



# BIOINORGANIC CHEMISTRY OF ZIP PROTEINS

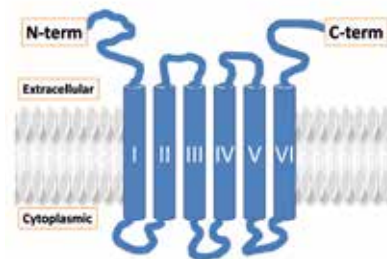


Fig. 1  
ZIP6 protein as an example of ZIP family transporters

THE NEWEST FINDINGS CONCERNING ZIP PROTEINS HAVE BEEN PRESENTED BELOW. WE STUDIED THREE DIFFERENT DOMAINS FROM THREE DIFFERENT PROTEINS. ALL OF THE RESULTS AND THUS CONCLUSIONS GAINED DURING OUR STUDIES, REVEALED NEW PROPERTIES OF THOSE INTERESTING LIGANDS AND SIMULTANEOUSLY SHED NEW LIGHT ON THE BIOCHEMICAL PROPERTIES OF SPECIFIC ZIP PROTEINS DOMAINS. WE TRULY BELIEVE THAT OUR STUDIES MAY GIVE AN ADDITIONAL CLUE FOR UNDERSTANDING THE ROLE OF METAL ION BINDING DOMAINS IN CRUCIAL MULTI-HISTIDINE AND CYSTEINE PROTEINS

ZIP proteins (Slc39a, Zrt/Irt-like Proteins) are metals transporters within the cells and different cellular compartments. They are transmembrane biological structures. The general role of those proteins is to increase the metal concentration within the cytoplasm [1-3]. Among mammalian ZIP family of proteins, one can distinguish 14 members which are expressed in different types or parts of the cells [4, 5]. They are built of 6-8 helical transmembrane domains (TMDs) and cytoplasmic/extracellular loops between them (Fig. 1).

The loops are very often rich in histidine residues, that are expected to act as metal binding sites [6, 7]. Till now, not much is known about the mechanism of action of those metal transporters. The transport of zinc by human ZIP1 and 2 does not require the presence of ATP and is induced by  $\text{HCO}_3^-$  what suggests a symport mechanism. Different kinds of approaches have been used to answer a number of questions concerning *e.g.* the role of histidine-rich domains (HRDs) present in the loops and many contradictory hypotheses have been put forward. The long flexible region in the cytoplasm between TMD III and IV is one of the most interesting. This loop contains the motif with a general formula  $(\text{HisX})_n$ , where X is any amino acid and n equals to 3-6 [8-10].

The mechanism of ZIP transporters and the function of their histidine rich sequences are currently under debate. Experiments are performed on both prokaryotic and eukaryotic cells. The structure of ZIP transporters is well conserved among variety of living organisms and thus results which are obtained for simple models are often valid for organisms placed higher in the evolutionary hierarchy. What is also worth to mention, they are evolutionarily related to prion proteins [11] (which are able to undergo transformation into the pathogenic form and become at least partially responsible for neurodegenerative disorders [12, 13]), what makes them even more interesting as a subject of study.

## ZIP13

Human ZIP13 has a very interesting, multi-cysteine, extracellular N-terminus. One can distinguish 2 isoforms of this transporter: 1 and 2, containing 371 and 364 amino acids, respectively. This protein is located mainly in osteoblasts, chondrocytes, pulpal cells, fibroblasts and Golgi apparatus of different cells [14]. It possesses eight transmembrane domains. The N-terminal sequence is interesting mainly from the chemical point of view as a multicysteine peptide ligand. Similar motifs are present naturally *e.g.* in metallothioneins

or bacterial chaperones, that is why they are those of great interest. From the same reason, studied N-terminus were examined as a ligand not only for divalent metal cations like  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$  (potential substrates for the whole protein), but also for  $\text{Bi}^{3+}$ , which may interact with similar motifs in bacterial chaperones loops *e.g.* in *H. Pylori* [15]. Metal complexes of  $\text{MPGCPCPGCG-NH}_2$  peptide has been investigated by potentiometry, mass spectrometry, NMR, CD, and UV-Vis spectroscopies [16]. Complete characterization of the formed complexes was possible by the correlation of the experimental data obtained from different methods. MS measurements provided the information on the stoichiometry of the interactions, UV-Vis and CD spectroscopies gave us some insights about the geometry of complexes, potentiometric titrations allowed us to determine the stability constants, whereas analysis of NMR showed the exact complex species binding sites formed in solution. What appeared straight forwards, all investigated metals ( $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Bi}^{3+}$ ) have similar binding modes, with the three cysteine residues side chains involved in metal ion binding; example showed on  $\text{Bi}^{3+}$  complex (Fig. 2).

