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STRUCTURAL DIVERSITY OF TRANSITION METAL COMPLEXES OF PEPTIDES CONTAINING CYSTEINE RESIDUE

Cysteine containing peptides are effective metal ion chelators. However, the coordination ability of these peptides significantly depends on the position of cysteine(s) and the presence of other side chain donor groups. In this paper we demonstrate the structural diversity of the complexes formed with peptides containing multiple metal binding sites. The results confirm that the thiolate functional group of cysteine in the peptides behaves as an effective metal binding site for nickel(II) and zinc(II). However, the other side chains of the peptide may significantly contribute to the metal binding depending on their position in the peptide chain.

The metal-peptide complexes are an excellent choice for mimicking the binding modes of the proteins and modelling the active site of metalloproteins and metalloenzymes. The metal binding affinities of these small biomolecules are rather selective and largely depend on the side chain donors of peptides and the character of the metal ions. Probably, the most important and widely-studied binding sites are the histidyl and cysteinyl side chains; however, specific coordinating side chains (such as the carboxyl group of aspartic- and glutamic acid) may also be involved in the coordination of the metal ion. The first studies on the quantitative description of metal complexes of peptides were reported in the 1980s. Recently, several studies have reviewed the general observations related to the metal-peptide interactions **[1-3]**. The coordination chemistry of multi-histidine peptides with transition metal ions are a subject of interest because various forms of neurodegenerative diseases are linked to these molecules. Furthermore, peptides containing cysteine residues have also been investigated; the most important publications concern the zinc transporter and zinc finger proteins and the nickel homeostasis of *Helicobacter pylori* **[4, 5]**. The number of the possible peptide sequences that can be synthesized is infinite because of the effective development of solid phase peptide synthesis. Therefore, systematic studies are needed in order to give deeper insight into the complex formation processes of peptides containing multiple and different binding sites. In our work, N-terminally free peptides containing aspartyl, histidyl or cysteinyl residues on the N-termini were synthesized and the contribution of the distant cysteinyl residue to the complex formation processes was investigated. Here we report the main results based on the thorough analysis of equilibrium, spectroscopic and theoretical data. The investigated peptides are shown in Scheme 1.

It is clear from Scheme 1, that the terminal amino group, the carboxylate of aspartyl, imidazolium of histidyl and the thiol of cysteinyl side chain are taking part in deprotonation processes. Moreover, thorough analysis of the NMR spectra offers a possibility to calculate the deprotonation microprocesses. It was shown, that the lowest pK value belongs to the deprotonation of carboxylate group, while the last

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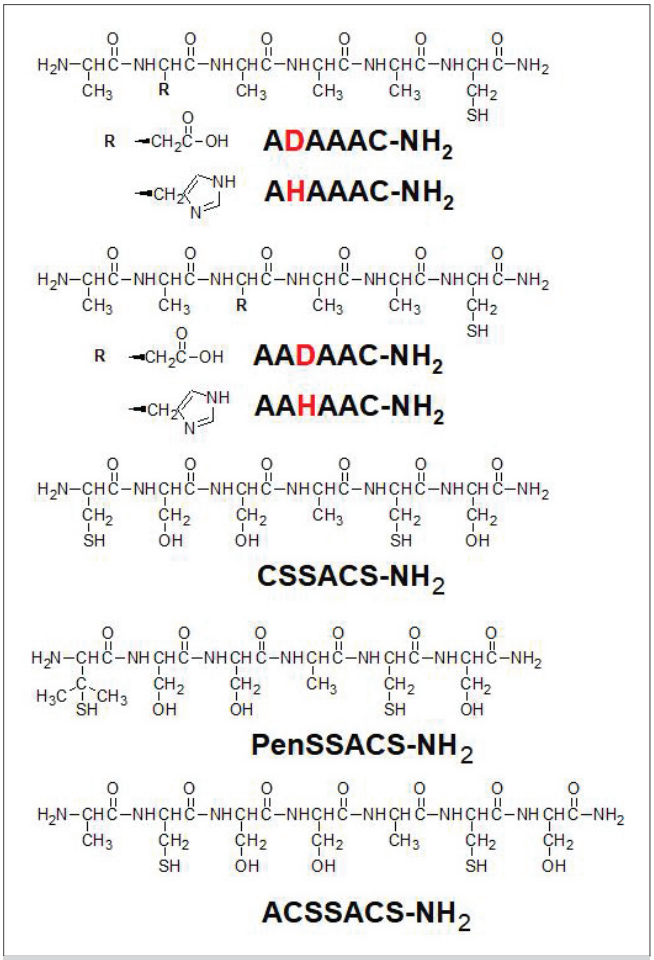

