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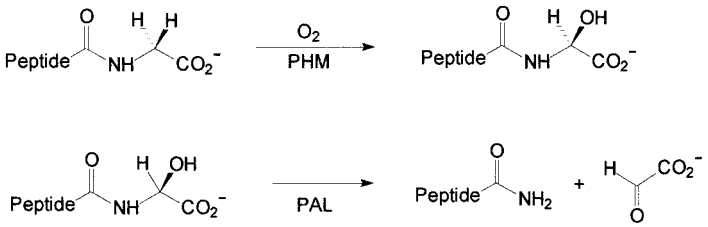
Selective α-Carbon Hydroxylation of Glycine in Nickel(II)–Cyclotetrapeptide Complexes by Oxygen**

Katharina Haas, Harald Dialer, Holger Piotrowski, Jan Schapp, and Wolfgang Beck*

Dedicated to Professor Ernst-Ludwig Winnacker on the occasion of his 60th birthday

The copper-containing bifunctional enzyme peptidylglycine α-amidating monooxygenase (PAM) catalyzes the bioactivation of peptide hormones by amidation.[1] In the first step the copper-containing peptidylglycine α-hydroxylating monooxygenase (PHM) catalyzes the stereospecific hydroxylation of the C-terminal glycine to give an α-hydroxyglycine peptide (Scheme 1). A second enzyme, the peptidyl-α-hydroxyglycine

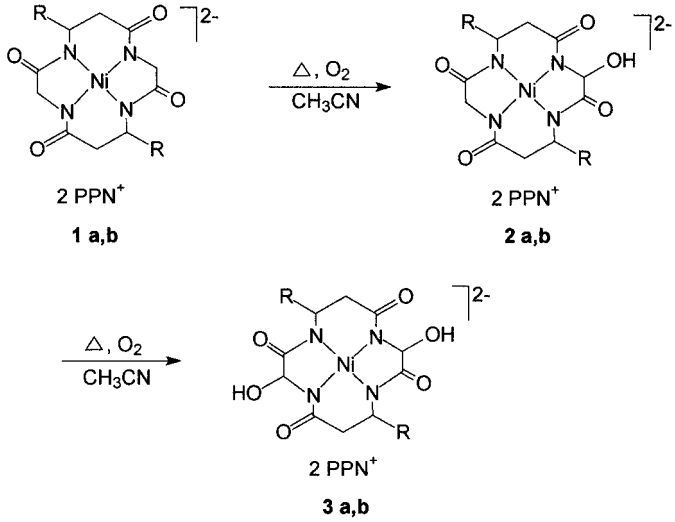


Scheme 1. Steps in the bioactivation of peptide hormones.

α-amidating lyase (PAL) generates the bioactive peptide amide and glyoxylate under α-C–N cleavage. Some reactions which are related to these transformations have been reported:

- The reactions of nickel(II) complexes of open-chain peptides with oxygen occur via nickel(III) intermediates to give oxidative cleavage of the terminal glycine α-C–N bond.[2]
- The nickel(II) complex [Ni^{II}(Gly-Gly-His)] is decarboxylated and α-C hydroxylated by oxygen via a nickel(III) intermediate.[3] This reaction can lead to the formation of DNA adducts.[4]
- α-C hydroxylation of the amino acid component in Ni^{II} Schiff base complexes from salicylaldehyde and α-amino acid esters which is followed by C–N cleavage was observed by Paul Pfeiffer,[5] one of the fathers of coordination chemistry.
- The copper(II)-mediated α-hydroxylation of an *N*-acylglycine,[6] the glycine-specific α-C–N cleavage of a dipeptide by nickel and copper peroxide,[7] and especially the α-hydroxylation of glycine-containing dipeptide ligands in a Co^{III} terpyridine complex by oxygen[8] are models for PAM.

