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Oligomeric structure of Opa proteins


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Background

During infection, Gc Opacity-associated (Opa) proteins trigger engulfment of the bacteria into human cells after binding human carcinoembryonic antigen-related cellular adhesion molecule (CEACAM) proteins. The NMR structures of an Opa from Neisseria gonorrhoeae purified and refolded from inclusion bodies published by our lab reveal an 8-stranded β-barrel with four extracellular dynamic loops, three of which contain regions of sequence that vary between Opa paralogs. Different Opa proteins selectively bind human CEACAM proteins – or subsets of CEACAM proteins – in vivo. Attempts to capture CEACAM specificity between different Opa-CEACAM complexes have conflicted with the established knowledge of the field, suggesting that the refolded monomeric Opa protein is missing some biological determinant of binding specificity.

Aim/Methods

To further understand Opa in a more native bacterial membrane context, we have optimized expression and purification from the E. coli outer membrane. The isolated Opa proteins were investigated with cro-EM, X-ray crystallography, and other biophysical and analytical approaches.

Results

Preliminary results reveal a higher order oligomer of Opa and putative intercellular Opa-Opa contacts that that are impacted by the presence of divalent cations. In addition to structural studies, binding affinities to CEACAM were determined using microscale thermophoresis.

Conclusions

These results establish a new molecular understanding of Opa and Opa-CEACAM binding interactions in the context of cell-cell interactions encountered during infection.