AsCy3 AS A PROBE OF PROTEIN CONFORMATION IN THE PROTEIN ANTHRAX LETHAL FACTOR

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Abstract: Anthrax toxin is an AB toxin constituted by three distinct proteins: the B component protective antigen (PA), the A component edema factor (EF), and lethal factor (LF). PA forms a membrane-spanning pore that allows either LF or EF to translocate into the cell. The pore includes a narrow iris called the phi-clamp that is only 6 Angstroms wide. Since the pore is very narrow, LF and EF need to completely unfold their protein structure to pass through the pore and enter the cell. Once EF and LF enter the cell, the proteins are able to refold back into their original structure. The process of unfolding, translocation, and refolding in the cell is not understood. To understand these processes, we have mutated residues 53, 54, 60, and 61 to cysteine (LFNC4), which will allow the binding of the fluorescent dye AsCy3. Although these residues are found outside the binding site for PA interaction, it is expected not to have any influence on stability or binding. AsCy3 is known to have a fluorescence emission maximum at 568 nm; however, when AsCy3 binds to 2 cysteine (Cys) pairs across two helical turns of an alpha-helix, the intensity of the fluorescence increases by a factor of six. The emission peak also is red shifted to 576 nm. These characteristics of AsCy3 provide the ability to investigate how LFn is able to refold in the cell because of the high fluorescence intensity from AsCy3. Our initial experiments show that AsCy3 can bind to LFNC4, which significantly changes the AsCy3 fluorescence. This probe can now be used in unfolding and refolding experiments and, because of its reversibility, in translocation experiments across lipid membranes.

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