The cyclocondensation of non-activated dipeptide esters at Cu^{II}, Ni^{II}, and Pd^{II} templates is a simple and attractive synthesis of cyclotetrapeptides.[9, 10] Cyclotetrapeptides are of interest for, among others, the formation of optically active, C-substituted cyclams. Following the method which was introduced by Neumann et al.[11] for the reduction of cyclopentapeptides, the carbonyl functions of cyclotetrapeptides can be reduced with $LiAlH_4$.[12] We have now found that α-hydroxylation of the glycine components of the cyclopeptide ligands in the nickel(II) complexes **1a**, **b** occurs stepwise on heating the complexes in acetonitrile in air to give the compounds **2** and **3** (Scheme 2). The monohydroxylated



Scheme 2. α-C hydroxylation of the cyclotetrapeptide complex **1** by atmospheric oxygen. **1a**–**3a**: R = H; **1b**–**3b**: R = CO_2Me.

species **2a**-$(PPN)_2$ (PPN = triphenyl[triphenylphosphoranylidene)amino]phosphonium) precipitates from the solution as orange crystals, which on heating in a saturated acetonitrile solution react with the oxygen in the air to afford the dihydroxylated complex **3a**-$(PPN)_2$. Because of the strong bonding of the cyclotetrapeptide to the Ni^{II} center an α-C–N cleavage does not take place and the rare α-hydroxyglycine unit remains intact.

In the mass spectra (FAB or ESI) of **2** and **3** the ions [M^{2-}] (171, **3a**) and [$M^{2-}+H^+-H_2O$] (M = **2a**, **b**, **3b**) were detected. The transformation of the C_2-symmetric complex **1a** to the

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asymmetric compound **2a** can be clearly detected in the ^{1}H and ^{13}C NMR spectra. Thus, for the amide carbon atoms two signals appear for **1a** and four signals for **2a** in the ^{13}C NMR spectra. The characteristic signal of the α-hydroxylated carbon atom appears downfield ($\delta = 90.2$ ppm). In the IR spectra intensive OH absorptions are observed at 3405 cm^{-1}. Because the compounds **2** are formed as enantiomers, the complexes **3** are formed as the corresponding diastereoisomers, as can be seen from the several overlapping sets of signals in the ^{1}H NMR spectra of **3a**.

The X-ray structure determination of **2a**-$(PPN)_2$[13] which was only successful after several attempts confirms the composition of the molecule. The nickel(II) ion is surrounded by the four N atoms of the cyclopeptide in a square-planar fashion. The hydroxy group, which results from the oxidation of the α-C atom, can be clearly detected (Figure 1). The complex anion crystallizes with two PPN counterions and with about 8.5 water molecules from the aqueous methanol solution. The solution of the structure was hampered by a low quality of the crystal and disorder of the complex in the crystal.

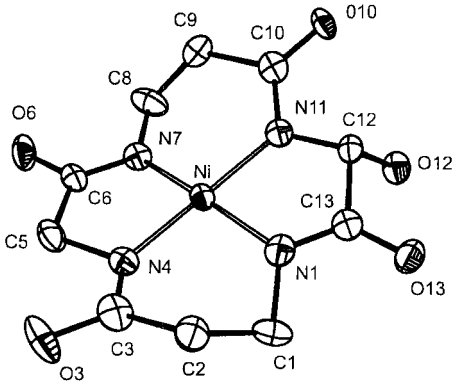
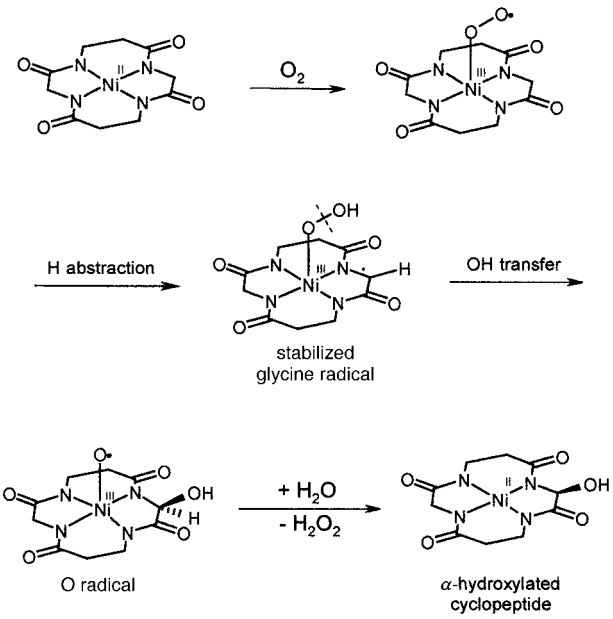


Figure 1. Molecular structure of the complex anion of **2a** (thermal ellipsoids set at 25% probability, hydrogen atoms omitted for clarity). The disordered molecule with the higher site-occupation factor is shown. The hydroxy group generated by oxidation is on C12. Selected bond lengths [pm] and angles [°]: Ni-N1 191.4(12), Ni-N4 188.1(12), Ni-N7 181.1(10), Ni-N11 187.7(12); N1-Ni-N4 94.2(5), N4-Ni-N7 86.6(5), N7-Ni-N11 97.6(5), N11-Ni-N1 82.3(5).

