Copper(II)-cis,cis-1,3,5-triaminocyclohexane complex-promoted hydrolysis of dipeptides: kinetic, speciation and structural studies

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Abstract The hydrolysis of glycylglycine (GlyGly), glycyl-L-leucine (GlyLeu), L-leucylglycine (LeuGly) and glycyl-DL-serine (GlySer) promoted by a copper(II)-cis,cis-1,3,5-triaminocyclohexane complex [Cu(II)-TACH] was investigated at 70 °C and pH 7–10, using HPLC. The observed pseudo-first-order rate constants ($k_{\text{obs}}$) and rate enhancing factors (REF) were as follows: $4.1 \times 10^{-3}$ h$^{-1}$ (REF=23) for GlyGly, $1.6 \times 10^{-3}$ h$^{-1}$ (REF=21) for GlyLeu, $5.1 \times 10^{-3}$ h$^{-1}$ (REF=64) for LeuGly and $9.2 \times 10^{-2}$ h$^{-1}$ (REF=47) for GlySer [pH 8.1, dipeptide 2 mM, copper(II) 2 mM and TACH 2 mM]. Based on the pH dependence and dipeptide concentration dependence of the initial rates and speciation of the Cu(II)-TACH-dipeptide system at 25 °C and $I=0.1$, the reactions proceed via the formation of a ternary complex [Cu(TACH)(dipeptide)]$^+$ as an intermediate followed by OH−-dependent and OH−-independent paths to give amino acid(s). GlyGly, GlyLeu and LeuGly preferred the OH−-dependent path, while GlySer preferred the OH−-independent path. The latter can be explained by the intramolecular attack of the amide carbonyl group coordinated with its oxygen atom by the OH group in the serine residue. The X-ray crystal structure of [Cu(TACH)(GlyGly)]BPh$_4$·MeOH confirmed that GlyGly coordinates to copper(II) ion with its terminal amino N and amide O atoms. The crystal structures of [Cu(TACH)(Gly)]BPh$_4$ and [Cu$_2$(TACH)$_2$(OH)$_2$]$(\text{ClO}_4)_2$·NaClO$_4$·H$_2$O are also reported. Electronic supplementary material to this paper can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00775-002-0368-9.

Keywords Copper(II) complexes · Triaminocyclohexane · Peptide hydrolysis · Kinetics and mechanism · Solution and crystal structures

Introduction

There has been great interest in designing artificial metallopeptidases that hydrolyze unactivated peptides in a site- or sequence-selective manner under mild conditions [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. The hydrolysis of unactivated peptide bonds is exceptionally slow at physiological pH (the half-life of a typical peptide bond is 7–350 years at neutral pH and 25 °C) [25, 26, 27], although this can be efficiently catalyzed by enzymes [28, 29]. To understand the mechanism of these remarkable hydrolysis reactions, and to mimic enzymatic processes, many studies have been carried out over several decades using a variety of model complexes of Cu(II) [7, 8, 9, 30, 31, 32, 33, 34, 35 (recent papers)], Zn(II) [22, 34, 35], Ni(II) [34, 35], Pd(II) [10, 11, 12, 13, 14, 15, 16, 17], Pt(II) [10, 17, 18], Co(III) [36, 37, 38 (recent papers)] and Ce(IV) [21, 23]. However, the hydrolysis of unactivated peptide bonds promoted by substitution-labile bivalent metal complexes such as Ni(II), Cu(II) and Zn(II) is generally too slow for detailed kinetic studies. Therefore, activated amides have often been used to investigate reaction mechanisms, instead of peptides themselves [4, 5, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41].
Recently, Burstyn and co-workers [9] reported that the copper(II) complex of the macrocyclic ligand
[9]aneN₃ can effectively hydrolyze both the unactivated dipeptide glycylglycine and proteins at near physiological pH. Although no reaction mechanism has been suggested, activation of the carbonyl group of the peptide bond by the metal center seems to play an important role in such a reaction [1, 2, 3, 4, 5, 6, 39, 40, 41]. An important aspect is the site selectivity of the hydrolysis, which has not yet been explored.

We have been studying several copper(II)-triamine complexes that effectively promote the hydrolytic cleavage of DNA [42, 43], as well as peptide bonds, and recently briefly reported the crystal structure of the glycylglycine complex of copper(II)-cis,cis-1,3,5-triaminocyclohexane [Cu(II)(TACH)] as a reaction intermediate [44]. In this paper, we discuss the mechanism of the hydrolysis of the dipeptides GlyGly, GlyLeu, LeuGly and GlySer with Cu(II)(TACH), together with the site selectivity of the serine residue [19, 20, 22], based on detailed kinetic and speciation studies and the crystal structures of the relevant complexes [Cu(TACH)(Gly-Gly)]BPh₄,MeOH, [Cu(TACH)(Gly)]BPh₄ and [Cu₂(TACH)₂(OH)₂](ClO₄)₂·NaClO₄·H₂O.

Materials and methods

Materials

All reagents were of analytical grade and used without further purification. GlyGly, GlyLeu, LeuGly, glycine (Gly), l-leucine (Leu), and l-serine (Ser) were purchased from the Peptide Institute. GlySer and serylglycine (SerGly) were purchased from Sigma. All reagents were of analytical grade and used without further purification.

