Interplay of multiple metal ion binding sites regulates the catalytic activity of metalloenzymes

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Oligohistidine tags are frequently applied for protein purification using immobilized metal ion affinity chromatography (IMAC). However, these tags may affect the structure and function of the target protein and thus, need to be removed before the further characterization of the properties of the proteins. The interference with the His-tag needs particular consideration in case of metalloproteins, since the histidines can also bind metal ions with various consequences. The metal ion may be e.g. removed from the active center, or ternary mixed ligand or mixed metal complexes may form.

Recently, we have published the purification of a DNA hydrolyzing enzyme, an NColE7 mutant with the aid of a C-terminal 6×His-tag, which was cleavable without any remaining extra amino acid residues after the IMAC step[1]. NColE7 binds Zn(II) ions in its active center possessing a ββα motif. Three His side-chains are coordinated to the metal ion, while the fourth position is reserved for the substrate or water binding. Further His side-chains are provided for metal ion binding by the enzyme itself, as well as by the His-tag. A complex effect of the affinity tag on the catalytic activity, influenced by the concentration of the Zn(II) ions, was observed in these experiments. Correlating the Zn(II) concentration dependent catalytic activity with the species distribution diagram, obtained from the corresponding mass spectrometric results, allowed a better understanding of the role of His-tag in the catalytic process[2].

Investigation of NColE7 and its various mutants in the presence of Mg(II), Cu(II), Ni(II) and Cd(II) metal ions lead us to elaborate fine tuning and regulation of the nuclease action. The effects of metal ion competition between the Zn(II) and non-native metal ions on the solution structure and catalytic activity were monitored by circular dichroism spectroscopy and agarose gel electrophoretic mobility shift assays, respectively. In our hope, this knowledge can be applied in the development of regulated artificial nucleases for nuclease-mediated genome editing to achieve safe action in complex biological matrices.

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