

Affinity capillary electrophoresis hyphenated to mass spectrometry: a tool to understand the role of protein sulfation in the CXCR4/SDF-1 bio-molecular interaction

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Sulfation is one of the most important 300 Post-Translational Modifications (PTMs). However, the mechanisms by which it may affect functions of biomolecules like carbohydrates, glyco-conjugates and proteins are still not well understood.

Proteins known to be sulfated are mostly cell receptors which, binding their ligands, trigger cellular signalization cascades in response to external stimuli. Among these receptors, CXCR4 attracts growing attention because of its implication in numerous physio-pathological processes (immune response, HIV infection). The extracellular domain of CXCR4 is important for the interaction with its specific ligand, the SDF-1/CXCL12 chemokine (Stromal cell-derived factor-1). Three tyrosine residues (Tyr) within the extracellular domain are known to be sulfated, but the role of this sulfation in this interaction remains to be established.

The main goal of this work is to investigate the role of the sulfation of CXCR4 extracellular domain on interaction with the SDF-1 chemokine. For that purpose we have used a synthetic peptide corresponding to the extracellular domain of the receptor (38 amino-acids) containing the three potential site of sulfation on Tyr residues (Tyr 7, 12, 21). This synthetic peptide, called P38, can be sulfated *in vitro* either enzymatically by TyrosylProteinSulfoTransferase-1 and -2 enzymes, or by total chemical synthesis.

The impact of both sulfate distribution and position of sulfate groups on the interactions between P38 and SDF-1 is studied by affinity capillary electrophoresis (ACE) hyphenated to electrospray mass spectrometry (ESI-MS). ACE enables the determination of association constants of these non-covalent complexes freely formed in solution and without partner immobilization. The coupling ACE-MS allows the separation of non-covalent complexes and their characterization by MS in native mode (stoichiometry).