HUMAN PHOSPHODIESTERASE 5A CATALYTIC DOMAIN: EXPRESSION AND PURIFICATION

Selbach, B.P., Fonseca, I.O., Basso, L.A. and Santos, D.S.

Centro de Pesquisas em Biologia Molecular e Funcional, IPB, PUCRS, Porto Alegre - RS - Brazil

Phosphodiesterases (PDEs) are a super family of enzymes which degrade the intracellular second messengers cGMP and cAMP. As essential regulators in cyclic nucleotide signaling with diverse physiological functions, PDEs are drug targets for the treatment of various diseases. Of the 11 PDE existing gene families, the cGMP-specific PDE5 is the principal cGMP-hydrolyzing activity in the human corpus cavernosum tissue. It is well known as the target of many drugs used in the treatment of erectile dysfunction, like sildenafil. This work presents the expression of the catalytic domain of the PDE5 gene in Escherichia coli Rosetta (DE3) cells using pET system and purification by FPLC. Future perspectives rely on enzyme immobilization using the Surface Plasmon Resonance technology. This technology exploits the evanescent-wave phenomenon to characterize molecule-analyte interactions. Binding of analytes in solution to surface-immobilized receptors alters the refractive index of the medium near the surface. This change can be easily monitored in a real time way and allows the accurate measurement of the amount of bound analyte, its affinity for the receptor and also the association and dissociation kinetics of the interaction for the search of natural products from the Brazilian biodiversity as possible inhibitors that may be used for the treatment of some human ailments which include erectile dysfunction.