HIGH-THROUGHPUT IDENTIFICATION AND CO-LOCALIZATION OF ANTIMICROBIAL PEPTIDES IN TISSUE SAMPLES BY IMAGING MASS SPECTROMETRY

Luciano Paulino Silva

Laboratório de Espectrometria de Massa, EMBRAPA - Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil

Traditionally the spatial localization of peptides and proteins expressed in tissues has been investigated for a limited number of compounds by using molecular probe-based strategies like *in situ* hybridization and immunohistochemistry. However, such techniques are not suitable to establish the accurate spatial distribution of compounds in several situations mainly due to the large numbers involved, their small sizes (e.g. unordered peptides), diffusion, and/or loss of molecules during tissue processing. Imaging mass spectrometry (IMS) has enabled the determination of the co-localization of hundreds of peptides and proteins in tissue samples by means of a single experiment. In the present study, some of these limitations have been addressed by developing a label-free method to investigate in large scale the co-localization phenomena. As a demonstration of its utility, this method was used to array the co-localization profile of bioactive peptides and proteins on skin fragments of frogs as prime model. Frog skin was chosen due to its unprecedented variety of components, mainly antimicrobial peptides, with molecular masses ranging from 1 to 9 kDa. The detection of proteomic profiles associated with specific tissue compartments as well as the detection of biomarkers of altered states (e.g. cold exposure) and post-translational modifications demonstrate the usefulness of this approach for rationalization of IMS co-localization data.

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