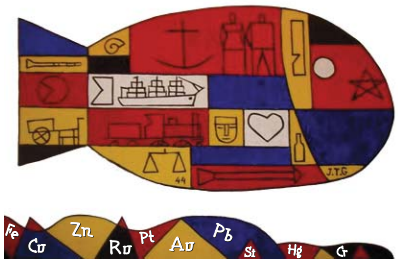
ISBN: 978-9974-0-0911-0



Abstracts and Proceedings of the 12th International Symposium on Metal Ions in Biology and Medicine

María H. Torre and Dinorah Gambino (Eds.)

11, 12 and 13 March 2013
Punta del Este, Uruguay

Park9 interaction with Manganese and other divalent cations

Zoroddu, M.A.[a*]; Remelli, M.[b]; Peana, M.[a]; Medici, S.[a]; Solinas, C.[a]

[a]*Department of Chemistry and Pharmacy, University of Sassari, Sassari (Italy),*
[b]*Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara (Italy),*
[*]*Email: zoroddu@uniss.it*

Abstract

Two peptide sequences from Park9 Parkinson's disease 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 studied in their interaction with Mn(II) and Zn(II) ions. These fragments lie from residue 1165 to 1171 and from 1184 to 1193 in the Park9 encoded protein, that can protect cells from manganese poisoning, an environmental risk factor for a Parkinson's disease-like syndrome called Manganism. The study was carried out through potentiometric and spectroscopic (UV-Vis, EPR, mono- and multidimensional NMR) techniques, to cast light on the details of metal binding at different pH values and different ligand to metal molar ratios.

Keywords: Parkinson, Manganism, divalent cations

Introduction

Recently, a connection between genetic and environmental causes of Parkinson's disease (PD) has been reported [1,2]. It was known that environmental and occupational exposure to Mn(II) [3] can lead to symptoms that resemble Parkinson's disease, called Parkinsonism or Manganism [4], but two articles appearing almost at the same time in the literature showed that a human PD gene, PARK9 (a member of the P5-type ATPase family, perhaps a metal transporter, considering its richness in coordinating residues), and its homologue in yeast, YPK9, can prevent manganese- induced PD and protect neurons and cells from manganese poisoning [1]. In fact, deletion of the YPK9 gene, which is 58% similar and 38% identical in its amino acid sequence to human PARK9, confers sensitivity to growth defects in the presence of cadmium, nickel, selenium, and manganese, suggesting that YPK9 protein may play a role in the sequestration of divalent heavy metal ions. In the same way, a mutation on PARK9 may expose humans to these cations, especially to manganese. We have thus decided to verify the possibility of metal binding to some simplified portions of PARK9 and YPK9, by choosing promising sites for metal interaction due to the presence of coordinating residues, far from the membrane-spanning and the alpha-helical rich domains not easily accessible by metals, and taking into account highly conserved binding motifs on both PARK9 and YPK9. We identified two interesting fragments and tested them for metal binding with manganese and zinc [5,6].

Materials and methods

Peptides were synthesized using solid-phase Fmoc chemistry in an Applied Biosystems Synthesizer. Peptides were N-terminally acetylated and C-terminally amidated in order to mimic this region of YPK9 within the full-length protein.

NMR experiments were performed on a Bruker Ascend^{TM} 400 MHz spectrometer equipped with a 5 mm automated tuning and matching broad band probe (BBFO) with z-gradients. Samples were 2.5 or 5 mM in concentration and dissolved in 90/10 (v/v) H_2O/D_2O solutions, at 298 K in 5 mm NMR tubes. NMR data were processed with TopSpin (Bruker Instruments) software and analyzed by Sparky 3.11 MestRe Nova 6.0.2 (Mestrelab Research S.L.) programs.

Potentiometric titrations were performed with Orion EA 940 and Orion 720A pH-meters equipped with a combined glass electrode (Metrohm EA125), Hamilton MicroLab M and MicroLab300 motor burettes, and Hamilton syringes. The temperature was kept at 298.2 ± 0.1 K by use of a thermostat. All measurements were carried out under nitrogen atmosphere.