Scheme 1 - Peptides involved in this study

two or three deprotonation processes (deprotonation of terminal amino group and side chain of cysteine residue(s)) significantly overlap. However, the terminal amino group is more acidic site than the thiol group of cysteine [6].

The complex formation reactions of the investigated peptides with nickel(II) indicate that in all cases, the terminal amino group is the primary metal binding site resulting in amino, amide coordinated species with high stability. In the case of aspartyl containing peptides, the complexes with (NH_2,N$^-$,β-COO-) or (NH_2,N-,N-,β-COO$^-$) donor sets are the major species in the slightly acidic and neutral pH range. Upon increasing the pH, additional base consumption process yields the binding of the thiolate group and the co-existence of coordination isomers. DFT calculations predict that the C-terminal part of ADAAAC-NH_2 is more effective metal binding site for nickel(II) than the N-terminal part, while nickel(II) is mainly coordinated to the N-terminal part in the case of AADAAC-NH_2 (Fig. 1).

This behaviour can be explained by the enhanced stability of the complex with (NH_2,N$^-$,N$^-$,β-COO$^-$) donor set. The peptides with aspartyl residues form complexes with zinc(II) due to the binding of (NH_2,β-COO$^-$,S$^-$) donors. These macrochelate complexes are able to suppress the hydrolysis of the metal ion, however, they cannot induce the deprotonation and coordination of amide functions to the metal ions [6].

The N-terminally free peptides with histidyl residues are effective metal chelators for nickel(II) and zinc(II). Moreover, AAHAAAC-NH_2 has outstanding nickel(II) binding ability. This is due to the formation of albumin-like coordinated species which prevents the binding of the thiolate group. This donor group is involved in the metal ion coordination, when nickel(II) is in excess. It is important to note, that this albumin-like coordination keeps copper(II) in solution and hinders the interaction between copper(II) and

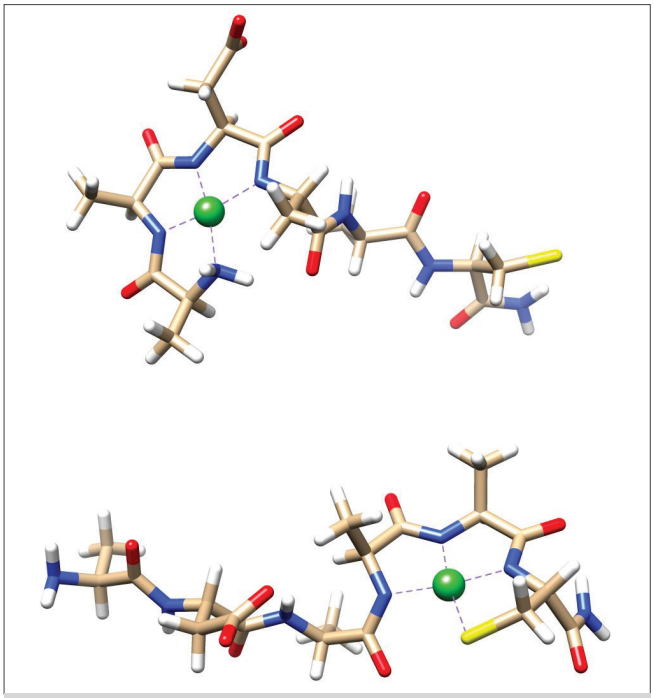

Fig. 1 - Energetically preferred coordination isomers formed in the Ni(II):AADAAC-NH_2 (top) and Ni(II):ADAAAC-NH_2 (bottom) systems calculated by DFT at B3LYP/*def2*-TZVP level of theory

