Identification of Motifs Necessary for Interaction between AB Toxins and PDI

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?This experiment's purpose was to elucidate mechanisms of the interaction between AB toxins (responsible for cholera, pertussis, ricin, etc.) and host protein, protein disulfide isomerase (PDI). AB toxins are transported to the endoplasmic reticulum (ER) by retrograde transport. Inside the ER, PDI unfolds the A1-subunit of the toxin. The A1-subunit, now mistakenly identified as a misfolded protein, hijacks the ER-associated degradation pathway (ERAD), exits the ER, and enters the cytosol. The A1-subunit escapes degradation because of its absence of lysine. Using a bacterial two-hybrid system in which the T18 and T25 fragments of the catalytic domain of Bordatella pertussis adenylate cyclase were fused to cholera toxin A-1 subunit (CTA1) variants and PDI polypeptides, direct interaction between CTA1 variants and PDI was detected. CTA1 variants containing single amino acid substitutions were isolated. Particular variants unable to interact with PDI provided insight into the process of PDI binding with CTA1. Variants then underwent the Miller Assay to quantify the two-hybrid results and determine the strength of the interaction. The Miller Assay results illustrated that some mutations greatly decreased the strength of interaction between CTA1 and PDI, while some did not affect the interaction. Based on the above considerations, a model of CTA1 was constructed depicting the binding areas and non-binding areas of PDI. Overall, it appears specific amino acids in the sequence of the A-subunit of AB toxins are absolutely required for binding with PDI. Understanding this interaction will allow for synthesis of novel therapeutic agents to inhibit the toxicity of AB toxins.

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