

MRJP1-Binding Proteins from Honeybee Brain

Cruz, G.C.N.¹, Garcia, L.^{1,2}, Ricart, C.A.O.¹, Sousa, M.V.¹

¹Department of Cell Biology, University of Brasilia, Distrito Federal, Brazil; ²Mass Spectrometry Group, Physics Department, CEADEN, Havana, Cuba.

Major royal jelly protein 1 (MRJP1) is the main protein component of honeybee (*Apis mellifera*) royal jelly, and is also present in its brain. MRJP1 expression changes during ontogenetic and behavioral development of honeybee worker subcastes. MRJP1 was 9-fold more abundant in nurse than in forager brain. MRJP1 was localized in the cytoplasm of brain cells, along filaments of the cytoskeleton, in the antennal lobe, optical lobe and mushroom body. MRJP1 was deposited on the rhabdom, a structure composed of numerous tubules. MRJP1 could be associated to proteins of filamentous structures. MRJP1 was also detected in intercellular spaces between cells in mushrooms bodies, indicating that it is a secreted protein. It was also found as a calcium/calmodulin-binding protein. However, its roles in the nervous system are still unknown. In this work, we looked for the possible MRJP1-binding proteins (M1BPs) from the brain extract of nurse honeybees. To this end, activated thiol-sepharose, with the ability to reversibly couple proteins, was used for preparing an affinity chromatography column. Purified MRJP1 was first immobilized to the resin to function as a bait. The nurse brain extract was incubated to this affinity column in order to bind the M1BPs. After washing, the whole MRJP1-M1BPs complex was eluted, and analyzed by 1D and 2D electrophoresis. Several polypeptides were retained by the column, and could be seen by 1D gel. These proteins were considered M1BPs since none of them was found to bind directly to the resin on the control experiment. The eluted complex was also separated by 2DE, and presented over 200 M1BPs spots, which are now under mass spectrometry analyses for identity determination.

Keywords: Affinity chromatography, *Apis mellifera*, honeybee, MRJP1, MRJP1-binding proteins.

Supported by: CAPES, CNPq and TWAS.