.e.* **2**). During controlled electrode-potential electrolysis the absorbance at 305 nm increased almost linearly for 2 h (0.017 absorbance units min^{-1}, 1 mmol dm^{-3} solution). EPR spectroscopy showed that the immediate products were Ni^{III} complexes with major peaks typical of a tetragonally-distorted octahedral complex,[10,11] $g_\perp$ 2.25, $g_\parallel$ 2.01; pH 6.5. No EPR signals were seen unless the solutions from electrolysis were frozen rapidly (77 K). Formation of Ni^{III} is a common feature in oxidations of square-planar Ni^{II} peptide complexes,[12] and similar EPR spectra have been reported to arise from

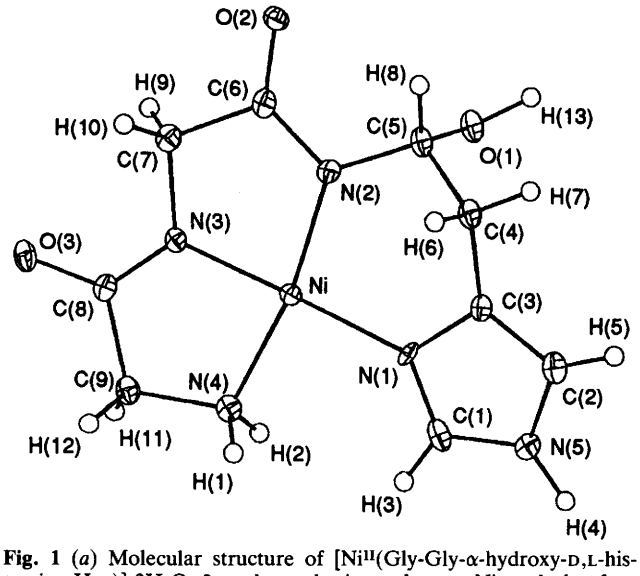

Fig. 1 (*a*) Molecular structure of [Ni^{II}(Gly-Gly-α-hydroxy-D,L-histamine-H_{-2})]·3H_2O **2** and numbering scheme. Ni and the four coordinated N atoms are planar to within ±0.023 Å; the two 5-membered rings are coplanar with the coordination plane to within 1.2°, and the imidazole ring is tilted by 14.3°. There is an extensive series of intermolecular H-bonds in the crystal lattice (not shown).

oxidation of **1** with $IrCl_6^{2-}$.[13] Electrochemical oxidation of the Cu^{II} complex of Gly–Gly–His also gives an α-hydroxyhistamine product by oxidative decarboxylation.[14,15]

Under the conditions of cyclic voltammetric measurements the Ni^{II}–Ni^{III} redox process was reversible, the difference between cathodic and anodic peaks for **1** (0.1 mol dm^{-3} phosphate, 1 mol dm^{-3} $NaClO_4$, pH 7 or 7.7) being proportional to the square-root of the scan rate.[16] The mid-point potential, 0.73 V (SCE), was similar to values reported previously.[17]

Further reactions of the autoxidized product **2** appeared to occur on long standing. For example, after 2 d ^1H NMR spectra show additional peaks, and weak EPR signals are seen with *g* values different from those seen for electrochemical solutions. Also in the UV spectrum, a new band appears at 370 nm over a period from 1–4 d.

We can begin to formulate a mechanism by which **2** is formed from **1**. It seems likely that complex **1** reacts initially with O_2 to generate Ni^{III} and superoxide, O_2^-. Dismutation of O_2^- could produce H_2O_2, and we have evidence for the production of both O_2^- and H_2O_2 during such reactions of **1**. Both **1** and solutions of air-oxidized **1** diminished the flux of O_2^- generated during the oxidation of hypoxanthine with xanthine oxidase [>50% at 0.1 mmol dm^{-3}, pH 7.4] as measured by cytochrome c reduction.[18] The formation of H_2O_2, as monitored by the production of a chromophoric product from *o*-dianisidine in the presence of horse radish peroxidase, was dependent on the concentration of **1**. Solutions of complex **1**, but not Ni^{2+} ions alone, were found to catalyse the rapid disproportionation of H_2O_2, even at low concentrations of **1** (5 μmol dm^{-3}), with release of O_2^- (reduction of nitroblue tetrazolium[19]), and O_2 production (Rank oxygen electrode).

Superoxide could act as a reductant for Ni^{III}, as proposed previously,[20] and disproportionation of H_2O_2 could occur *via* a $Ni^{IV}O$ intermediate, a potential source of hydroxyl radicals. Hydroxyl radical-mediated abstraction of a hydrogen atom from the α-carbon of amino acids is a common oxidative pathway.[21] However, our mannitol scavenging experiments suggested that release of hydroxyl radicals was minimal, in agreement with the findings of Cotelle *et al.*[22] Also in ^1H NMR studies of the autoxidation of **1** in D_2O, deuterium does not appear to be incorporated at His Cα. Loss of CO_2 from the initial Ni^{III}–superoxide complex (which we observed to be stoichiometric) to form a His Cα-centred free radical, followed by peroxide formation seems more likely. Such a peroxide might undergo oxygen atom abstraction to yield **2**, or H_2O_2 elimination to give a double bond at His Cα-Cβ. Indeed a crystalline product with the latter structure has been isolated from autoxidation of $[Cu^{II}(GGH\text{-}H_{-2})]^-$,[23] and ^1H NMR spectra[24] of the product from reaction of **1** with excess O_2 suggest that under these conditions the latter pathway can predominate for nickel.

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Footnotes

† *Crystal data* for $C_9H_{19}N_5O_6Ni$, M = 352.0, triclinic, a = 8.777(3), b = 8.994(2), c = 10.358(3) Å, α = 64.59(2), β = 86.00(3), γ = 69.35(2)°, space group $P\bar{1}$, Z = 2, U = 687.9 $Å^3$, D_c = 1.699 g cm^{-3}, $F(000)$ = 368, μ(Mo-Kα) = 20.285 cm^{-1}. Data collection: unit cell dimensions and intensity data were obtained at 293 K using an Enraf-Nonius diffractometer and area detector with graphite monochromated Mo-Kα radiation, following previously described procedures[25] (D = 50 mm, $2\theta_D$ = 20°). A total of 2173 reflections were measured, of which 1917 were unique. Solution of structure: the heavy method[26] was used and refined by full matrix least squares methods (SHELX-93).[27] Absorption corrections were applied at the isotropic refinement stage using the DIFABS procedure[28] adapted for FAST geometry.[29] H atoms were allowed to ride on their parent carbon atoms in their calculated positions (C–H = 0.96 Å); those of water molecules which were located in the difference map were refined [the W(O)–H distance was constrained to 1 Å and H–W(O)–H angle to 108°]. The final $R1$ and $wR2$ values are 0.034 and 0.089, respectively.

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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