Mass Spectrometry and Metallomics: binding site location in the Cyt *c*-CDDP model system

Elena Michelucci[1], Giuseppe Pieraccini[1], Gloriano Moneti[1], Chiara Gabbiani[2], Alessandro Pratesi[3], Luigi Messori[3]

[1] *Mass Spectrometry Center (CISM), University of Florence, via Ugo Schiff 6, 50019 Sesto Fiorentino, FI, Italy; elena.michelucci@unifi.it*
[2] *Department of Chemistry and Industrial Chemistry, University of Pisa, via Moruzzi 13, 56124 Pisa, Italy*
[3] *MetMed, Department of Chemistry, University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, FI, Italy.*

Filter aided sample preparation (FASP)/bottom-up high resolution mass spectrometry (HR-MS) approach was applied to the cytochrome *c*-*cis*-diamminedichloridoplatinum(II) (Cyt *c*-CDDP) model system in order to identify the Pt binding sites on this protein. The binding site location was accomplished in an automated way by using Mascot search engine: the potential coordinating amino acid residues M, C, H, K, W, T, S, E, D and Y (S-, N- and O-donors) were included in the search files as modified residues with mass gains relative to the possible CDDP fragments Pt^{2+}, $[Pt(NH_3)]^{2+}$, $Pt[(NH_3)_2]^{2+}$ and $[Pt(NH_3)_2Cl]^{+}$ and considering the charge brought by each of them. The platinated peptides found with Mascot were manually assessed to positively confirm the presence of the characteristic Pt isotopic profile in the HR-MS full scans of the precursor ions and in their MS/MS spectra.[1-3] The following ten binding sites were identified: T58, W59, K60, E61, E62, T63, M65, E66, Y67, M80. Among them, E61, E62, T63, M65 and M80 have already been reported in literature[4-7] while T58, W59, K60, E66 and Y67 have been detected for the first time. Due to the small number of characteristic b and y fragments obtained, probably related to Pt binding on protein, all these binding sites result equally probable and no one of them can be excluded at this level of our investigation. Anyway, the FASP/bottom-up approach used here has demonstrated its ability to highlight the remarkable selectivity of Cyt *c*-CDDP binding since only two specific portions of the protein (T58, W59, K60, E61, E62, T63, M65, E66, Y67 in peptide 56-73 and M80 in peptide 80-86) resulted involved. The critical issues of FASP/bottom-up HR-MS approach applied to metallomics are highlighted.

References

[1] J. Will, D. A.Wolters, W. S. Sheldrick, *ChemMedChem*, **3**, 1696-1707 (2008).
[2] I. Moraleja, E. Moreno-Gordaliza, M. L. Mena, M. M. Gómez-Gómez, *Talanta*, **120**, 433-442 (2014).
[3] I. Moraleja, E. Moreno-Gordaliza, D. Esteban-Fernández, M. L. Mena, M. W. Linscheid, M. M. Gómez-Gómez, *Anal. Bioanal. Chem*., **9**, 2393-2403 (2015).
[4] E. Moreno-Gordaliza, B. Cañas, M. A. Palacios, M. M. Gómez-Gómez, *Talanta*, **88**, 599-608 (2012).
[5] G. Ferraro, L. Messori, A. Merlino, *Chem. Commun*., **51**, 2559-2561 (2015).
[6] N. Zhang, Y. Du, M. Cui, J. Xing, Z. Liu, S. Liu, *Anal. Chem*., **84**, 6206-6212 (2012).
[7] T. Zhao, F. L. King, *J. Am. Soc. Mass Spectrom.*, **20**, 1141-1147 (2009).