Available online at www.sciencedirect.com
ScienceDirect
Journal of Inorganic Biochemistry 102 (2008) 1438–1448
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Copper(II), nickel(II) and zinc(II) complexes of *N*-acetyl-His-Pro-His-His-NH_2: Equilibria, solution structure and enzyme mimicking

Noémi I. Jakab [a], Attila Jancsó [a], Tamás Gajda [a], Béla Gyurcsik [a,*], Antal Rockenbauer [b]

[a] *Department of Inorganic and Analytical Chemistry, University of Szeged, P.O. Box 440, H-6701 Szeged, Hungary*
[b] *Chemical Research Center, Institute of Chemistry, P.O. Box 17, H-1525 Budapest, Hungary*

Received 10 October 2007; received in revised form 29 December 2007; accepted 2 January 2008
Available online 8 January 2008

Abstract

The copper(II), nickel(II) and zinc(II) binding ability of the multi-histidine peptide *N*-acetyl-His-Pro-His-His-NH_2 has been studied by combined pH-potentiometry and visible, CD and EPR spectroscopies. The internal proline residue, preventing the metal ion induced successive amide deprotonations, resulted in the shift of this process toward higher pH values as compared to other peptides. The metal ions in the parent $[ML]^{2+}$ complexes are exclusively bound by the three imidazole side chains. In $[CuH_{-1}L]^{+}$, formed between pH 6–8, the side chains of the two adjacent histidines and the peptide nitrogen between them are involved in metal ion binding. The next deprotonation results in the proton loss of the coordinated water molecule ($CuH_{-1}L(OH)$). The latter two species exert polyfunctional catalytic activity, since they possess superoxide dismutase-, catecholase- (the oxidation of 3,5-di-*tert*-butylcatechol) and phosphatase-like (transesterification of the activated phosphoester 2-hydroxypropyl-4-nitrophenyl phosphate) properties. On further increase of the pH rearrangement of the coordination sphere takes place leading to the $[CuH_{-3}L]^{-}$ species, the deprotonated amide nitrogen displaces a coordinated imidazole nitrogen from the equatorial position of the metal ion. The shapes of the visible and CD spectra reflect a distorted arrangement of the donor atoms around the metal ion. In presence of zinc(II) the species $[ZnL]^{2+}$ forms only above pH 6, which is shortly followed by precipitation. On the other hand, the $[NiL]^{2+}$ complex is stable over a wide pH range, its deprotonation takes place only above pH 8. At pH 10 an octahedral $NiH_{-2}L$ species is present at first, which transforms slowly to a yellow square planar complex.

Keywords: Histidine-rich peptides; Peptide complexes; Equilibrium studies; SOD activity; Catecholase activity

1. Introduction

Recently, it has been shown that the genetic constraints upon diversification and evolution resulted in proteins with amino acid runs containing Ala (A), Gly (G), Pro (P) and His (H) amino acids [1]. Among these, proteins with H/P-rich sequences exert diverse biological functions, including growth regulating proteins, like the human brain specific homeobox and other transcription factors [1–3]. The histidine–proline-rich glycoproteins (HPRG) are multidomain plasma proteins that have been shown to regulate a number of important biological processes, such as angiogenesis, cell adhesion, migration, fibrinolysis and coagulation [4]. These proteins contain different combinations of H and P amino acids in their sequences offering a multi-imidazole environment as potential binding site for different metal ions. Indeed, several functions of H/P-rich proteins are related to zinc(II) binding. A well known example is the zinc(II) dependent interaction of HPRG with heparin or tropomyosin on endothelial cells, which is believed to mediate angiogenesis and cell proliferation [4].

Histidine–proline-rich peptides have also been utilized for the enhanced bacterial bioaccumulation of toxic metal ions in bacteria displaying a short GHHPHG sequence at

* Corresponding author. Fax: +36 62 420 505.
E-mail address: gyurcsik@chem.u-szeged.hu (B. Gyurcsik).

0162-0134/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2008.01.006

their surface [5–7]. Moreover, H and H/P-rich sequences like hexahistidine or GHPHHG have been playing important role in anchoring and purifying proteins on metal ion-affinity chromatography [8–10].

The recent literature on the coordination properties of multihistidine peptides [11–18] serves the lesson on their highly versatile coordination properties and their ability to catalyze oxidative reactions [11]. However, the enzyme-like function of these complexes, possessing HXH segment(s) within their peptide chain (X ≠ P), is strongly restricted by the high affinity of copper(II) toward amide nitrogens, i.e. by the formation of the stable $\{N_{im}, N^-_{amide}, N^-_{amide}, N_{im}\}$ type coordination. The presence of adjacent histidine units in N-Ac-HHGH-OH shifted the formation of this binding mode above pH 8 [11]. Therefore, the insertion of a secondary amino group containing, and thus more rigid, proline residue into the peptide sequence containing adjacent histidines could be a suitable way to decrease the number of copper(II)-bound amide groups around pH 7.

