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An unprecedented *trans*-oriented product from the cleavage of a dipeptide

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An unusual *trans* cleavage reaction was observed when *trans*-[Co(3,2,3-tet)Cl_2]Cl {3,2,3-tet = *N*,*N*′-bis(3-aminopropyl)ethylenediamine}, was allowed to react with β-alanyl-L-histidine (a bioactive dipeptide) in an aqueous medium at pH ~7.5 and 45 °C for 6 h.

Metal complexes that can cleave peptide bonds under mild condition are of potential interest due to their usefulness for structure–activity studies of proteins, investigation of protein structural domains and in converting large proteins into smaller fragments that are more amenable for sequencing.[1,2] Moreover, such reagents are also useful for the chemical modification or heavy atom labeling of proteins.[2,3] The cleavage reaction either follows an oxidative or a hydrolytic pathway and the latter is preferred because the oxidative process involves the high risk of damaging the protein chain. Cobalt(III) complexes are known to induce the hydrolysis of peptides and model studies were carried out to investigate the mechanism of the peptide cleavage reaction by using small peptide molecules.[4] Those studies involve *cis*-Co(III) complexes at a slightly basic pH which proceed by a *cis*-coordination to the peptide molecule and, subsequently, the peptide bond undergoes cleavage by the attack of the hydroxide anions.[5] The unsuccessful attempt to cleave the peptide bond by the use of *trans*-Co(III) complexes are reported in literature.[6] In this communication, we report a peptide bond cleavage reaction which is promoted by an unusual *trans*-Co(III) complex (*trans*-[Co(3,2,3-tet)Cl_2]Cl {3,2,3-tet = *N*,*N*′-bis(3-aminopropyl)ethylenediamine} (see ref. 7 for structure) and follows a different reaction pathway.† To the best of our knowledge, this is the first *trans*-oriented product obtained from the cleavage of peptide bonds. These results are important for a better understanding of the cleavage reaction mechanism(s) and should provide useful ideas for designing new active metal complexes for sequential and specific cleavage of protein matrices.

β-Alanyl-L-histidine (*carnosine*, found in relatively high concentrations in several body tissues—most notably in skeletal muscle, heart muscle, and brain, acts as an antioxidant and promotes wound healing)[8] was allowed to react with a half molar amount of *trans*-[Co(3,2,3-tet)Cl_2]Cl at pH ~7.5 and 45 °C for 6 h. The reaction mixture was spotted on a silica gel plate and eluted with CH_3OH–H_2O (1:1) mixture; the cobalt containing species remained on the starting line but the L-histidine fragment moved in the normal manner, which has been compared with the movement of known L-histidine solutions. The *trans* oriented cleavage product [Co(3,2,3-tet)(H-β-alanine)$_2$]$^{3+}$ **1** was isolated as the perchlorate salt from the solution. The L-histidine residue was collected by column chromatographic separation techniques and was charaterised by ^{1}H NMR[9] and electron spray mass spectroscopy [M + 1 = 156.1]. The reaction yield (checked by ^{1}H NMR) of the cleavage product based on the isolated L-histidine is 75%; however, we could isolate only 30% of the [Co(3,2,3-tet)(H-β-alanine)$_2$]-$(ClO_4)_3$ in crystalline form due to its high solubility. Continued concentration of the mother liquor results to a highly viscous material. This was redissolved in water and subjected to electron spray mass spectroscopy. The spectrum shows only one signal at 409 that corresponds to [Co(3,2,3-tet)(β-alanine)$_2$]$^+$, which illustrates the exclusive formation of **1** in this reaction.

In a control experiment an aqueous solution of β-alanyl-L-histidine was kept at similar conditions (at pH ~7.5 and 45 °C for 6 h) without any *trans*-[Co(3,2,3-tet)Cl_2]Cl. No cleavage products were formed as evidenced by ^{1}H NMR spectroscopy, TLC work and electron spray mass spectroscopy. To check the possibility of a peptide bond cleavage, under the influence of silica gel, used for column chromatography, we further ran the solution through the column and eluted it with water. The ^{1}H NMR shows the identical spectrum as that known for β-alanyl-L-histidine.[10] The intact β-alanyl-L-histidine was further checked by electron spray mass spectroscopy which shows a single peak at [M + 1] = 227.2.