EPR spectra were recorded with an X-band (9.4 GHz) Bruker EMX spectrometer, 100 kHz field modulation, at 120 K on neat aqueous solutions or mixed with few drops of ethylene glycol, at different ligand to metal molar ratios, ranging from 1:0.2 to 1:1, pH 7 and 1 mM peptide concentration.

Results and discussion

Peptide 1, $P_1D_2E_3K_4H_5E_6L_7$

Only mono-nuclear complexes have been potentiometrically detected in the systems containing Mn(II) and the investigated peptides, and the speciation diagrams are in agreement with the results found with NMR, UV-vis and EPR measurements.

Mn(II) is a paramagnetic ion, thus the effect of its addition to peptide 1 and 2 was monitored through the NMR line broadening and/or disappearance it causes on the resonances of the free ligand (Fig.1).

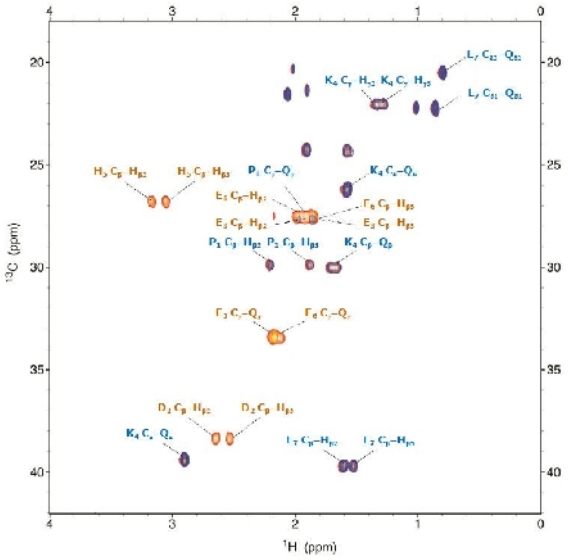


Fig. 1 Selection of aliphatic regions in ^{1}H-^{13}C HSQC NMR spectra for the Ac-$P_1D_2E_3K_4H_5E_6L_7$- Am peptide, 2.5 mM, pH 6.2, T 298 K in the absence (orange) and in the presence (blue) of 0.1 equivalents of Mn(II).

As expected whenever a histidine residue is present on a peptide fragment coordinating a metal, this was the favoured binding site for both Mn(II) and Zn(II) ions with peptide 1.

From the different sets of experiments performed, it was clear that once His_5 has anchored the peptide at slightly acidic pH, other residues are involved in coordination as the pH is raised. Mn(II) progressively affected the relaxation rates of the amidic HN protons from the backbone together with the complete disappearance of Asp_2,

Glu_3 and Glu_6 aliphatic protons signals in the 2D TOCSY spectra, and a decrease or total loss of their proton–carbon correlations in the 2D ^{1}H-^{13}C HSQCs.

Moreover, the analysis of the aliphatic HSQC region revealed that Qγ protons of Glu_3 and Glu_6 vanished upon Mn(II) addition, indicating that glutamic carboxylic groups are involved in the coordination, a fate shared also by the aspartate residue. All the other signals remained unaffected upon manganese addition. From the analysis of the data thus collected, the donor groups involved in metal coordination might be the Nε nitrogen from the imidazole ring of His_5, and the γ-O from three carboxylic moieties of Asp_2, Glu_3 and Asp_6 side-chains (Fig 1).

EPR spectra of the Mn(II) complexes reflect the symmetry of the electronic environment of the ion which is imposed by the ligands in the primary coordination sphere. Changes in the composition or geometric arrangement of the ligands in the coordination sphere of the metal ion lead to changes in the position of the EPR spectral lines. The Mn(II)–peptide 1 spectra are almost indistinguishable from those of free Mn(II). The spectral identity indicated that the interaction, in aqueous solution, between the ligand and Mn(II) ions does not affect the electronic structure of the metal ion itself. Thus, it is possible that the peptide does coordinate to Mn(II) by displacement of aqua ligands; however, the six donor set and coordination geometry is almost maintained so that the zfs parameters remain nearly unchanged. The spectra are in agreement with an octahedral or distorted octahedral geometry, probably involving bidentate interaction of carboxyl groups carboxyl groups (Fig. 2b).

