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The copper(II) binding properties of the cyclic peptide *c*(HGHK)

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Abstract

The new cyclic tetrapeptide *c*(HGHK) was synthesised in the solid phase and its complexes with copper(II) were studied in aqueous solution at various pH values by means of potentiometric and spectroscopic methods (UV, EPR, CD). Six mononuclear coordination species were clearly identified within the pH range 3–11. Spectroscopic data strongly suggest sequential formation of N, 2N, 3N and 4N equatorial donor sets around the copper(II) centre from the lowest to the highest pH, involving both imidazole nitrogens and amide nitrogens. A detailed comparison with the copper(II) binding properties of HGHG and Ac-HGHG ligands is also reported.

Keywords: Copper (II); Cyclo-peptides; Complex

1. Introduction

The His–Xaa–His motif in the peptide sequence is commonly used as a model for the copper(II) binding site of superoxide dismutase [1]. The multi-His sequences are also basic for the copper(II) binding motifs in other proteins like, e.g., APP (amyloid precursor protein) [2–4] or SPARC (secreted protein, acidic and rich in cysteine) [5]. However, scarce data are presently available on copper(II) complexes of cyclopeptides despite the fact that suitably tailored ring sizes may mimic enzymatic cavities. Recently, our group carried out the synthesis of new constrained cyclotetrapeptides bearing various side chains on the core nucleus [6] by using a rapid and flexible method based on solid phase peptide synthesis (SPPS). The synthesis was specifically addressed to the preparation of *cyclen* scaffolds which are of great interest as chelating agents of radioisotopes for radiodiagnostic and/or radiotherapeutic purposes [7]. However, cyclopeptides containing histidine residues are per se interesting for studies of copper(II) complexation.

We have prepared here a series of cyclic tetrapeptides bearing two histidine residues plus an additional side chain. Peptides were prepared by SPPS working in the pseudo-dilution conditions.

The following sequences were obtained: *c*(HGHG) [6], *c*(HGHK), *c*(HGHD) and *c*(HGHE). All these sequences contain two His residues; basic and acid side-chains have been then added to the lead sequence. To the best of our knowledge, the last three cyclopeptides were not previously synthesised.

The copper(II) binding properties of these cyclopeptides were preliminarily tested by some rapid spectrophotometric assays. We found that the *c*(HGHK) sequence is the best one in terms of solubility in water,

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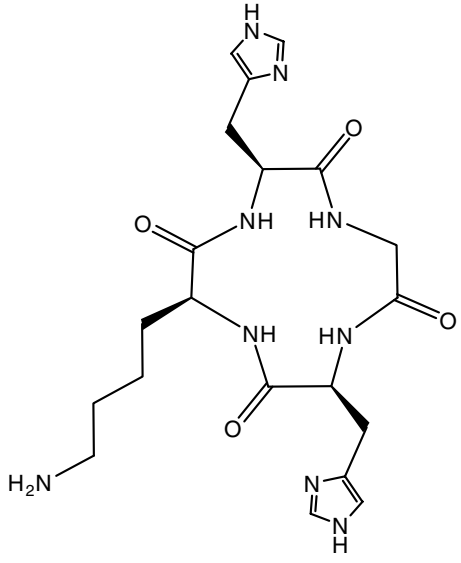
in the other cases, extensive precipitation of the copper complex is observed – especially around physiological pH – that limits the pharmaceutical use of these substances.

Accordingly, we have focused our attention, primarily, on the *c*(HGHK) species (1) (Scheme 1). We report here the investigation of the copper(II) binding properties of this cyclic tetrapeptide as a function of pH, based on potentiometric and spectroscopic measurements.

2. Experimental

2.1. Ligand synthesis

Cyclotetrapeptides were synthesized on a PLS 4×4 manual synthesizer (Advanced ChemTech, Louisville, KY) following the SPPS method and using the orthogonal Fmoc/Trt/allyl protection scheme. The synthesis started from anchoring the imidazole group of the protected amino acid Fmoc–His–OAl to a trityl-type resin (500 mg, 0.46 mmol/g). Deprotections of amino acids were performed by 20% piperidine in DMF (2×5 mL) for 15 min. The amino acids residues were introduced according to the TBTU/HOBt/NMM method with formation of active esters. The coupling reactions were performed by using an excess of the amino acids, HOBt and TBTU (4 mole equiv.) and of NMM (8 mole equiv.) in DMF, under vortex mixing for 1 h. After each coupling, the resin was washed with DMF (3×5 mL) and DCM (2×5 mL). Coupling reactions were controlled by the Kaiser test. After treatment of the on-resin peptides with $PhSiH_3$ (24 equiv.) and $Pd(PPh_3)_4$ (0.25 equiv.) in dry DCM under argon (2×40 min) (allyl removal), and then with 20% piperidine in DMF (Fmoc-deprotection), the linear peptides were obtained with the free amino and carboxy functions ready for final cyclization. Thus, in pseudo-dilution conditions, the on-resin cyclization was performed with TBTU/DIPEA in 4 h at r.t., with no evidence of oligomerization by-products. The crude peptides were cleaved from the resin, simultaneously with the removal of the trityl group, by treating with 95% TFA, 2.5% H_2O and 2.5% TIS (8 mL) for 3 h. The filtered TFA solutions were concentrated under nitrogen fluxing and the products were precipitated with cold diethyl ether and centrifuged. The peptides were then dissolved in water and lyophilized. The solid products were re-precipitated several times from MeOH/diethyl ether and, finally, dissolved in water and lyophilized. After then, the peptides were purified by solid phase extraction (SPE) with an C18-E Strata^{TM} from Phenomenex and using H_2O/CH_3CN as eluent, affording colorless products (136 mg of **1**, 59% yield).



Scheme 1. The ligand *c*(HGHK)**1**.

ESI-MS of compound 1: *m/e* calcd. $[M + H]^+$ 460.2, found 460.5.

2.2. Potentiometric measurements

Stability constants were calculated from titration curves, carried out at 25 °C using sample volumes of 1.5 mL. Alkali was added by using a 0.25-mL micrometer syringe calibrated by weight titration of standard materials. The ligand concentration was 1.5×10^{-3} M, and the metal-to-ligand ratio was 1:1. The pH-metric titrations were performed in 0.1 M KNO_3 on a MOLSPIN pH-meter system using a Metler Toledo InLab 422 semi-micro combined electrode calibrated in hydrogen ion concentration using HNO_3. Stability constants [3] $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$ and stoichiometry of complexes were calculated with the aid of SUPERQUAD [8] program. Standard deviations quoted were also computed by SUPERQUAD and referred to random errors only. There is, however, a good indication of the importance of particular species in the equilibrium.

2.3. Spectroscopic measurements

Absorption spectra were recorded on a Perkin–Elmer Lambda Bio 20 spectrophotometer, circular dichroism (CD) spectra on a JASCO J 715 spectropolarimeter in the 850–200 nm range and electron paramagnetic resonance (EPR) spectra on a Bruker ESP 300E spectrometer at X-band frequency (9.4 GHz) at 120 K. [Cu(II)] was 3×10^{-3} M and the metal-to-ligand ratio was 1:1.

3. Results and discussion

The cyclopeptide *c*(HGHK) shows three protonation constants connected with the presence of two histidyl