It is noteworthy, that the hydrolytic cleavage reaction occurs irrespective of a 1:1 or a 1:2 molar ratio between *trans*-[Co(3,2,3-tet)Cl_2]Cl and β-alanyl-L-histidine. In the latter case, a higher yield of the crystalline material [Co(3,2,3-tet)(H-β-alanine)$_2$]$(ClO_4)_3$ is obtained.

The X-ray analysis[11] of the perchlorate salt of **1** shows the *trans*-coordination of the (bis-β-alanine) residues to the cobalt center through oxygen atoms of the carboxylate groups, while the protonated amine groups remain dangling. The basal plane is constructed by the four nitrogen atoms of the 3,2,3-tet ligand. An ORTEP[12] diagram of **1** is presented in Fig. 1. The Co–N bond distances span the range of 1.952–1.992 Å. One of the Co–O bonds (1.952 Å) is longer than the other (1.909 Å). The bond angles and the torsion angle are in normal ranges.

β-Alanyl-L-histidine possesses multiple coordinating centers, and the type of complex formation depends on the metal ion, ligand : metal ratios and pH of the solution.[13] Dihalo complexes of Co(III), are known to be highly labile and react with water to form aquo-hydroxo complexes, specially if the solutions are neutral or slightly basic.[4–6] The crystal structure **1** indicates the substitution of OH^-/H_2O by the carbonyl oxygen of the peptide linkage leading to the cleaved product (Scheme 1). The coordination of the carbonyl oxygen to the metal centre promotes attack by the hydroxide ion to the carbonyl carbon atom and, thus, causes the cleavage of the peptide bond.

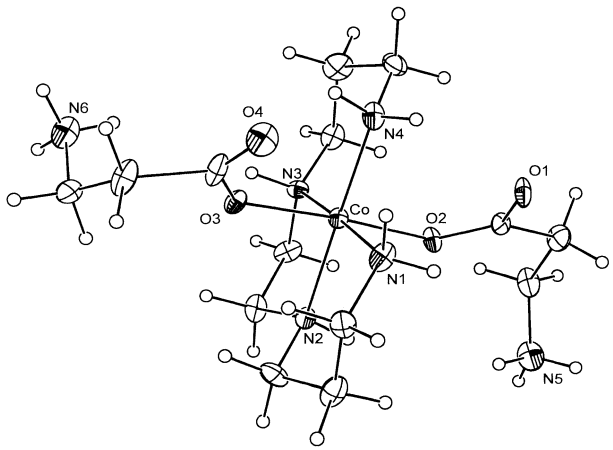

Fig. 1 An ORTEP view of **1**.

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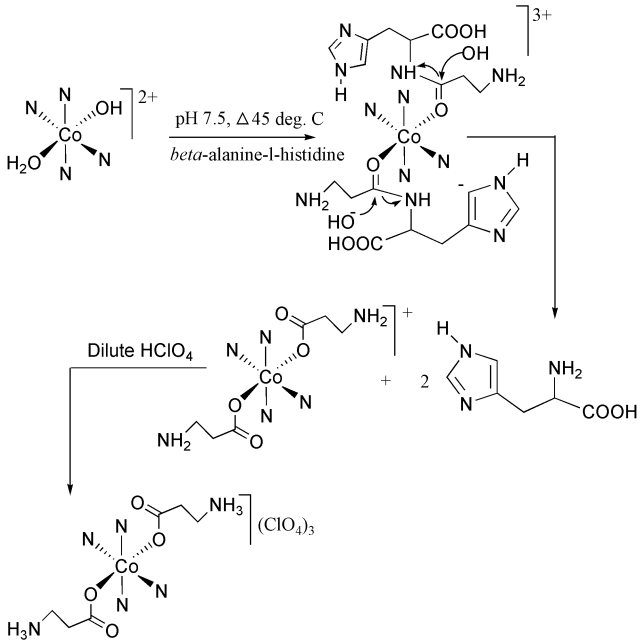

Scheme 1 Reaction pathway for formation of [(3,2,3-tet)Co(H-β-alanine)$_2$]$^{3+}$.

