(1) Submission ID#1527817

Structure of anti-gonococcal lipooligosaccaride antibody bound to mimetic peptide vaccine informs therapeutic and vaccine strategies

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Background

Neisseria gonorrhoeae (Ng) is the etiologic agent of gonorrhea, which causes local genital infection and disseminated disease. Mouse monoclonal antibody (mAb) 2C7 recognizes a widely expressed glycan epitope of Ng lipooligosaccharide (LOS), called the 2C7 LOS epitope, and elicits complement-dependent bactericidal activity. Previously, we identified a cyclic peptide called CP2 that mimicked the 2C7 LOS epitope. Immunization of mice with CP2 configured as a tetrameric multi-antigen peptide, TMCP2, attenuated Ng vaginal colonization.

Aim/Methods

We used biophysical approaches to characterize the interactions between the therapeutic mAb candidate 2C7 and the multi-antigenic mimetic peptide vaccine candidate TMCP2. To this end, we employed small-angle X-ray scattering, native mass spectrometry, NMR spectroscopy, X-ray crystallography and molecular dynamics (MD) simulations to characterize complexes of mAb and Fab 2C7 with tetrameric and monomeric peptides TMCP2 and CP2.

Results

Small-angle X-ray scattering experiments with Fab 2C7 identified changes in the radius of gyration and shape upon forming complexes with CP2 and TMCP2. Native mass spectrometry with mAb and Fab 2C7 displayed different oligomeric structures bound to tetrameric TMCP2. Saturation transfer difference and transferred nuclear Overhauser effect NMR spectroscopy showed that the chemical environments of primarily hydrophobic CP2 residues changed with mAb 2C7 binding. The high-resolution crystal structure of Fab 2C7 in a complex with CP2 revealed that the peptide formed a beta-hairpin and confirmed that the Fab bound via hydrophobic interactions. A second crystal structure of Fab 2C7 alone showed that there were no substantial conformational changes in the Fab upon peptide binding. Finally, NMR studies, crystallographic thermal factors, and molecular dynamics simulations revealed the dynamic conformational behavior of CP2 when bound to mAb/Fab 2C7.

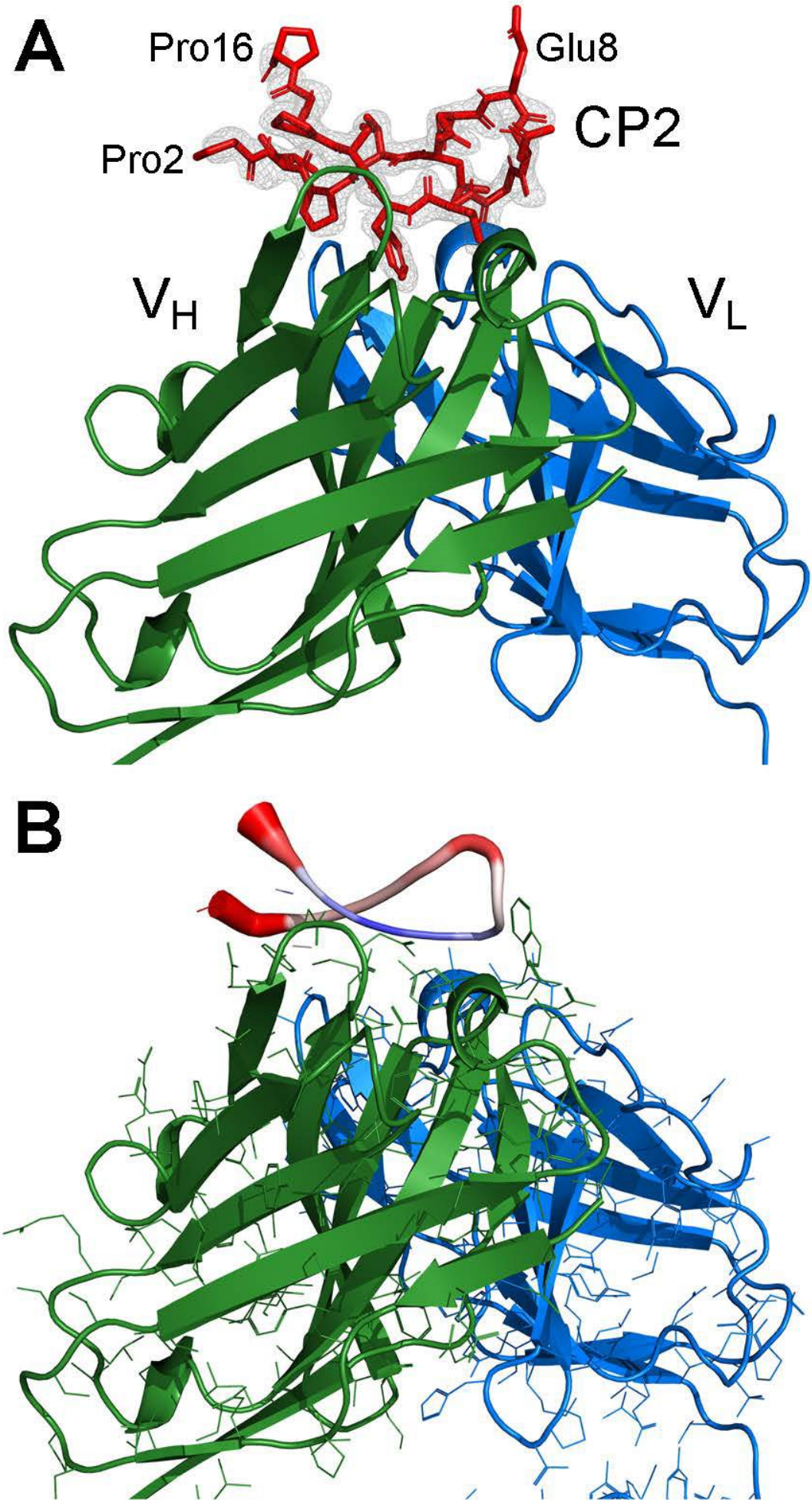
Conclusions

Using a combination of biophysical approaches, we mapped the CP2/TMCP2 epitope and characterized the interactions between mAb 2C7 and CP2. These studies, in conjunction with MD simulations, identified the bioactive conformations of CP2 when bound to Fab 2C7. Collectively, our studies will guide strategies for humanizing mAb 2C7 for use as a therapeutic antibody against gonococcal infection, and for optimizing CP2 as a gonococcal vaccine candidate.

Uploaded File(s)

Supplemental Document Upload

Structure of mimetic peptide CP2 (red) bound to Fab fragment of therapeutic mAb 2C7 (green and blue).


A
Pro16
Glu8
CP2
Pro2
V_H
V_L
B