APPLICATION OF 'INTENSITY ION FADING' APPROACH FOR SEARCHING INHIBITORS OF TYROSINASE IN COMPLEX MIXTURES

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Inhibitors of tyrosinase are attractive from the point of view of the cosmetic industry for the reason of their prospective application in the treatment of the symptoms of skin hyperpigmentation. Plants and other natural products constitute an important source of biologically active substances. During our work we evaluate an application of 'intensity ion fading' mass spectrometry (IF-MS) approach to find the specific interactions between tyrosinase and the component(s) of the plants with minimal pre-treatment of the extract. IF-MS, described for the first time in 2003 by the group of Professor Francesc Aviles[1], was shown to be a useful, relatively easy and efficient method to study protein-ligand interactions in the complex mixtures. IF-MS methodology is based on the observation of the formation of specific protein-ligand complexes leading to the decrease ('fading') of the ion response of the MS-accessible ligand in the analyzed sample (comparing to the reference without the target)[2]. Our IF-MS approach is based on the application of immobilized protein as the target in studied sample. This approach seems to be more versatile comparing to the application of 'free' enzyme, since protein-ligand complex covalently connected with solid support may be easily separated from the solution and subjected to 'ligand recovery' step to confirm the initial 'ion fading' results. Both original protocol and further works in this domain utilize MALDI-MS as the leading tool[3,4] and as such is not suitable for studying small molecules. In our laboratory we synthesized core-shell type silica-coated iron oxide magnetic nanoparticles (MPs) having high protein loading capacity. MPs turned out to be very efficient support for matrix-free surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) both in a raw state and also with attached proteins. It opened the possibility of studying interactions of low-mass molecules with protein target by conducting MPs-assisted comparative analysis both in solution (by ESI-MS) and directly on MALDI plate, avoiding in the latter case the background of the matrix. During the presentation we will discuss the application and critical steps of IF-MS methodology for recognizing inhibitors of tyrosinase, on the basis of our work on standards (kojic acid, glabridin) and real samples: extract of liquorice (*Glycyrrhiza glabra*).

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