Coordination through other sites of the dipeptide requires a *cis* coordination to the metal ion through one of peptide linkage atoms in order to form a chelate ring, a process that will induce polarity to the peptide bond. However, a *cis* attack to the metal centre is restricted by the rigidity of the 3,2,3-tet ligand. The above results demonstrate that a *trans*-oriented cleavage reaction can take place when a polydentate ligand to the metal complex shows strong rigidity towards the *trans*-orientation. The *cis* orientation and the chelate formation are not an essential condition to polarize the peptide linkage. Further investigations are in progress to provide more information on the *trans*-oriented cleavage reaction. The fact that the 3,2,3-tet ligand can be forced to the rearrange to a *cis*-β- product by a strong enough *cis*-binding ligand has been observed in this group.[14]

Notes and references

† *Experimental section*: for column chromatography, the silica used came from the Davison Chemical Division of Grace Chemical with its pH in water at *ca.* 5.5. [(3,2,3-tet)$CoCl_2$]Cl was prepared by the known literature method.[15] 0.17 g (0.05 mol) of [(3,2,3-tet)$CoCl_2$]Cl was dissolved in 100 cm^3 water. The pH of the solution was adjusted to 7.5 by adding an aqueous solution of LiOH. A 10 cm^3 aqueous solution of 0.26 g (0.1 mol) of β-alanyl-L-histidine was added slowly and the reaction mixture was set to 45 °C, with continuous stirring, for 4 h. The mixture was allowed to cool and the solution was added to a silica gel chromatographic column and eluted by water. The histidine fragment was eluted out first, followed by the single dark red band, which was collected. The solution was reduced to 25 cm^3. 3 cm^3 of 10% H(ClO_4) was added to it and after seven days crystals were obtained. ^{1}H NMR spectra were recorded at 300 MHz on a GE QE-300 spectrometer in deuterated water. The solvent signal (δ 4.8 ppm) was used as internal standard. Electron spray mass spectra in aqueous solution were recorded with an LCQ DECA XP mass spectrometer. The cleavage product, L-histidine, shows the same peak positions for ^{1}H NMR spectra as recorded in the literature[9] and shows a single molecular ion peak at [M + 1] = 156.1. The solution obtained by mixing β-alanyl-L-histidine in absence of metal complex, then run through silica column, shows a signal at [M + 1] = 227.2, corresponding to uncleaved β-alanyl-L-histidine. The ^{1}H NMR spectra is the same as that reported in the literature.[10]

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11 *Crystal data*: $C_{14}H_{36}N_6O_{16}Cl_3Co$, M_w = 709.8, orthorhombic, space group $Pbn2_1$, a = 11.696(3), b = 14.531(3), c = 16.561(4), V = 2815.03 Å^3, Z = 4, D_c = 1.67 g cm^{-3}, μ = 0.975 mm^{-1}, Mo-Kα , $F(000)$ = 1471.7, θ range of data collection 2.1–26.0, final R = 0.034, wR_2 = 0.086, GOOF = 1.015 for 352 parameters and 3327 unique reflections, [$R_{(int)}$ = 0.039] of which 2148 are observed, positive and negative peaks in ΔF map 0.340 and −0.464 e Å^{-3}. the data set was collected on a Enraf Nonius CAD4 system equipped with Mo-Kα radiation (λ = 0.71073 Å) at room temperature. The structure was solved by Patterson and Fourier analyses and refined by full-matrix least squares based on F^2 using SHELX 97.[16] All the hydrogen atoms were placed in their geometrically ideal positions with isotropic temperature factors 1.2 times those of the attached non-hydrogen atoms. All calculations were performed using the WinGX system (ver 1.64).[17] CCDC 187487. See http://www.rsc.org/suppdata/cc/b2/b202587k/ for crystallographic data in CIF or other electronic format.

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