

Design specific ELISA kit based on DNA aptamer for detection of epsilon toxin

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Abstract

Epsilon toxin produced by *Clostridium perfringens* is a robust bio-threat agent. This toxin is known as the third dangerous toxin after tetanus toxin and botulinum toxin and is used in biologic warfare as potent biological agents. In addition, this toxin leads to a fatal enterotoxemia in domestic animals. Also, epsilon toxin causes pulmonary edema, renal failure, and cardiovascular collapse. The lethal dose for human is estimated at 7 µg parenterally. Designing the rapid, facile and available methods for detection of Epsilon toxin can be useful to prevent from its harms. Currently, there is only one commercial ELISA kit based on monoclonal antibody to detect this toxin. Monoclonal antibody production technology is a complex and costly process. Aptamers can be an appropriate alternative to monoclonal antibodies. In addition to high specificity and high sensitivity in diagnosis, aptamer commercialization process is cheaper and easier. In this study, to isolate single strand aptamers, DNA library comprising 82 nucleotides were designed using Oligo software. To purify the specific ssDNA oligomers, positive affinity chromatography columns containing Cyanogen bromide sepharose 4b gel were prepared and the epsilon toxin bound to the column. Then the appropriate aptamers will be selected by SELEX procedure. Finally, aptamers were purified by ethanol precipitation method. Then, the indirect ELISA (Enzyme-linked aptamer sorbent assay) were performed to analyze four rounds for assessing ssDNA pool-binding ability. The selected aptamers were cloned into *E. coli* DH5-α and each clone was analyzed by specificity test. Among 11 clones, three of them were selected with the higher specificity and affinity. In conclusion, for the first time, in current study, the specific ssDNA aptamer with appropriate affinity was isolated against epsilon toxin and is useful to design ELISA kit for this toxin.

Keywords: Epsilon Toxin; Biological agent; ssDNA aptamer; SELEX