Solutions of **1** in acetonitrile turn deep red on exposure to the air. In the first step nickel(III) complexes are formed (Scheme 3)—confirmed by cyclic voltammetry—as also observed for the oxidation of Ni^{II} complexes with deprotonated open-chain peptide ligands.[14] The cyclic voltammogram of **1a** in acetonitrile shows perfect reversible redox behavior with the Ni^{II}/Ni^{III} event at a normal potential $E^0 = 615$ mV. For the oxidation of Ni^{II} complexes of open-chain peptides E^0 values of 800–850 mV were measured in aqueous solution.[15] For the PHM reaction it was demonstrated by $^{18}O_2$ experiments that the hydroxy groups originate from air oxygen and not from water[1, 16] which could attack an *N*-acyl imine or a glycine α-C cation. Mass spectra of the product of **1a** and $^{18}O_2$ containing air[17] point to a complex of the composition $[Ni(cyclo\text{-}\alpha\text{-OH-Gly-}\alpha\text{-OH-}\beta\text{-Ala} - 4H^+)_2(NCCH_3)_2]^{2-}$ with ^{18}O-containing hydroxy groups. In relation to the suggested mechanism of the PHM transformation[1] we formulate the



Scheme 3. Proposed mechanism for the α-hydroxylation of **1**.

hydroxylation of **1** as shown in Scheme 3 in which a glycine radical functions as an intermediate. Glycine radicals have been frequently observed, for example, in polypeptides.[18] We have found that complex **1** is an active catalyst for the disproportionation of hydrogen peroxide and thus **1** could exhibit superoxide bismutase activity.[19] Unexpectedly hydroxylation of the glycine component of the copper(II) complex $[Cu(cyclo\text{-Gly-}\beta\text{-Ala-Gly-}\beta\text{-Ala} - 4H^+)](PPN)_2$[9] could not be observed.

Experimental Section

2a-$(PPN)_2$: **1a**-$(PPN)_2 \cdot 5H_2O$ (74 mg; 0.5 mmol) was dissolved in CH_3CN (10 mL). The solution was heated in air to 80 °C for 5 min and on cooling to room temperature orange needle-shaped crystals formed. The crystals were collected and recrystallized from methanol/water, washed with water, and dried in vacuo at 60 °C, yield 61%. IR (KBr): $\tilde{\nu} = 3405.7$ (vs, br OH), 1576.7 (vs), 1569.0 (vs), 1539.7 cm^{-1} (vs; amide-I); ^{1}H NMR (400 MHz, CD_2Cl_2): $\delta = 7.70 - 7.47$ (m, 60H, PPN^+), 5.39 (s, 1H, OH), 4.91 (s, 1H, CH), 3.36 (s, 2H, CH_2), 2.79 (m, 2H, CH_2CH_2), 2.70 (m, 2H, CH_2CH_2); 1.96 (m, 2H, CH_2CH_2), 1.91 ppm (m, 2H, CH_2CH_2); ^{13}C NMR (68 MHz, CD_2Cl_2): $\delta =$ 180.2, 179.5, 177.8, 175.9 (CONR), 84.2 (CHOH), 55.1 (CH_2), 38.9, 39.3, 39.5, 39.8 ppm (CH_2CH_2); MS (ESI): *m*/*z* (%): 309 (24) $[M^{2-}+H^+ - H_2O]$, 327 (100), 328 (52), 329 (38) $[M^{2-}+H^+]$; 864, 865, 866 $[M^{2-}+PPN^+]$; elemental analysis calcd (%) for $C_{82}H_{72}N_6O_5P_4Ni \cdot 6H_2O$ (1512.192): C 65.13, H 5.59, N 5.55; found: C 65.08, H 5.56, N 5.54.

