

Raman Spectroscopy Investigation on Metal-Metallothionein Complexes

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Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins with an exceptional heavy metal coordination capacity. Because of their ability to bind metals and to scavenge oxidant radicals, MTs are considered to play a role in metal homeostasis, metal detoxification and control of the oxidative stress. Their high heterogeneity, metal binding abilities and primary structure suggest very diverse functional specializations. Structural and functional studies have been mainly devoted to vertebrate MTs. Participation of metal ligands other than Cys and the presence of secondary structure elements in metal-MT complexes are fairly unknown, especially in non-vertebrate MTs. Recently, it has been shown that other ligands can participate in metal coordination; two main ligand types have been identified: endogenous, as His residues and exogenous, as inorganic ions (i.e. S^{2-} or Cl^-).

In vivo-synthesized Zn-MTs, representative of different families (mollusc, insect, nematode, echinoderm, vertebrate and plant, enclosing the mammalian MT1 isoform), were heterologously synthesized in *E.coli* and studied by analytic and spectroscopic techniques. The examined MTs (CeMT2, MeMT, SpMTA, MtnB, QsMT and MT1) contain 43-73 a.a., among which at least a 30% are Cys. To evaluate the influence of the metal on the MT structure, also the Cd-QsMT was synthesised and analysed in comparison with Zn-QsMT. Almost all the MTs considered are devoid of aromatic residues (only Phe in SpMTA and QsMT, Tyr in CeMT2, and His in CeMT2 and QsMT) and contain variable amounts of metal and S^{2-} ions, quantitatively evaluated by acid ICP-AES and GC-FPD measurements. The formation of more than one species (S^{2-} -containing and S^{2-} -devoid complexes), revealed by ESI-MS spectra, was evident for all the MTs.

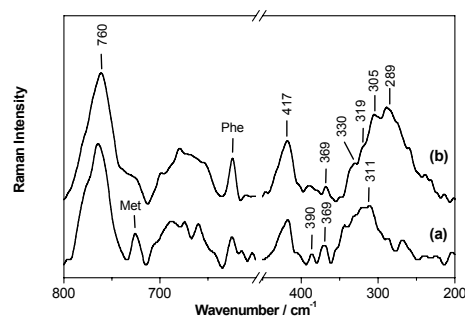


Fig. 1: Raman spectra of (a) Zn-QsMT and (b) Cd-QsMT.

The Raman spectra gave information about the structure of the metal-MT aggregates. In particular, the presence of secondary structure elements was determined: for the examined MTs isoforms, a relevant contribution of β -sheet and β -turns was shown, whereas the α -helix content resulted almost negligible. As regards Cys sulfurs, almost all Cys were involved in the metal coordination, as indicated by several bands attributable to metal-S stretching modes at low wavenumbers ($< 500 \text{ cm}^{-1}$) (Fig. 1). In particular, the high number of ν M-S bands, as well as their broadening, suggest the formation of different metal centres. Raman bands markers of sulfide bridging ligands were also identified. In Zn-

CeMT2 and Zn-QsMT the eventual participation of the His residue in metal binding was evaluated through a curve fitting analysis of the $1630\text{-}1565 \text{ cm}^{-1}$ region (Fig. 2). By considering the integrated intensity of the bands, it can be concluded that His residues are mainly coordinated in Zn-CeMT2 ($\approx 90\%$), whereas in Zn-QsMT His is mainly present as free tautomer ($\approx 90\%$). The combination of analytical and spectroscopic techniques has been highly informative for the analysis of *in vivo*-synthesized metal-MT complexes; Raman studies revealed one of the most promising experimental strategies to provide new data on the knowledge of the metal binding behavior of MTs from the most diverse organisms.

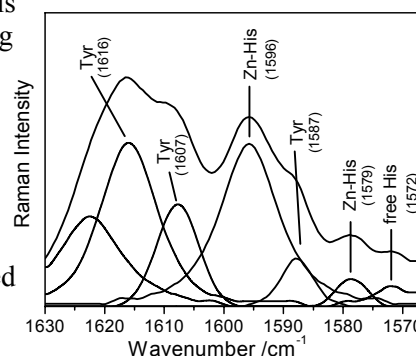


Fig. 2: Curve fitting analysis of His-containing Zn-CeMT.