Graduate Category

Poster Title:

Identifying and Eliminating the Deoxyribonuclease Activity of the Diphtheria Toxin Mutant CRM197

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Abstract

The carrier protein Cross Reactive Material 197 (CRM197) is a non-toxic mutant of diphtheria toxin (DT) that is currently being used in FDA-approved conjugate vaccines, e.g. Prevnar 13 against Pneumococcal pneumonia. CRM197 contains a single point mutation (Gly52Glu) that eliminates the ADPRT activity and results in CRM197 being ~1 million-fold less toxic than DT. CRM197 still retains the metal dependent deoxyribonuclease (DNase) activity of DT since the DNase active site is remote to the ADPRT active site. This DNase activity has been suggested to be cytotoxic in cell culture so eliminating this activity could lead to safer vaccines. The X-ray crystal structures for both DT and CRM197 have been solved and a potential metal binding site was identified using METSITE containing: Ser109, Thr111, and Glu112. These mutations (S109A, T111A, and E112Q) as well as combinations of these mutations reduced of DNase activity. The location of the potential DNA binding pocket was identified in a cleft adjacent to the predicted metal binding site: Lys103, Glu116, Thr120, Glu122, Phe123, Lys125, and Arg126. T120A, E122A, F123A, K125A, and R126A mutations as well as the E116A/E122A, E116A/R126A, E122A/R126A, and E116A/E122A/R126A combination mutants appeared to reduce DNase activity more significantly than the metal binding mutations.