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Title: Conformational analysis of opacity proteins from *Neisseria meningitidis*.

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Abstract: Opacity-associated (Opa) proteins are outer membrane proteins which play a critical role in the adhesion of pathogenic *Neisseria* spp. to epithelial and endothelial cells and polymorphonuclear neutrophils. The adherence is mainly mediated by the CD66-epitope-containing members of the carcinoembryonic-antigen family of human cell-adhesion molecules (CEACAM). For the analysis of the specific interactions of individual Opa proteins with their receptors, pure protein is needed in its native conformation. In this study, we describe the isolation and structural analysis of opacity proteins OpaJ129 and OpaB128 derived from *Neisseria meningitidis* strain H44/76. When the Opa proteins were produced with the *phoE* signal sequence in *Escherichia coli*, they were localized at the cell surface and the recombinant bacteria were found to specifically interact with CEACAM1. For refolding and purification, the proteins were overproduced without their signal sequences in *E. coli*, resulting in its cytoplasmic accumulation in the form of inclusion bodies. After solubilization of the inclusion bodies in urea, the proteins could be folded efficiently *in vitro*, under alkaline conditions by dilution in ethanolamine and the detergent n-dodecyl-N,N-dimethyl-1-ammonio-3-propanesulfonate (SB12). The structure of the refolded and purified proteins, determined by circular dichroism, indicated a high content of β -sheet conformation, which is consistent with previously proposed topology models for Opa proteins. A clear difference was found between the binding of refolded vs. denatured OpaJ protein to the N-A1 domain of CEACAM1. Almost no binding was found with the denatured Opa protein, showing that the Opa-receptor interaction is conformation-dependent.[ABSTRACT FROM AUTHOR]

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