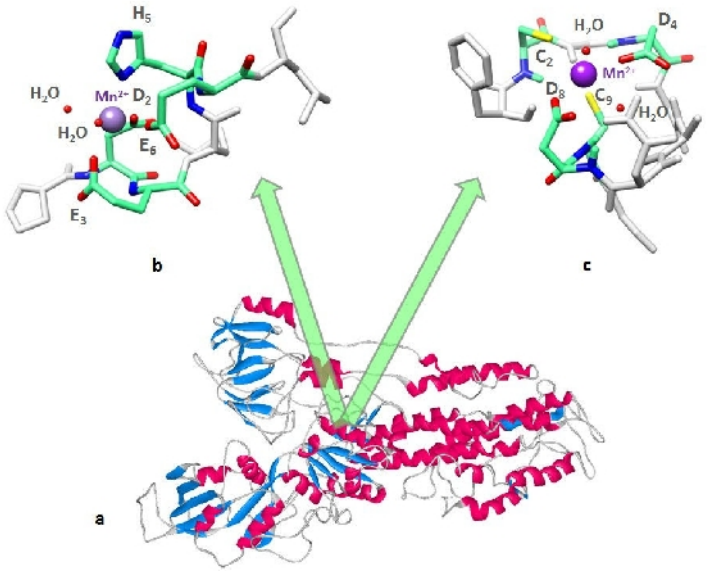


Fig. 2 a) The model of the YPK9 protein built with ESyPred3D using the 3D crystal structure of the sodium-potassium pump (PDB 3B8E chain A) as the template, and the 3D structural models proposed for b) the Mn(II) ion complexed with Ac-$P_1D_2E_3K_4H_5E_6L_7$-Am and c) with the Ac- $F_1C_2G_3D_4G_5A_6N_7D_8C_9G_{10}$-Am peptide.

Diamagnetic zinc ion was studied in order to have more information about the coordination behaviour of both investigated peptides. In fact, we were able to observe that the signals affected by diamagnetic shift under zinc interaction were the same which experienced paramagnetic broadening under manganese interaction, suggesting that the donor atoms involved in the coordination with peptide 1 are the same for both metal ions. In addition, a

change in the resonances for HN and Hγ2 of Lys_4, and for HN of Leu_7 shows that these two residues, although not directly involved in the complex formation, experience a new electronic environment after metal coordination.

Peptide 2, $F_1C_2G_3D_4G_5A_6N_7D_8C_9G_{10}$

Coordination of manganese to peptide 2 starts with the binding to a cysteine residue, as evidenced by the broadening of its signals already at very low metal concentration. So, in the absence of a histidine residue, cysteine acts as the anchoring site for the metal ion. In fact, Mn(II) progressively affected the relaxation rates in the resonances of both Cys_2 and Cys_9 with a complete disappearance of their signals in the 2D TOCSY spectra and the total loss of their proton–carbon correlations in the 2D ^{1}H-^{13}C HSQCs (Fig. 3).

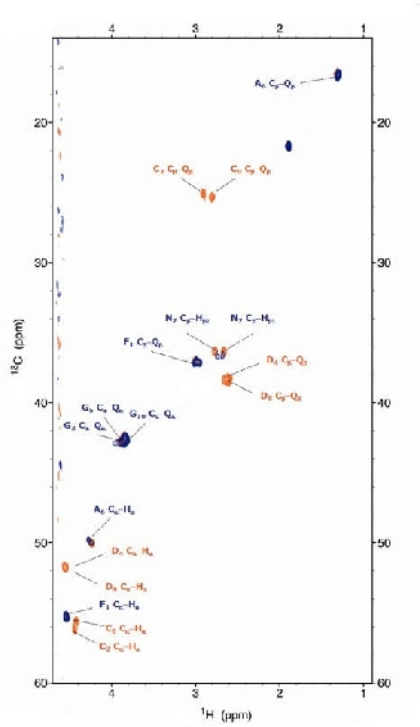


Fig. 3 Aliphatic regions of ^{1}H-^{13}C HSQC NMR spectra of the Ac-$F_1C_2G_3D_4G_5A_6N_7D_8C_9G_{10}$-Am peptide, 2.5 mM, pH 6.3, T 298 K, in the absence (orange) and in the presence (blue) of 0.1 equivalents of Mn(II).

