Protein-Protein Interactions Involved in the Assembly of Bacterial Nanoinjectors Defined by EPR Spectroscopy

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Introduction

The goal of this project is to use EPR to determine the protein-protein interactions of two proteins from *Yersinia pestis*: LcrV and LcrG. These proteins are essential in the pathogenesis of *Yersinia pestis*, the causative agent of bubonic plague. Contrary to what was expected for LcrG – that it forms a coiled coil, our NMR results indicated that LcrG lacks a tertiary structure and consists only of secondary alpha helical structures [1]. However, the current hypothesis in the literature is that LcrG forms a coiled coil upon binding to LcrV (**Fig.1**) [2]. Our NMR analysis could not identify if the two helices are in close contact with each other [1], hence, we used EPR. In our last year's report, the EPR results demonstrated the presence of a population of LcrG in a 'closed' conformation. Our recent EPR results suggested that when complexed with its binding partner (LcrV), LcrG assumes an open conformation. This model of how LcrG binds to LcrV is somewhat unexpected as the dogma in the field is that LcrG will assume a 'closed' conformation (see **Fig.1**) when bound to LcrV. EPR is thus changing our perspective on how these virulence proteins work. We are currently drafting a manuscript to report our results.

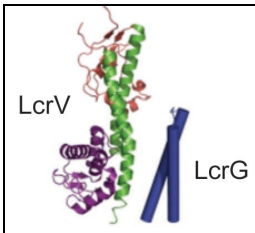

Fig.1 LcrG is expected to bind to LcrV in a closed conformation (image from ref. 1).

Experimental

Site-directed cysteine mutants of LcrG recombinant proteins were expressed and purified following published methods [1]. MTSL spin labels (R1) were attached to LcrG and LcrV proteins following published protocols [3]. EPR experiments have been carried out at the NHMFL using a Bruker E680 spectrometer and a HiPER spectrometer.

Results and Discussion

Here, we used EPR to determine the model of the protein-protein complex formed by LcrG and LcrV. Both proteins were spin labeled with MTSL (R1, see **Fig.2**). EPR results indicate that LcrG assumes an 'open' conformation, when bound to LcrV.

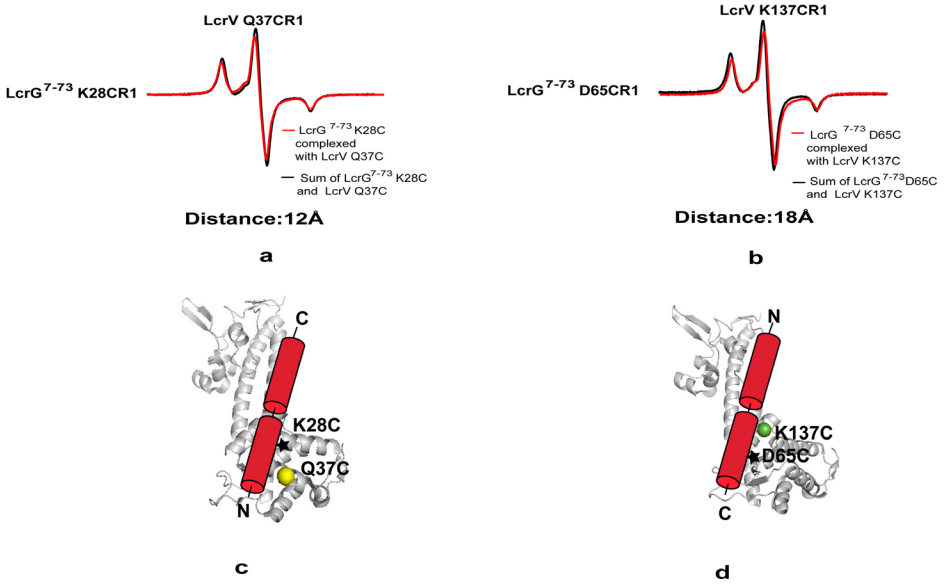

Fig.2 EPR data on **(a)** LcrV Q37C - LcrG K28C and **(b)** LcrV K137C - LcrG D65C indicate that LcrG binds in an 'open' conformation with LcrV. Two models **(c, d)** of the complex are proposed.

Conclusions

The EPR model of LcrG-LcrV interaction will change the current dogma in the literature (**Fig.1**) about the LcrG-LcrV complex.

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References

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