Potentiometry as a tool to study metal ion coordination to metallothioneins

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The aquatic fungus *Heliscus lugdunensis* was found to be able to survive in a polluted spring on a former mining area in Germany, which shows high metal ion concentrations. The fungus produces a number of different thiolate-containing molecules, among them the metallothionein Neclu_MT1[1].

MTs are a large super-family of small metalloproteins (10 electron configuration. Neclu_MT1 is with only 24 amino acids one of the smallest MTs, but features with eight Cys residues the same high Cys content as the vertebrate forms. In addition, Neclu_MT1 contains a single C-terminal His residue, which is one of its most interesting features since it was shown to be a Cd(II)-specific MT, both on the protein and on the gene transcription level.[2] Based on the available ligands two types of clusters can be envisioned, $Cys_8M^{II}_3$ or $Cys_8HisM^{II}_3$, which both would be of great interest, since none of them has been found in MTs before.

In order to better understand the role of the His residue, an arginine mutant was produced (Neclu_H24R_MT1). The binding capacity of Neclu_H24R_MT1 is comparable to the wild-type: both are able to complex three Cd(II) or Zn(II) ions. With potentiometric measurements we were able to calculate the pK_a values of all titratable residues of wild-type and mutant apo-Neclu_MT1. Furthermore, complexation studies are being carried out on the two systems with both Cd(II) and Zn(II).

The obtained data is intended to provide information about the binding ligand pattern and the specific role of the His residue. UV-visible, circular dichroism and mass spectrometry experiments complement the potentiometric data.

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[2] J. Loebus, B. Leitenmaier, D. Meissner, B. Braha, G. Krauss, D. Dobritzsch & E. Freisinger. The major function of a metallothionein from the aquatic fungus *Heliscus lugdunensis* is cadmium detoxification. J. Inorg. Biochem. 127, 253-260 (2013).