Asp_4 and Asp_8 residues experience the main difference in the observed Mn(II)-induced effects for the carbon and proton frequencies, indicating that they take part, together with the cysteine residues, in the complex formation, most likely through their carboxyl groups (Fig. 2c). The presence of the paramagnetic metal provides a signal relaxation also for HN protons of Gly_5, Ala_6 and Asn_7 residues. This behaviour could be due to an indirect paramagnetic line broadening effect caused by a through-space relaxation of these residues along the peptide chain which happen to be close to the Mn(II) centre.

The EPR spectra of Mn(II)–peptide 2 complex and of free Mn(II) ion in aqueous (glass) solution show that they are qualitatively the same in the resolved sextet region, but the overall breadth of the pattern is a little different, indicating that the axial zfs parameter is different for the Mn–peptide species. This can be explained by a decrease in the number of water ligands around Mn(II) in the presence of the peptide fragment, suggesting their replacement

by oxygen and sulphur donor atoms. Diamagnetic NMR with Zn(II) ions evidenced that the residues involved in metal coordination are those experiencing the strongest chemical shift differences: Cys_2 and Cys_9. Other residues appear to be indirectly interested in the complex formation by slight perturbations: Phe_1, Gly_3, Asp_8 and Gly_{10}. Gathering the results from the NMR experiments we are able to infer that zinc coordination to peptide 2 involved the sulphur donors from the two cysteine side chains, forming a macrochelate complex. It is interesting to point out that the involvement of the two carboxylic groups from Asp_4 and Asp_8, in contrast to manganese coordination, has not been evidenced for Zn(II) coordination, thus indicating that, in this case, the behaviour of peptide 2 is different as a function of the different metal ion.

Conclusions

The preliminary results of the study on these two Park9 protein fragments show that both metals are able to bind them in an effective way, although with different coordination features depending on the fragment chosen, evidencing that portions of the same protein can interact in different ways with different ions. Although the fragments used are small and only represent a part of the entire protein, nevertheless this kind of study may provide insight in the divalent cations trafficking (transportation, detoxification) within the cell, at the base of the connection between genetic and environmental Parkinson's disease causes. Further investigations, involving bigger domains of PARK9/YPK9 protein, are planned to shed light on the role of the entire protein in sequestering manganese and in the involved detoxification mechanism.

References

1) Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat Genet. 2009; 41; 308-15

2) Schmidt K, Wolfe DM, Stiller B, Pearce DA. Cd^{2+}, Mn^{2+}, Ni^{2+} and Se^{2+} toxicity to Saccharomyces cerevisiae lacking YPK9p the orthologue of human ATP13A2. Biochem Biophys Res Commun. 2009 ; 383; 198-202

3) Covy JP, Giasson BI. α-Synuclein, leucine-rich repeat kinase-2, and manganese in the pathogenesis of Parkinson disease. Neurotoxicology. 2011; 32; 622-9

4) Perl DP, Olanow CW. The neuropathology of manganese-induced Parkinsonism. J Neuropathol Exp Neurol. 2007; 66; 675-82

5) Medici S, Peana M, Delogu LG, Zoroddu MA. Mn(II) and Zn(II) interactions with peptide fragments from Parkinson's disease genes. Dalton Trans. 2012 ;41; 4378-88

6) Remelli M, Peana M, Medici S, Delogu LG, Zoroddu MA. Interaction of divalent cations with peptide fragments from Parkinson's disease genes. Dalton Trans. 2012; DOI: 10.1039/C2DT32222F

Acknowledgments

This work was also supported by the Regione Autonoma Sardegna FSE Sardegna 2007–2013 L.R.7/2007 "Promozione della ricerca scientifica e dell'innovazione tecnologica in Sardegna" programme, the L.R.7/2010 programme and the Fondazione Banco di Sardegna.