Synthesis of [Cu(TACH)(Gly-Gly)]BPh₄,MeOH (1)

CuSO₄·5H₂O (1 mmol), TACH·3HCl (1 mmol) and GlyGly (1 mmol) were dissolved in distilled water (20 mL) and stirred. A 0.1 M NaOH solution was added to the reaction mixture to adjust the pH to 8.0, and then NaBPh₄ (50 mmol) in methanol (10 mL) was added, resulting in a blue precipitate. A complex material was obtained by filtration, washed with water and air dried. Complex 1 was obtained in 60% yield as blue crystals grown by slow evaporation of a MeOH/H₂O solution. Anal. calcd for C₃₅H₄₆BCuN₅O₄: C 62.27, H 6.87, N 10.17, Cu 9.38%; found: C 62.41, H 6.94, N 10.37, Cu 9.46.

Synthesis of [Cu(TACH)(Gly)]BPh₄ (2)

CuSO₄·5H₂O (1 mmol), TACH·3HCl (1 mmol) and Gly (1 mmol) were dissolved in distilled water (20 mL) and stirred. A 0.1 M NaOH solution was added to the reaction mixture to adjust the pH to 8.0, and then NaBPh₄ (50 mmol) in methanol (10 mL) was added, resulting in a blue precipitate. A complex material was obtained by filtration, washed with water and air dried. Complex 2 was obtained in 60% yield as blue crystals grown by slow evaporation of a MeOH/H₂O solution. Anal. calcd for C₅₃H₆₅BCuN₅O₁₅: C 65.58, H 6.71, N 9.56, Cu 10.85%; found: C 65.64, H 6.75, N 9.46, Cu 10.62%.

Synthesis of [Cu₂(TACH)₂(OH)₂](ClO₄)₂·NaClO₄·H₂O (3)

TACH·3HCl (0.5 mmol) in water (10 mL) was added dropwise to a MeOH/H₂O (1:1) solution (20 mL) containing copper(II) perchlorate hexahydrate (0.5 mmol) and sodium perchlorate (2 mmol), and stirred. The pH of the reaction solution was adjusted to 8.0 with 0.1 M NaOH solution, and the resulting solution was stirred for 2 h at room temperature. After removing the solvent in vacuo, the blue material was extracted with acetonitrile (15 mL), from which blue crystals suitable for X-ray single-crystal diffraction studies were deposited one week later. The blue crystals were filtered off, washed with methanol and dried in vacuo; yield, 30%. Anal. calcd for C₁₂H₁₄Cl₂Cu₂N₂NaO₁₅: C 18.99, H 4.52, N 11.07, Cu 16.75%; found: C 18.63, H 4.46, N 11.07, Cu 16.58%.

HPLC measurements

A JASCO HPLC system (LCSS-905) equipped with PU-980 pumps, an LU-980-03 solvent selection unit, an AS-959-10 auto-sampler and an FP-920 fluorescence detector was used for HPLC measurements. The columns were a JASCO AApak NaII-H (6.0×80 mm) and a JASCO AECPakII (4.6×50 mm), and the samples were eluted with JASCO HPLC buffers (citrlic acid, sodium citrate, and sodium perchlorate) and 0.2 M NaOH. The fluorescence of the eluate was detected at 455 nm with excitation at 345 nm, after reacting with OPA and 2-mercaptoethanol in 0.2 M potassium borate buffer (pH 10.5) at 60°C in a reaction coil (0.5×2 m) [46]. The retention times at a flow rate of 0.6 mL min⁻¹ were as follows: GlyGly = 18 min, GlySer = 14 min, SerGly = 16 min, GlyLeu = 22 min, LeuGly = 21 min, Gly = 11 min, Ser = 7 min and Leu = 16 min. The extent of peptide hydrolysis was calculated from the peak areas of the peptide and amino acid(s) produced in each run, using a calibration curve for each peak. During hydrolysis, the HPLC peak of a diketopiperazine-type compound was not detected.

Kinetic measurements

In a typical experiment, a solution (10 mL) containing CuSO₄ (2.0 mM), TACH·3HCl (2.0 mM), peptide (2.0 mM) and buffer (75 mM) was prepared, and the pH was adjusted to 8.1 with 10 mM NaOH (aq) at 25°C. The solution was heated and stirred at 70±0.1°C in a water bath equipped with a thermostat. After the reaction, the pH of the solution was measured again to confirm that it had been kept constant. The pH values of the solutions before and after the reaction were within ±0.15 pH unit. The hydrolysis of dipeptides was monitored by HPLC. In a typical example, samples (500 μL) of the reaction solution were taken at intervals and added to 100 μL of Na₂EDTA (20 mM) to quench the reaction. Next, 10 μL of the solution was subjected to HPLC. Duplicate measurements were made, and the reported data represent mean values. The hydrolysis of peptides in the absence of the Cu(TACH) complex was measured in the same way as in the presence of the Cu(TACH) complex.

Speciation

Protonation and Cu(II) complex formation equilibria were investigated by potentiometric titration in aqueous solution (T = 0.1 M, NaClO₄, and T = 25 ± 0.1°C) in an automatic titration set that included a Dosimat 665 (Metrohm) autoburette, an Orion 710A pH meter, a digital pH meter, and an IBM-compatible PC. An Orion