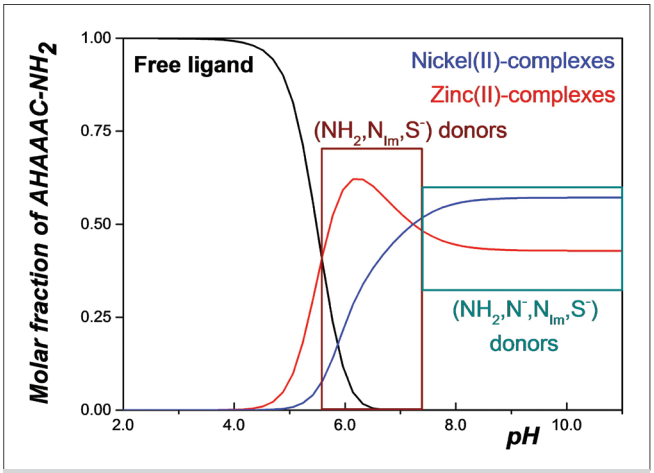

Fig. 2 - Calculated species distribution of the complexes in the Ni(II):Zn(II):AHAAAC-NH_2 1:1:1 system. c_L = 2 mM

the thiolate group, thus preventing redox processes in neutral pH range [7]. For zinc(II), the primary metal binding site is the thiolate group and imidazole-N of histidine in the slightly acidic pH range. Additional base consumption process is observed due to the binding of terminal amino group and the macrochelate complex is dominant in the basic pH region.

Nickel(II) and zinc(II) complexes of AHAAAC-NH_2 differ from those of AAHAAC-NH_2. This is due to the presence of the histidyl residue in the secondary position. Therefore,, the major species for nickel(II) is the complex with (NH_2,N^-,N_{Im}) donor set at physiological pH. However, this coordination sphere is unsaturated and the square-planar geometry of the metal ion is suitable to accommodate the thiolate group of the distant cysteine residue to form (NH_2,N^-,N_{Im},S^-) coordinated complex. In the case of zinc(II), the major complex is the (NH_2,N_{Im},S^-) coordinated species. Its outstanding stability is well demonstrated by Fig. 2, where the theoretical species distribution of the zinc(II) and nickel(II) complexes formed with AHAAAC-NH_2 is shown. Upon increasing the pH, both metal ions are able to induce the deprotonation and coordination of amide nitrogen and the stability order of zinc(II) and nickel(II) complexes revers because this type of reaction is more favoured for nickel(II) than for zinc(II) [8].

The complex formation processes of N-terminally free peptides containing two cysteinyl residues were characterized in the presence of zinc(II) because the formation of polymer complexes hindered the determination of the stability constants in the nickel(II) containing system. Both ligands have outstanding zinc(II) binding ability due to the formation of macrochelate complexes. In the case of CSSACS-NH_2, the amino terminus is the primary metal binding site *via* the (NH_2,S^-) 5-membered chelate that is supported by macrochelation due to the internal cysteinyl residue. This binding mode prevents the hydrolysis of the metal ion and hinders the formation of bis-complexes when the ligand is in excess. In contrast, the formation of bis-complexes was observed in the case of ACSSACS-NH_2. This behaviour can be explained by considering that the thiolate groups are the primary metal binding sites for zinc(II) and the binding of the terminal amino group is unlikely. In the case of equimolar samples, the terminal amino group and the peptide nitrogen are also involved in the metal ion coordination and the formation of the complex with (NH_2,N^-,S^-,S^-) donor sets occurs in the slightly basic pH range. The zinc(II) induced deprotonation and coordination of peptide nitrogen has already been described in various peptides containing histidine in the secondary position, however, this is the first example for the formation of Zn-N(peptide nitrogen) bond in the case of cysteine containing peptides. The complex formation processes of cadmium(II) with the above mentioned cysteine containing peptides are similar to those observed for zinc(II) [9].

