Possible naturals substrates for human kallikrein 13 obtained from solid phase combinatorial peptide library

Andrade, D.[1]; Oliveira, J.R.[1]; Alves, F.M.[1]; Hirata, I.Y.[1]; Blaber, M.[2]; Blaber, S.[2]; Juliano, L.[1]; Juliano, M.A.[1]
[1]Departamento de Biofísica, Instituto Nacional de Farmacologia, Universidade Federal de São Paulo, São Paulo Brazil; [2]Departament of Biomedical Sciences, Florida State University, Tallahassee, Florida EUA.

Combinatorial chemistry has revolutionized both drug discovery and fundamental approaches to the elucidation of various processes in the biological as well as the physical science. The development of the portion-mixing synthesis methodology has made possible the rapid generation of millions of compounds for high throughput screening of peptide libraries. The recent development of solid supports such as TentaGel and PEGA allowed the screening of libraries to be performed directly on resin-bound compounds. One our research goals is the application of solid phase combinatorial methodology to characterize proteolytic enzymes. In the present work, were synthesized two libraries, the library (I) K(Dnp)-X6X5X4X3X2X1K(Abz)-PEGA1900 and library (II) K(Dnp)-k-X7-X6-X5-X4-X3-X2-X1K(Abz)-k-PEGA1900. These two libraries were incubated with the human kallikrein 13, a secreted serine protease, trypsin-like expressed in various tissues including central nervous system (NS). In this screening the sequences isolated from libraries were analyzed by BLAST and several possible naturals substrates were found. One of them was the Myelin Basic Protein (MBP), the second most abundant protein in NS, responsible for adhesion of the cytosolic surfaces of multilayered compact myelin. The digestion of the MBP by hK13 was performed and it was extensively and quickly degraded (two times faster than hK6). This result suggests that the deregulated hK13 expression and/or activity in NS may play a pivotal role in demyelinating diseases like multiple sclerosis.

Supported by CNPq and FAPESP