The molecular structure of the interface of the DA receptor containing heteromers.

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Biological mass spectrometry has emerged as an important tool for the study of noncovalent complexes (NCX). One of the main motivations for these studies is the possibility that the structure, stability, and conformations of NCX gas-phase ions may provide information pertaining to their formation in biological systems. Our previous work has highlighted the role of certain amino acid residues, mainly two or more adjacent arginine on one peptide and two or more adjacent glutamate, or aspartate, or a phosphorylated residue on the other in the formation of NCXs between peptides. In this work, we employ mass spectrometry to investigate the formation and stability of NCXs formed between peptides and proteins, through an electrostatic interaction between arginine residues and phosphorylated residues.

Methods: In this work, two mass spectrometers were used to study NCXs. A MALDI-TOF/TOF 4700 Proteomics Analyzer was used in this study for both MS and MS/MS analysis. Data was also acquired with a periodic focusing MALDI- Ion mobility (IM)-TOFMS instrument in positive ion mode. A mobility resolution of 30 (FWHM) and a mass resolution of 3000 for *m/z* 1000 are routinely achieved. MALDI-IM-MS allows for the instantaneous 2D analysis of biological families such as lipids, peptides, and nucleotides, which are separated from each other by differences in their IM drift times and separates multiple charge states, which is particularly useful in the elimination of chemical noise and the simplification of the distribution of molecular ions (MH^+) of peptides and proteins

Results: Electrostatic interactions between a highly conserved arginine rich epitope (basic motif), found in the third intracellular loop of Dopamine D_2, D_3 and D_4 receptors, and a phosphorylated serine/threonine (acidic motif) on the Adenosine A_{2A} receptor are involved in receptor heteromerization. We have demonstrated that this arginine-phosphate electrostatic interaction possesses "covalentlike" stability. Hence, these bonds can withstand fragmentation by mass spectrometric collision-induced dissociation at energies similar to those that fragment covalent bonds and they show very slow dissociation of the noncovalent complex by plasmon resonance. However the complex is disrupted by addition of calmodulin which displaces the Adenosine A_{2A} epitope and forms a noncovalent complex with the basic motif.
Our work also highlights the importance of phosphorylation-dephosphorylation events in the modulation of electrostatic attraction. Phosphorylation of the acidic motif by casein kinase on one receptor makes it available for interaction with the basic motif on the other. On the other hand, phosphorylation of serine and/or threonine residues downstream from the basic motif, by protein kinase A or C slows down the attraction between the epitopes. Although analyzed here in the frame of receptor heteromerization, the arginine-phosphate electrostatic interaction most likely represents a general mechanism in protein-protein interactions.

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