3a-$(PPN)_2$: **2a**-$(PPN)_2$ (45 mg, 0.03 mmol) was dissolved in CH_3CN (10 mL). The solution was heated in air to 80 °C for 5 min and on cooling to room temperature an orange, crystalline precipitate formed which was separated, recrystallized from methanol/water, washed with water and dried in vacuo at 60 °C. Orange crystals, yield 61%. IR (KBr): $\tilde{\nu} = 3408$ (vs, br; OH), 1575.8 (vs), 1535.8 cm^{-1} (vs, br, amide-I); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.72 - 7.51$ (m, 60H, PPN^+), 5.13 (s, 2H, OH), 5.10 (s, 2H, OH), 3.65 – 3.55 (m, 2H, CHOH), 3.16 – 3.08 (m, 1H, CH_2CH_2), 2.92 – 2.83 (m, 1H, CH_2CH_2), 2.61 – 2.59 (m, 1H, CH_2CH_2), 2.15 – 1.99 ppm (m, 3H, CH_2CH_2); MS (ESI): *m*/*z* (%): 171 (4) $[M^{2-}]$, 325 (35) $[M^{2-}+H^+ - H_2O]$; 343 (100), 344 (67), 345 (51) $[M^{2-}+H^+]$, 880, 881, 882 $[M^{2-}+PPN^+]$; elemental analysis calcd (%) for $C_{82}H_{72}N_6O_6P_4Ni \times 5H_2O$ (1510.175): C 65.21, H 5.47, N 5.56; found: C 65.11, H 5.50, N 5.17.

2b-$(PPN)_2$: **1b**-$(PPN)_2 \cdot 7H_2O$ (20 mg, 0.012 mmol) were dissolved in CH_3CN (5 mL) and the solution heated to reflux several times. Needles

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of **2b** were formed as orange crystals, yield 5%; MS (negative ion FAB): *m/z* (%): 443 (19), 444 (12), 445 (8) [$M^{2-}+H^+$].

3b-$(PPN)_2$: **2b**-$(PPN)_2$ (20 mg, 0.012 mmol) were dissolved in CH_3CN (5 mL) and the solution heated to reflux several times, whereby the orange red solution turns dark red. On cooling the solution crystals of **3b** formed as orange needles, yield 69%; MS (negative ion FAB): *m/z* (%): 459 (100), 460 (31), 461 (9) [$M^{2-}+H^+$].

Labeling experiment: **1a**-$(PPN)_2$ (0.013 mmol, 20 mg) were dissolved in CH_3CN (5 mL) and the solution heated to reflux several times under a $^{16}O_2$/$^{13}O_2$ atmosphere. Orange needlelike crystals were obtained after removal of the solvent. MS (negative ion FAB): *m/z* : 461, 462, 463.

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[13] X-ray structure determination **2a**-$(PPN)_2$. Kappa-CCD (Bruker-Nonius), rotating anode, $Mo_{K\alpha}$ (graphite monochromator, $\lambda =$ 71.069 pm), direct methods (SIR97), refinement with full matrix on F^2 (SHELXL), H atoms at C in calculated positions, H atoms at O not considered. Geometrical analysis and graphics with PLATON and ORTEP. **2a**: $[NiC_{10}H_{12}N_4O_5][C_{36}H_{30}NP_2]_2 \cdot 8.5\,H_2O$, $M_r = 1557.18$, crystal size $0.34 \times 0.14 \times 0.02$ mm, triclinic, $P\bar{1}$, $a = 14.3402(2)$, $b =$ 14.7447(2), $c = 19.3789(3)$ Å, $\alpha = 79.0455(5)$, $\beta = 75.3656(6)$, $\gamma =$ 74.7252(5)°, $V = 3790.92(9)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.36423(3)$ g cm^{-3}, $T =$ 200 K, $\mu = 0.410$ mm^{-1}, 49 829 reflections collected, $2\theta_{max} = 45°$, 9877 unique reflections, Lp-correction, $R_{int} = 0.1105$, absorption correction (6 faces, min/max transmission = 0.9116/0.9918), average $\sigma(I)/I =$ 0.0629, 8165 reflections with $I > 2\sigma(I)$, $w^{-1} = \sigma^2(F_o) + (0.0718P)^2 +$ 26.8552*P*, $3P = \max(F_o^2; 0 + 2F_c^2)$, 1 molecule of water disordered, 2 molecules of water with site occupation factors (SOFs) < 1, the complex anion is disordered. The disorder can be described by a rotation of the ligand molecule around an axis through Ni perpendicular to the coordination plane. 986 parameters, no geometrical restraints, $R_1 = 0.1006$, $R_w(F^2) = 0.2311$, $S = 1.082$, max. residual electron density 1.295 e Å^{-3}. The high residual electron density near the second α-C atom presumably originates from traces of the dihydroxylated species.CCDC 179782 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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