The stability of the metal complexes changes in the following series  $\text{Bi}^{3+} \gg \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+}$ , clearly the strongest being for bismuth ( $\log \beta =$

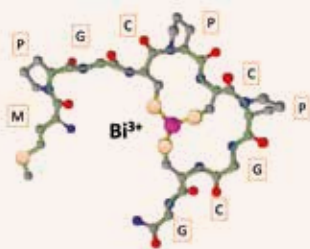


Fig. 2

Proposed structure of BiL complex; L is the N-terminus of ZIP13 (MPGCPCPGCG-NH<sub>2</sub>). For clearer imaging, the hydrogen atoms were removed; colours: silver - carbon; blue - nitrogen; red - oxygen; orange - sulphur; green - backbone. Figure prepared in ACDLABS 12.0

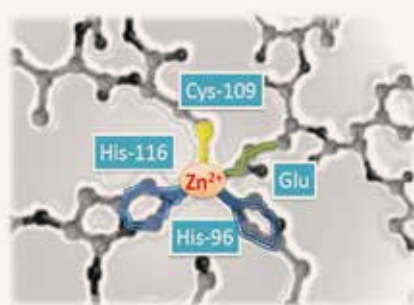


Fig. 3

The coordination of Zn<sup>2+</sup> ion in the complex with Ac-(95)MHVLPDSFEM-LSSICLEENPWHK(117)-NH<sub>2</sub> peptide

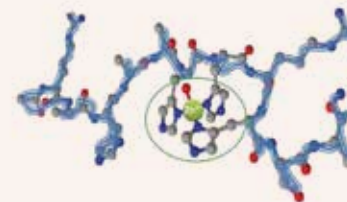


Fig. 4

The representation of Zn<sup>2+</sup> binding site in Ac-(185)RAHAAHRRHSH(195)-NH<sub>2</sub> peptide (HRD)

29.39, 19.34, 15.96 and 12.73, respectively). In the BiL complex, the binding mode does not change in all pH range. Cysteine residues coordinate at very low pH and do not change when pH is rising. In the case of ZnL and CdL complexes, the fourth coordination site is the amine group from Met residue. Ni<sup>2+</sup> binding yields the diamagnetic complex species with three thiolates involved in the metal coordination. No further binding group has been identified by NMR spectroscopy, so we assumed that the fourth coordination group is water; three thiolate sulfurs provide enough energy to maintain pseudoplanar geometry of Ni<sup>2+</sup> ion. The coordination of the N-terminal amino group stabilizes additionally Zn<sup>2+</sup> complexes when compared to Ni<sup>2+</sup>. As expected, Cd<sup>2+</sup> complexes are thermodynamically more stable than Zn<sup>2+</sup> species. The multi-cysteine fragment of the human metallothionein (MDPETCPC-NH<sub>2</sub>) and a histidine and cysteine containing peptide from *H. Pylori* (A-AC-CHDHKKH-NH<sub>2</sub>) were chosen for the comparison studies. Such 2 and 3 Cys containing systems comparative studies further confirm the involvement of all three thiol groups in metal binding [16]. The obtained results not only provide some insight into the biological chemistry of Cys-rich peptide fragments, but also give information about the coordination modes associated with them.

### IRT1

Iron-regulated transporters (IRTs) are ZIP family proteins. IRT1 was the first gene identified among this family and till now widely studied [17-20]. The protein is located in the roots of many plants and acts as a regulator

of an iron uptake from the rhizosphere. The transporter possesses eight TMDs. Its specificity toward iron ions is not very high; under favourable conditions it transports also other divalent metal ions like essential Zn<sup>2+</sup>, Mn<sup>2+</sup> or even toxic Cd<sup>2+</sup> [21, 22]. IRT1 from *Arabidopsis thaliana* is a protein of 347 residues and 36.7 kDa. The protein contains very interesting, selectivity-responsible regions, where even one amino acid exchange causes a dramatic change in whole protein specificity toward various metal ions. As such example, one can recall the replacement of Glu-103 with alanine that leads to a form of protein, which transports Fe<sup>2+</sup>, Mn<sup>2+</sup> and Cd<sup>2+</sup>, but is not able to deal with Zn<sup>2+</sup> ions. The deletion of some particular residues, results in no transport activity; example might be His-96 in the loop between the TMD II and III.

Crystallographic data or NMR structures of the ZIP proteins are not available unfortunately, but bioinorganic investigations carried out on the domains crucial for transport, may provide us valuable information. Very interesting example is the extracellular loop of IRT1 located between the II and III TMD. Zinc complexes with the Ac-(95)MHVLPDSFEM-LSSICLEENPWHK(117)-NH<sub>2</sub> peptide revealed surprisingly high thermodynamic stability [23]. Multicysteine, N-terminal fragment of human ZIP13 zinc transporter (MPGCPCPGCG-NH<sub>2</sub>, ZIP13) was chosen for the comparison studies. Surprising was the fact, that at pH around physiological, the stability of IRT1-Zn<sup>2+</sup> and ZIP13-Zn<sup>2+</sup> is comparable. An interesting coordination mode was observed for the IRT1-Zn<sup>2+</sup> complex, in which two imidazoles from histidine residues (His-96 and His-116),

a cysteine thiolate (Cys-109) and one of a glutamic acid carboxyl group are bound (Fig. 3). Glutamic residue is involved in binding starting from low pH (NMR data). The impact of Glu residue is of great importance for the increased stability of IRT1-Zn<sup>2+</sup>. What is also known from different studies, without Glu-103, this particular transporter is not able to transport Zn<sup>2+</sup> [21]. The observation, that glutamic acid side chain is involved in the coordination of Zn<sup>2+</sup> ions and the high impact of this residue on stability enhancement, may provide some insight into the biology of many other proteins. Examples where glutamic or aspartic acids may possibly take part in metals coordination are:

