BIO_05 - Development of a peptide mapping protocol with post-translational modifications detection for the recombinant human erythropoietin by LC-MS/MS-based proteomics

Jose Roberto Aparecido dos Santos Pinto[1]; Maíra Peixoto Pellegrini[1]; Ana Cláudia Bergamo[1]; Ana Carolina Magalhães Andrade[1]; Simone Ferreira Rodrigues Fernandes[1].
[1]Fiocruz/Bio-Manguinhos

Introduction: Peptide mapping is an analytical approach used by the biopharmaceutical industry to assess the identity confirmation of a therapeutic protein. In addition, this approach offers the advantage of providing site- specific information regarding post-translational and chemical modifications such as oxidation or deamidation that may arise during production, processing, or storage. Here a mass spectrometry-based proteomics protocol for peptide mapping and post-translational modifications (PTMs) detection in biopharmaceuticals was reported. For this purpose, the recombinant human erythropoietin (rhEPO) was used.

Objectives: Development of a peptide mapping strategy for monitoring the primary structure of biopharmaceuticals.

Methodology: The rhEPO samples (1 mg/mL) were provided by Center of Molecular Immunology, Havana, Cuba. For in-solution digestion, 100 µg of rhEPO were solubilized in 50 mM ammonium bicarbonate, pH 7.9, containing 7.5 M urea. Proteins were reduced with 10 mM DTT at 37oC for 60 min and alkylated with 40 mM iodoacetamide for 60 min in the dark. The samples were treated with the following two proteolytic enzymes: trypsin and Glu-C/V8 protease (1:20) at 37°C for 16 h. The digested samples were desalted and submitted to LC- MS/MS analyses (ESI Q-TOF, 6545XT, Agilent). Mass Hunter Workstation 11.0 software was used to control the data acquisition over the mass range of m/z 100-3000. MS/MS spectra were interpreted, and peak lists were generated using BioConfirm Analysis 11.0 software. Peptide identification was performed against FASTA database containing the rhEPO protein sequence (accession code P01588) with a false discovery rate (FDR) of less than 1%. Carbamidomethyl was specified as a fixed modification, while methionine oxidation and deamidation were specified as variable modifications.

Results: The sequence assignment of 100% of the rhEPO was obtained using shotgun proteomic approach with two different proteolytic enzymes – trypsin (91.6% coverage) and Glu-C (92.8% coverage). PTMs such as oxidation (M54) and deamidation (N47 and N147) were detected and confirmed by spectra interpretation. These PTMs have been described as the most common degradation pathway for pharmaceuticals; and yet to impact structure and biological activity of EPO.

Conclusion: Peptide mapping and PTMs detection are important concerns in drug development. Thus, the proteomic strategy demonstrated here offers an efficient approach for monitoring primary structure of rhEPO and other biopharmaceuticals.

Keywords: Biopharmaceuticals; Mass spectrometry; Shotgun proteomic approach