

عنوان مقاله:

Cloning, Expression and Purification of SNAP-25 Proterin

محل انتشار:

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خلاصه مقاله:

Background & Objectives: Clostridial neurotoxin inhibits neurotransmitter release by selective and specific intracellular proteolysis of synaptosomal associated protein of 25KDa (SNAP-25), synaptobrevin/VAMP-2 and syntaxin. SNAP-25 is one of the components that forms docking complex in synaptic ends. This protein is substrate for botulinum neurotoxins types A,C, and E. Each of these toxin serotypes specifically cleaves SNAP-25 in a particular position and thereby block docking and synaptic vesicle membrane fusion and finally prevents neurotransmitter exocytosis and transition of neurotic signals. Recombinant production of SNAP-25 in the laboratory can be used as a substrate for the detection of clostridium botulinum types A, and E neurotoxins. **Materials & Methods:** In order to use the protein as a substrate for detection of different types of clostridium neurotoxins in-vitro the protein was produced by recombinant technique. The cDNA from SNAP-25 was synthesized from total RNA purified from frozen Rattus norvegicus brain. and amplified by RT-PCR The amplified fragment was cloned into pET32a expression vector. The identity of recombinant protein was confirmed by Western blot using specific antibody and finally the recombinant protein was purified through an affinity column chromatography (Ni-NTA). **Results:** The optimum conditions of expression of SNAP-25 were found to be IPTG(1mM) and incubation at 37°C for 5 hours. The recombinant protein was isolated and purified using Ni-NTA column with imidazole at a concentration of 250mM. Using enterokinase to cut the fision at 37°C comparatively yielded better results than room temperature. **Conclusion:** The protein retained its structure during the purification process being suitable for cutting and further tests. The purified protein we obtained can be used as substrate for .detection of clostridium botulinum types A, and E toxins

کلمات کلیدی:

Botulinum neurotoxin types A, and E, Recombinant protein, SNAP-25

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