Advances in Biological Chemistry, 2012, 2, 123-131 ABC
http://dx.doi.org/10.4236/abc.2012.22015 Published Online May 2012 (http://www.SciRP.org/journal/abc/)

Two disulfide mutants in domain I of *Bacillus thuringiensis* Cry3Aa δ-endotoxin increase stability with no effect on toxicity*

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Received 28 January 2012; revised 1 March 2012; accepted 10 March 2012

ABSTRACT

To increase protein stability and test protein function, three double-cysteine mutations were individually introduced by protein engineering into the cysteine-free Cry3Aa δ-endotoxin from *Bacillus thuringiensis*. These mutations were designed to create disulfide bonds between α-helices 2 and 5 (positions 110 - 193), and α-helices 5 and 7 (positions 195 - 276 and 198 - 276). Comparison of the CD spectra of the wild-type and the double-cysteine mutant proteins indicates a tighter helical packing consistent with formation of at least two of the disulfide bonds between the central and the outer helices. Thermal stability analysis indicates that potential covalent linkages between the central α-helix 5 and the other helices increase resistance to thermal denaturation by 10˚C to 14˚C compared to the thermal stability of the wild-type protein. Spectroscopic analysis of the disulfide-specific absorbance band indicates that the double mutant proteins are more stable to temperature and denaturant (guanidine hydrochloride) than the wild-type protein, as a result of the formation of two of the disulfide bridges. These results indicate that the double mutations $M_{110}C/F_{193}C$ and $A_{198}C/V_{276}C$ successfully established disulfide bonds, resulting in a more stable structure of the entire toxin. Despite the increase in stability and structural changes introduced by the disulfide bonds, no effect on toxicity was observed. A possible mechanism involving the insertion of all of domain I of Cry3Aa toxin into the target membrane accounts for these observations.

*Engineered disulfide bridges in Cry3Aa.
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Keywords: Disulfide Bonds; CD Spectra; Cry3Aa; Site Directed Mutagenesis

1. INTRODUCTION

Protein engineering is a powerful tool for modifying the properties of polypeptide molecules. One particular application of protein engineering is sequence alteration to enhance protein stability in order to broaden their utility in commercial and medical applications. It is known that the tertiary structure of native proteins is defined by a number of weak interactions including: hydrophobic interactions [1], salt bridges [2], weakly polar interactions [3], and hydrogen bonding [4]. Additionally, disulfide bonds can make a substantial contribution to the overall protein resistance to adverse environmental factors [5], and can play an important role in specific aspects of structural stability [6].

There have been reports in which protein stability of Cry1Aa protein has been improved by introducing artificial disulfide bridges [7,8]. Introduction of new disulfide bridges in proteins does not always result in increased stability to thermal or chemical inactivation, however. It has been suggested that one reason for this is that disulfide bridges in native proteins have special geometries which may be difficult to achieve in engineered proteins [9].

Several mechanisms have been proposed for the insertion of δ-endotoxins into the insect plasma membrane. The "penknife" [10] and "umbrella" models [11] are supported by changes in the distribution of the hydrophobic faces of the helices in domain I. Both models involve separation of the α-helices from the α-helical bundle, followed by their insertion into the target membranes resulting in the formation of ion pores. Other