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Research paper

Impact of histidine spacing on modified polyhistidine tag – Metal ion interactions

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ABSTRACT

Histidine rich sequences are chosen both by nature and by molecular biologists due to their high affinity towards metal ions. In this work, we examine the affinity and binding modes of Cu^{2+}, Ni^{2+} and Zn^{2+} towards two histidine tags, the common His_6-tag (Ac-HHHHHH-NH_2) and its modified sequence, which also contains six histidines, but separated with two alanine residues (Ac-HAAHAAHAAHAAHAAHAA-NH_2). The spatial separation of histidines has an important impact on its coordination properties. Cu^{2+} and Ni^{2+} complexes with Ac-HHHHHH-NH_2 are more stable than those with Ac-HAAHAAHAAHAAHAAHAA-NH_2; the contrary is observed for Zn^{2+}. In a narrow range of pH, Cu^{2+}-Ac-HHHHHH-NH_2 and Ni^{2+}-Ac-HHHHHH-NH_2 can even compete with the albumin-like binding of the respective metals.

1. Introduction

Understanding the relationship between metal ion binding, structure and function is an indispensable pillar of bioinorganic chemistry. Histidine rich proteins, chosen both by nature and by molecular biologists as metal chelators due to their high affinity towards metal ions, are a particularly interesting case of ligands. Histidine rich sequences, or, precisely speaking, a His_6-tag, present at the C- or N-terminus of a protein which is meant to be purified, is commonly used in immobilized metal affinity chromatography (IMAC) as a molecular 'anchor' that binds to a metal ion (usually nickel), immobilized by chelation with nitrilotriacetic acid (NTA) bound to a solid support.

Poly-His motifs have been found in a variety of biologically significant proteins [1], such as bacterial nickel chaperones [2–6], metal transporters [7,8], prion proteins [9–12], Zn-finger domains of transcription proteins [13–15], histidine-rich glycoproteins (HRG) [16,17], snake venoms [18,19] and antimicrobial peptides [20,21].

Our previous studies on different 'poly-His' regions show that they can form stable complexes with metal ions, such as Cu^{2+}, Ni^{2+} and Zn^{2+} [22–25], forming so-called polymorphic binding states, with the metal "moving back and forth" along such regions [22,23]. In several cases, metal ion binding induces the formation of an α-helical structure [23].

The results of our recent work [22], in which a series of experimental and theoretical techniques are used to study the interactions of Ac-HHHHHH-NH_2 (His_6-tag) with Cu^{2+}, strongly encouraged us to pursue further studies on the topic of metal ion-poly-His region interactions.

Metal affinities, pH-dependent speciation and changes that occur due to metal binding in Ac-HAAHAAHAAHAAHAAH-NH_2 and Ac-HHHHHH-NH_2 are of particular interest of this study. Examining Ni^{2+} and Zn^{2+} coordination to His_6-tag, as well as understanding the influence of spatial separation of histidines in the designed Ac-HAAHAAHAAHAAHAAH-NH_2 fragment on its coordination abilities towards Cu^{2+}, Ni^{2+} and Zn^{2+} are the major focus of this study. Will the separation of histidines by two alanine residues have a large influence on affinity of this peptide towards Cu^{2+}, and how will the structure of this complex change, with respect to the Cu^{2+}-His_6-tag one? How will these parameters change in the case of Zn^{2+} and Ni^{2+} complexes of both of these ligands? Such knowledge will be an important input to the bioinorganic chemistry of the studied metals that allows a better understanding of the proper design of His-tags.

2. Experimental section

2.1. Materials

Ac-HAAHAAHAAHAAHAAH-NH_2 and Ac-HHHHHH-NH_2 peptide (98% purity) were purchased from KareBio and used without further purification.

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Cu^{2+}, Ni^{2+}, and Zn^{2+} chlorides were extra-pure products (Sigma-Aldrich). The concentrations of stock solutions of these salts were determined by inductively coupled plasma mass spectrometry. The carbonate-free stock solution of NaOH (0.1 M) was purchased from Sigma-Aldrich and then potentiometrically standardized with potassium hydrogen phthalate as a primary standard. The HCl stock solution was prepared by diluting concentrated HCl (Sigma-Aldrich) and then standardized with NaOH. All of the sample solutions were prepared with freshly doubly distilled water. The ionic strength (I) was adjusted to 0.1 M by addition of KCl (Sigma-Aldrich).

2.2. Potentiometric measurements

Potentiometric measurements were performed at a constant temperature of 25 °C under an argon atmosphere using a Molspin pH meter equipped with a Mettler-Toledo InLab semi-combined electrode and a micrometer syringe with a volume of 0.5 cm^3. Before each measurement, the electrode was calibrated by titration of HCl having a concentration of 4×10^{-3} M with NaOH (0.1 M) [26]. Stability constants for proton, Cu^{2+}, Ni^{2+}, and Zn^{2+} complexes were calculated from potentiometric titration curves registered over the pH range 2.5–11. The pH-metric titrations were performed in an aqueous solution of HCl at 0.1 M KCl. The titrant was a carbonate-free standard solution of NaOH. The ligand concentration was about 1×10^{-3} M, and the metal-to-ligand ratio was 1:1.1. The exact concentrations and the purities of the ligand solutions were determined by the Gran method [27]. HYPERQUAD and SUPERQUAD programs were used for the stability constant calculations [28,29]. Standard deviations were given by the program itself and refer to random errors only. The speciation and competition diagrams were computed with the HYSS program [30].

