Development Of An Proteomic Platform for GPCRs Analysis By Using Animal Peptide Toxins As Ligands

Santos, L.D., Sales, F.P., Pinto, J.R.A.S., Palma, M.S.

Lab. Structural Biology and Zoochemistry, Center of Studies of Social Insects, UNESP, Rio Claro, SP, Brazil.

G-protein coupled receptors (GPCRs) form a large family of proteins that plays important roles in many physiological and pathophysiological processes. Some mastoparan peptides are responsible for releasing multiple pro-inflammatory mediators through the activation of GPCRs in different cell types. Therefore, the subject of this study is the development of an affinity chromatography platform by using the mastoparan peptide Protopolybia-MP III, previously reported as specific ligand of GPCRs from rat mast cells. The peptide ProtopolybiaMP III was synthesized by using F-moc strategy and then, coupled to the chromatographic resin Sepharose 4B, in order to set up an affinity chromatography protocol; the column (12 x 2 cm) was built in TRIS-HCl buffer pH 8.0. Rat peritoneal mast cells were collected and lysed with NaCl 1M; the membrane extract was reconstituted into proteolipossomes of 300 nm of diameter. The elution of proteolipossomes suspension was carried-out at a flow rate of 0.5 mL/min and monitored at 280 nm; fractions of 1 mL were collected. The liganded proteins (under proteolipossome form) were removed under salt gradient from 0 to 1 M NaCl in the same equilibration buffer. The proteins eluted in each proteolipossome fraction were extracted with a solution of 0.02% (w/v) SDS and submitted to 1-D SDS-PAGE, stained with 0.025% (w/v) Coomassie Brilliant Blue and submitted to proteomic analysis. As result, the protein profile showed five protein bands with molecular weights of 18 to 66 kDa, which were excised from gel, processed and sequenced by ESI-IT-MS/MS. This strategy permitted the identification of some GPCRs from mast cells. Financial Support: FAPESP/BIOprospecTA (2006/57122-7; 2007/01330-3) and CNPq (INCT-iii).