Dependence of Proline Isomerization on the Kinetics of Folding of Anthrax Lethal Factor

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The death that can come within a few days after exposure to the anthrax toxin from *Bacillus anthracis* is due to the presence and effects of anthrax lethal factor (LF). LF is a zinc metalloproteinase whose function depends upon the translocation of LF from the endosome of a host cell into the cytosol, where it cleaves mitogen-activated protein kinase kinases and disrupting cell signaling pathways. The translocation requires LF to unfold as it transits through the narrow channel of the pore, formed by the anthrax protective antigen (PA). Unfolding of LF has been shown to be pH-dependent, but little is known regarding the kinetics of unfolding and refolding of LF. Specifically, refolding of LF must occur fairly rapidly within the cell cytosol to prevent degradation of the protein by cellular proteases. The N-terminal PA binding domain of LF, LFN, has a single cis-proline residue (Pro16, and we hypothesized that this cis proline must isomerize to trans during the unfolding and translocation process and that refolding would be slow, and perhaps dependent on cellular prolyl isomerases for refolding. To this end, we have performed a detailed kinetic refolding/unfolding study of LFN from urea solutions. Our preliminary experiments indicate that LFN refolding occurs rapidly (within 1 second), suggesting that Pro166 does not isomerize to an appreciable extent in the unfolded state, or if it does isomerize, that isomerization back to cis is a fast process. The implications of these experiments on the mechanism of anthrax toxin lethality will be discussed.