Refining the Ensemble Conformation of Opa Extracellular Loops

Kieber, M., Columbus, L. (U. of Virginia, Chemistry); Hays, J. (U. of Virginia, Biomedical Engineering); Kasson, P.M. (U. of Virginia, Biological Physics and Molecular Biophysics and Biomedical Engineering) and Song, L. (NHMFL)

Introduction

Opa proteins from *Neisseria gonorrhoeae* and *N. meningitidis* pose an intriguing biological riddle: How can a structural scaffold that is tolerant to sequence diversity bind to the same family of receptors, and yet also provide receptor selectivity among the receptor variants? Opa is a class of outer membrane proteins that bind to various host receptors to induce engulfment of the bacterium. The Opa receptor specificity is determined by two segments (hypervariable, HV, 1 and 2) in extracellular loops 2 and 3 (**Fig.1**) [1]. These hypervariable regions have high sequence diversity. A structural motif for receptor engagement and selectivity has not been identified. The overarching goal is to determine how Opa proteins interact with their cognate human receptors, what is the mechanism of molecular recognition, and what are the specific molecular determinants of Opa-receptor interactions? Then, knowledge of the structure, dynamics, and specific interactions of Opa proteins and receptors will be used to design targeted liposome delivery to human cells. The Opa_{60} structure and dynamics data indicate the extracellular loops are disordered, yet sample a restricted volume such that frequent short-lived transient interactions occur between the loops on the nanosecond timescale [2]. The aim of this proposal is to combine EPR-derived distance distributions with molecular dynamics to refine the ensemble of the extracellular loops in order to propose a model for receptor engagement.

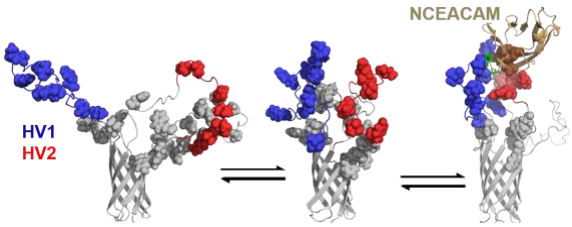

Fig.1 Opa_{60} structure (light gray) and hypothesis that the loop ensemble samples conformers that interact with CEACAM.

Experimental

Double-labeled Opa_{60} proteins in detergent micelles were measured using pulsed EPR with a W-band HiPER Spectrometer at the NHMFL. All samples were prepared to a final protein concentration between approximately 100 and 200 µM with 10% deuterated glycerol. The samples were loaded into plastic capillaries with a 3 mm od x 2 mm id (Adtech, Glos, UK) and were flash frozen in liquid nitrogen. Dipolar evolution data were processed using DEERAnalysis2016 software using Tikhonov regularization to generate distance distributions.

Results and Discussion

DEER data were collected for thirteen double mutants to assess signal and quality. Two of these samples were identified as highly informative pairs in the molecular dynamics simulations and further optimized to increase the dipolar evolution time in order to have reliable distance distributions. Although two well-chosen DEER pairs are not yet sufficient to refine the conformational ensemble of Opa_{60}, the restrained-ensemble simulations developed yield preliminary hypotheses regarding how Opa_{60} may bind its partner CEACAM1. Analysis of the loop-loop contacts in restrained-ensemble simulations yields four well-separated clusters.

Conclusions

Although preliminary, the methodology developed has powerful implications in refining dynamic proteins and has refined the Opa_{60} loop ensemble to generate two alternate structural hypotheses for receptor engagement.

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