- 1)  $\alpha$ -synuclein, a protein involved in the formation of the Lewy bodies typical for Parkinson's disease; the domain from 96 to 140 residue is negatively charged at physiological pH because of the presence of many acidic side chains (Asp and Glu), which also may have an impact on the stability of binding;
- 2)  $\beta$ -amyloid peptide, the main component of plaques characteristic of Alzheimer's disease. It is commonly accepted that the fragment (1)DAEFRHDSGYEVHHQK(16) coordinates many metal ions [24-27];
- 3) Hpn and Hpn-like, *H. pylori*'s proteins involved in the homeostasis of Ni<sup>2+</sup> ions. They possess Glu residues, which can easily serve as a donors for metal cations.

### TjZNT1

Most ZIP proteins have a long, unstructured loop between III and IV TMD. This region is probably located in the cytoplasm and is po-

stulated to be the metal ion binding site. We looked at the thermodynamic properties of Zn<sup>2+</sup>, Ni<sup>2+</sup> and Cu<sup>2+</sup> complexes with the histidine-rich motif Ac-(185)RAHAAHRRSH(195)-NH<sub>2</sub> (HRD), from the yeast TjZNT1 protein, located between TMDs III and IV [28] (Fig. 4).

The sequence is highly conserved among different species, also in higher plants, e.g. in *Thlaspi japonicum*. As it arises from the results, the stability of complexes increases in the series Ni<sup>2+</sup> < Zn<sup>2+</sup> < Cu<sup>2+</sup>. The geometry of complexes and ligand specificity toward different metal ions is very different - only in the case of Zn<sup>2+</sup> complexes, high specificity in binding is observed. Moreover, in this work, the stability of HRD-Cu<sup>2+</sup> complexes was compared with the five His residues containing ligand from Hpn protein (*H. pylori*). The studied metal complexes of the HRD peptide revealed very interesting chemical properties. Very informative technique in this project was 2-dimensional NMR. Upon the addition of 1 eq. of Zn<sup>2+</sup> ions, the NMR shift of 3 His residues was observed on TOCSY spectrum. Those 3 histidines are in the middle of the sequence, what suggests strongly their participation in binding. From potentiometric data we can state, that Ni<sup>2+</sup> complexes are the weakest. Looking at the NMR measurements at pH 10 informs us, that Ni<sup>2+</sup> ions have more than one binding site in this short fragment of TjZNT1 protein. Shifts observed for diamagnetic Ni<sup>2+</sup> complexes in the TOCSY proton correlation spectra evidence this physicochemical phenomenon. Arg-192 and Ser-194 suggest that preferable His residues are those at the C-terminus of the peptide, but simultaneously Ala residues shifts suggest other equally acceptable binding site. Performed competition diagrams based on potentiometric data revealed that Zn<sup>2+</sup> complexes have higher stability when compared to Ni<sup>2+</sup> complexes. Cu<sup>2+</sup> complexes are not so easy to study, since they are strongly paramagnetic. The most helpful region from TOCSY spectra was so-called fingerprint region, where one can observe correlations of separated spin systems. We detected broadening on residues Arg-192, Ser-194 together with His-193 and His-195 what suggested the C-terminus being a preferential binding site. We also studied the possibility of replacement of one metal by another. As it arises from the results, neither Zn<sup>2+</sup> nor Ni<sup>2+</sup> ion can substitute Cu<sup>2+</sup>; when we titrated Cu<sup>2+</sup>-HRD complex with the solution of Zn<sup>2+</sup> or Ni<sup>2+</sup> ions,

we could not detect any change of CD spectra. The reason is probably the nearly 8 orders of magnitude difference on cumulative stability constant (β) of ZnL and CuL. Another reason might be also the ligand length and the limited number of possible anchoring sites located in close proximity.

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### Chimica bioinorganica di proteine ZIP

In questo articolo vengono presentati i risultati più recenti in materia di proteine ZIP. Sono stati studiati tre domini differenti da tre proteine diverse. Tutti i risultati, e quindi le conclusioni acquisite durante la ricerca, hanno rivelato nuove proprietà di questi interessanti ligandi e, contemporaneamente, hanno fatto nuova luce sulle proprietà biochimiche di specifici domini di proteine ZIP. Questi nostri studi possono dare un indizio supplementare alla comprensione del ruolo di metallo ioni leganti proteine cruciali multi-istidina e cisteina.

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