To suppress the formation of polymer species, the cysteine residue was replaced to penicillamine (see Scheme 1, PenSSACS-NH_2). This bulky side chain may hinder the formation of polymer species and the complex formation processes could be characterized in the presence of nickel(II). The complex formation of nickel(II) with this peptide is similar to those of the peptides containing cysteine in the first position. Thus, the terminal amino group with the thiolate side chain is the primary metal binding site and the coordination sphere is completed by the binding of the amide nitrogen and the distant cysteinyl residue in equimolar solution. However, the complex formation processes of zinc(II) with this peptide showed similarities to the cysteine containing counterpart, although, the formation constants of

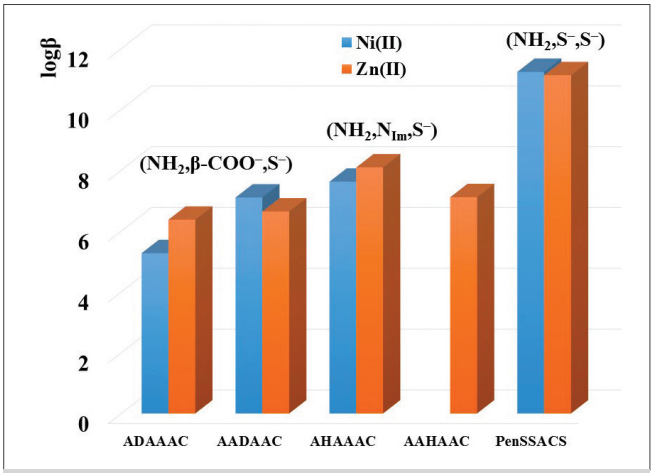

Fig. 3 - Stability constants (logβ) and coordination modes of the ML complexes formed in the investigated systems

the zinc(II)-PenSSACS-NH_2 complexes are 1-2 order of magnitude smaller than the corresponding zinc(II) complexes formed in the CSSACS-NH_2 system. This is most likely the consequence of the presence of the bulky methyl groups in penicillamine **[10]**.

In general, the thiolate functional group of cysteine containing peptides behaves as an effective metal binding site for nickel(II) and zinc(II), but the other side chains of the peptide may significantly contribute to the metal binding. This effect is clearly demonstrated in Fig. 3 where the stability constants of the ML complexes (M=Ni(II) or Zn(II)) are depicted as a function of coordinating donor groups. Fig. 3 clearly shows the outstanding stability of the thiolate coordinated complexes and it is also obvious from the data that the thiolate coordination is more favourable for zinc(II) than nickel(II) and the nickel(II) complexes have enhanced stability when the amide nitrogen is involved in the metal ion coordination. Moreover, the coordination of thiolate group can be hindered when the coordinating side chain of amino acid is placed in the third position of the peptide chain.

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Diversità Strutturale dei Complessi di Metalli di Transizione di Peptidi contenenti Residui di Cisteina

I peptidi contenenti cisteina sono buoni chelanti per gli ioni metallici. Tuttavia, la capacità di coordinazione di questi peptidi dipende in modo significativo dalla posizione della cisteina e dalla presenza di altri gruppi donatori in catena laterale. Questo articolo descrive la diversità strutturale dei complessi formati con peptidi contenenti più siti di legame per i metalli. I risultati confermano che il gruppo funzionale tiolato della cisteina nei peptidi si comporta come un efficace sito di legame per il nichel(II) e per lo zinco(II). Tuttavia, le altre catene laterali del peptide possono contribuire in modo significativo alla chelazione del metallo, in funzione della loro posizione nel peptide.