In order to better understand the metal binding ability of H/P-rich peptide sequences, and to explore the enzyme-like functions of their complexes, in this paper we report equilibrium and solution structural study on copper(II), zinc(II) and nickel(II) complexes of an N- and C-terminally protected minimum H/P-rich peptide without HXH (X ≠ P) motif: *N*-acetyl-His-Pro-His-His-NH_2 (HPHH) and the potential of its copper(II) complexes to promote oxidative (SOD- and catecholase-like activity) and hydrolytic (against an activated phosphate ester substrate) reactions.

2. Experimental

2.1. Materials

Copper(II), nickel(II) and zinc(II) chloride (Fluka) solutions were standardized complexometrically. pH-metric titrations were performed by NaOH (Fluka) standard solution. Fmoc-His(Trt)-OH (Novabiochem), Fmoc-Pro-OH (Novabiochem), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (Novabiochem), *N*-hydroxybenzotriazole (HOBt) (Novabiochem), *N*,*N*-diisopropylethylamine (Sigma), triisopropylsilane (Aldrich), pyridine (Merck), 1-methyl-2-pyrrolidone (NMP) (Molar Chemicals), piperidine (Aldrich), acetic anhydride (Fluka), trifluoroacetic acid (TFA) (Riedel-de Haën), *N*,*N*-dimethyl-formamide (DMF) (Molar Chemicals), dichloromethane (Molar Chemicals), methanol (Molar Chemicals), acetonitrile (Scharlau), 3,5-di-*tert*-butylcatechol (Sigma), 2-[*N*-morpholino]ethanesulfonic acid (MES) (Sigma), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (Fluka), 2-[cyclohexylamino]-ethanesulfonic acid (CHES) (Sigma) and tris(hydroxymethyl)aminomethane (TRIS) (Aldrich) were used without further purification. The barium salt of 2-hydroxypropyl-4-nitrophenyl phosphate (hpnp) was prepared according to the literature procedure [19].

2.2. Synthesis of N-acetyl-His-Pro-His-His-NH_2 (HPHH)

The peptide HPHH was prepared by solid phase peptide synthesis using the Fmoc methodology (Fmoc = 9-fluorenylmethoxycarbonyl). Rink Amide AM resin (200–400 mesh) (Novabiochem) was used as a solid support. The amino acid building blocks were applied in 3-fold excess over the capacity of the resin. The amino acid residues were coupled to each other (and to the resin) by applying HBTU (3 eq./building block), HOBt (3 eq./building block) and *N*,*N*-diisopropylethylamine (6 eq./building block) in NMP. The Fmoc-protecting groups were removed by using a solution of 20% piperidine in NMP. The usual coupling reaction time was 0.5–1 h and the attachment of each amino acid residues was monitored by Kaiser test [20]. After the last coupling step the Fmoc group was cleaved from the amino group of the N-terminal amino acid (histidine) which was acetylated afterwards with the mixture of acetic anhydride, dichloromethane and pyridine (10–85–5%). Cleavage of the peptide from the resin was performed in the mixture of TFA, H_2O and triisopropylsilane (90–5–5%). The ligand was purified by RP-HPLC using a Phenomenex Jupiter 5 μ C18 300A (250 × 10 mm, 5 μm) column. The compound was eluted by using the mixtures of water and acetonitrile containing 0.1% trifluororacetic acid (TFA) (Eluent A: 95–5% H_2O–CH_3CN, 0.1% TFA, Eluent B: 5–95% H_2O–CH_3CN, 0.1% TFA) applying the following gradient program: 0–5 min, 100% A; 5–30 min, 100–70% A (linear gradient); 30–35 min, 70–100% A (linear gradient); 35–40 min, 100% A; $R_t = 9.7$ min.

The peptide was identified by ESI-MS. The mass spectrometric measurements were obtained on a Finnigan TSQ-7000 triple quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA) equipped with a Finnigan electrospray ionization (ESI) source. The instrument was operated in positive ion mode, the ESI needle was adjusted to 4.5 kV and N_2 was used as a nebulizer gas. Analytical data: $m/z = 568.2$ $[M + H]^+$ and $m/z = 284.3$ $[M + 2H]^{2+}$. The calculated monoisotopic molecular mass is: 567.27 Da.

The ligand was obtained after purification as a trifluoroacetate salt. The concentration of its stock solution was determined by potentiometric titrations. (The purity was also confirmed by potentiometry.)

2.3. pH-metric measurements

The protonation and coordination equilibria were investigated by potentiometric titrations in aqueous solution ($I = 0.1$ M NaCl, and $T = 298.0 \pm 0.1$ K) under argon atmosphere, using an automatic titration set including a PC controlled Dosimat 665 (Metrohm) autoburette and an Orion 710A precision digital pH-meter. The Metrohm Micro pH glass electrode (125 mm) was calibrated [21] *via* the modified Nernst equation:

$$E = E_0 + K \cdot \log[H^+] + J_H \cdot [H^+] + \frac{J_{OH} \cdot K_w}{[H^+]}$$