Volume: 2
Year: 2012
Symposium Edition: XXIII

ISMEC GROUP SERIES
http://mat520.unime.it/ismecacta/
ISSN: 2239-2459

ISMEC2012

International Symposium on Metal Complexes

Lisbon 18-22 June

* Acta of the International Symposia on Metal Complexes

INSTITUTO SUPERIOR TÉCNICO
Universidade Técnica de Lisboa

Interaction of divalent cations with Park9 protein fragments

Maurizio REMELLI,[a] Massimiliano PEANA,[b] Serenella MEDICI,[b] Roberto ANEDDA,[c] Maria Antonietta ZORODDU,[b]

[a] Department of Chemistry, University of Ferrara, via L. Borsari 46, I-44121 Ferrara; maurizio.remelli@unife.it
[b] Department of Chemistry and Pharmacy, University of Sassari, Via Vienna 2, I-07100 Sassari
[c] Porto Conte Ricerche, S.P. 55 Porto Conte/Capo Caccia, Km 8.400 Loc. Tramariglio, I-07041 Alghero (SS)

Two peptide sequences from Park9 Parkinson's disease (PD) gene, -$P_1D_2E_3K_4H_5E_6L_7$- (**1**) and -$F_1C_2G_3D_4G_5A_6N_7D_8C_9G_{10}$- (**2**) have been tested for Mn(II), Zn(II) and Cu(II) binding. Park9 encoded protein can protect cells from manganese poisoning, which is an environmental risk factor for a Parkinson's disease-like syndrome [1-4]. In fact, Park9 belongs to a family of ATP-ases involved in metal coordination and transportation; familial gene mutations may result in early development of PD. The chosen fragments are located from 1165 to 1171 and from 1184 to 1193 residues in the Park9 sequence, and are highly conserved in a number of organisms, going from yeasts to humans. Potentiometric, UV-vis experiments together with mono- and multidimensional NMR spectroscopy have been used to understand the details of metal binding sites at different pH values and at different ligand to metal molar ratios, showing that the three metals are able to effectively bind the examined peptides.

From NMR measurements Mn(II) and Zn(II) coordination with peptide **1** involves imidazole N_ε or N_δ of His_5 and carboxyl γ-O of Asp_2, Glu_3 and Glu_6 residues. Six donor atoms participate in Mn(II) binding, resulting in a distorted octahedral geometry, possibly involving bidentate interaction of carboxyl groups; four donor atoms participate in Zn(II) binding, resulting in a tetracoordinated geometry. Potentiometric data show that soluble, hydroxylated Zn(II) species are formed in the alkaline pH range. The formation of Cu(II) complexes with peptide **1** starts below pH: only mononuclear complexes have been potentiometrically detected also in the presence of excess of ligand (see Figure 1). Imidazole nitrogen of His residues acts as first Cu(II) anchoring site; as pH is raised, ligand coordination proceeds with deprotonation and binding of neighbouring amide nitrogens of the peptidic backbone. UV-vis spectra agree that the main species at neutral pH is a $\{N_{im}, 2N^-, O\}$ complex, where the oxygen atom most likely belongs to an equatorially coordinated water molecule.

Cu(II), Mn(II) and Zn(II) coordination involves the cysteine residues with peptide **2** and complex-formation invariably starts at lower pH with respect to ligand **1**.

Mn(II) accepts additional ligand bonds from D_4 and D_8 to complete the coordination sphere; the unoccupied sites may contain solvent molecules. When the ligand is in excess, both Zn(II) and Cu(II) ions form bis-complexes. The two metal ions behave in a very similar way and the stoichiometry of main species at physiological pH depends on the metal/ligand ratio: $[ML]^{2-}$ in equimolar solution or $[MHL_2]^{5-}$ for the 1:2 ratio. Potentiometric data suggest for the former a {2S, 2O} and for the latter a {3S, 1O} coordination without any participation of amide nitrogens, as usually found for Cu(II)/peptide complexes.

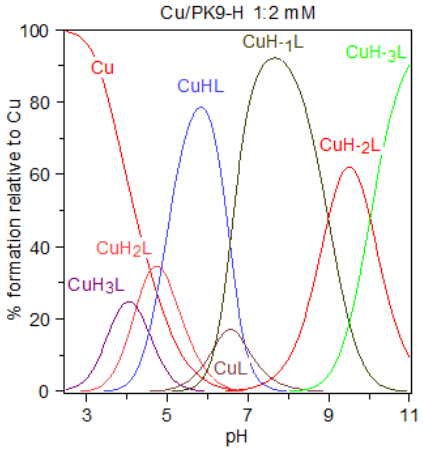

Fig. 1
Exemplificative distribution diagram for the system Cu(II)/peptide **1**

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