2.3. Spectroscopic studies

The absorption spectra in the UV−Vis region were recorded at 25 °C on a Varian Cary 300 Biospectrophotometer in 1 cm path length quartz cells. Circular dichroism (CD) spectra were recorded on Jasco J 715 spectropolarimeter over the 190–800 nm range using different path lengths (1 and 0.1 cm). The concentrations of solutions used for UV−Vis and CD spectroscopic studies were similar to those employed in the potentiometric experiments. Electron paramagnetic resonance (EPR) spectra were recorded in liquid nitrogen on a Bruker ELEXSYS E500 CW-EPR spectrometer at X-band frequency (9.5 GHz) and equipped with an ER 036TM NMR teslameter and an E41 FC frequency counter. The ligand pHAA was prepared in an aqueous solution of HCl at $I = 0.1$ M (KCl). The concentration of Cu^{2+} was 1×10^{-3} M, and the M:L molar ratio was 1:1.1. Ethylene glycol (30%) was used as a cryoprotectant for EPR measurements. The EPR parameters were analyzed by computer simulation of the experimental spectra using WIN-EPR SIMFONIA software, version 1.2 (Bruker). The pH was adjusted with appropriate amounts of HCl and NaOH solutions.

2.4. Mass spectrometric measurements

High-resolution mass spectra were obtained on BrukerQ-FTMS and Bruker MicrOTOF-Q spectrometers (Bruker Daltonik, Bremen, Germany) equipped with an Apollo II electrospray ionization source with an ion funnel. The BrukerQ-FTMS spectrometer was used for measurements on Cu^{2+}−pHAA complexes in the range of positive values of mass-to-charge ratio (m/z) from 150 to 1500. The instrumental parameters were as follows: scan range, m/z 400–1600; dry gas, nitrogen; temperature, 170 °C; capillary voltage, 4500 V; ion energy, 5 eV. The capillary voltage was optimized to the highest signal-to-noise ratio. Small changes in voltage (±500 V) did not significantly affect the optimized spectra. The Bruker MicrOTOF-Q spectrometer was used for measurements on Ni^{2+}−pHAA, Ni^{2+}−His_6-tag, Zn^{2+}−pHAA and Zn^{2+}−His_6-tag complexes in the positive ion mode over the m/z range from 200 to 1700. The instrumental parameters were as follows: scan range, m/z 250–2000; dry gas, nitrogen; temperature, 200 °C; ion source voltage, 4500 V; collision energy, 10 eV. The Cu^{2+}, Ni^{2+}, and Zn^{2+} complexes (metal:ligand stoichiometry of 1:1.1, $[ligand]_{tot} = 5 - \times 10^{-4}$ M) were prepared in a 1:1 MeOH/H_2O mixture at pH 4.5–5.0. The samples were infused at a flow rate of 3 μL/min. Before each experiment, the instrument was calibrated externally with the Tunemix mixture. Data were processed by application of the Compass DataAnalysis 4.0 program (Bruker Daltonic).

3. Results and discussion

Structural and thermodynamic properties of Cu^{2+}, Ni^{2+} and Zn^{2+}−pHAA and Ni^{2+} and Zn^{2+}−His_6-tag complexes were studied by mass spectrometry, potentiometry, and a variety of spectroscopic techniques. Mass spectrometric measurements provided the information on stoichiometry of the interactions, potentiometric titrations were the basis for the determination of precise stability constants and pH-dependent species distribution diagrams and combined UV–Vis, CD and (in the case of Cu^{2+}) EPR spectroscopic results allowed to conclude the binding mode of nickel(II) and copper(II) and the geometry of these species formed in solution. A combination of all used methods allowed to explain coordination geometries and perform a detailed thermodynamic analysis.

3.1. Protonation constants of the Ac-HAAHAAHAAHAAH-NH_2 (pHAA) peptide

The pHAA peptide (protected in the *N*-terminus by acetylation and in the C-terminus by amidation) contains six possible sites of protonation – all of them are assigned to the six histidine residues (Table 1). The p*Ka* values obtained from the potentiometric study (in the range 7.53–4.8) are typical values of histidine residues in similar poly-His systems [23,24,26]. Protonation constants of His_6-tag are described elsewhere [22].

3.2. Metal binding stoichiometry

Electrospray ionization mass spectrometry (ESI-MS) confirmed the purity of the studied ligands and showed the metal binding stoichiometry at pH 5. M/z values at 796.87 and at 531.59 correspond to $[L + 2H]^{2+}$ and $[L + 3H]^{3+}$ ligand forms, respectively. Only equimolar species were present under the studied conditions (m/z values at 827.33, 827.84 and 824.84 correspond to $[CuL]^{2+}$, $[ZnL]^{2+}$ and $[NiL]^{2+}$ complex forms, respectively) (Fig. S1).

3.3. Cu^{2+}-pHAA system

Experimental and simulated MS signals are in good agreement and m/z values at 827.33 and at 551.89 correspond to $[CuL]^{2+}$ and $[CuL + H]^{3+}$ complexes (Fig. S1 A).

Table 1
Protonation constants of pHAA peptide at 298 K and $I = 0.1$ M (KCl). The standard deviations are reported in parentheses as uncertainties on the last significant figure.

	logβ	p*Ka*
HL	7.52(2)	7.52
H_2L	14.17(1)	6.65
H_3L	20.71(2)	6.54
H_4L	26.69(2)	5.68
H_5L	32.49(1)	5.80
H_6L	37.75(2)	5.26