doi:10.1017/S1431927616006504
Microsc. Microanal. 22 (Suppl 3), 2016

Structural Significance of EmaA Glycosylation in *A. actinomycetemcomitans*

Alison Watson[1], Grace Tang-Siegel[1], Claire J. Brooks[1], Michael Radermacher[1], Keith P. Mintz[2], and Teresa Ruiz[1]

[1]Department of Molecular Physiology & Biophysics, University of Vermont, Burlington, VT
[2]Department of Microbiology & Molecular Genetics, University of Vermont, Burlington, VT

The Gram-negative bacterium, *Aggregatibacter actinomycetemcomitans* is an oral pathogen associated with periodontal disease, as well as other systemic diseases. The ability of the bacterium to bind collagen, the principal component of the extracellular matrix, is mediated by the extracellular matrix protein adhesin A (EmaA). EmaA forms antennae-like appendages on the bacterial cell surface that are comprised of three monomers. The functional domain, of approximately 30 nm in length, is located at the distal end of the adhesin and is subdivided into three subdomains (SI-SIII) [1]. Glycosylation of EmaA adhesins is critical for collagen binding, and it has been demonstrated that this post-translational modification exploits the same pathway as the O-polysaccharide (O-PS) of the lipopolysaccharide [2]. However, it is still unclear how glycosylation facilitates collagen binding. In this study we have analyzed the 3D structure of the functional domain of the EmaA adhesin from mutant strains with a disrupted glycosylation mechanism (*rmlC* and *waaL* mutants). The *rmlC* mutant strain lacks the rhamnose epimerase and the *waaL* mutant strain does not express the O-antigen ligase, WaaL, an essential component of the O-PS glycosylation pathway. Structural comparison of the glycosylated and non-glycosylated adhesins will help to determine if this modification promotes a structural conformation that is required for collagen binding.

EmaA adhesins from both the *rmlC* and the *waaL* mutant strains were analyzed by electron tomography of whole-mount negatively-stained preparations of the bacteria as previously described for other strains [3]. Bacteria were adsorbed on carbon-coated grids pretreated with a colloidal gold solution and negatively stained with Nano W (2% methylamine tungstate). Tomographic single-axis tilt series were acquired over a ±64° angular range in 2° intervals with a calibrated 3.08 Å pixelsize at the specimen scale. Single-axis tilt series were processed using the IMOD processing software to generate tomograms. EmaA adhesins were selected from the tomograms by marking two points on their axis. For each selected adhesin, a tilt series of subprojections was extracted and subvolumes were calculated with the adhesin's axis approximately parallel to the Y-axis using algorithms in both Spider and EMIRA [4,5]. These subvolumes were visualized in Chimera [6] and further aligned to a reference subvolume of the wild-type EmaA [1]. Lower quality EmaA subvolumes were removed from further processing (<10% subvolumes removed). Probabilistic Principal Component Analysis (PPCAEM) implemented in EMIRA was used to assess differences between all the EmaA subvolumes and estimate the missing data [5,7]. The 3D reconstructions were divided into groups of similar structures. The subvolumes were further aligned to a reference selected from the volumes present in each group. Chimera [6] was used to generate an average subvolume for each group.

The 3D electron microscopy analysis of the EmaA adhesins from the mutant strain suggests that glycosylation is important to maintain the overall structural stability of the adhesin and, specifically, the proper conformation of the functional domain. Mutant strains exhibit far fewer adhesins on the bacterial surface than the wild-type strain. A result that is consistent with previous observations, where EmaA was quantified in both strains using protein immunoblot and mRNA expression analysis [2]. Averages

from all the *waaL* mutant strain groups demonstrate that the adhesins lack the three-fold symmetry characteristic of the wild type strain and manifest a higher degree of flexibility. In addition, the members of Groups IV and II (Fig. 1) display a strong curvature along the whole length of the functional domain, with the majority of the adhesins containing bends in places beyond the characteristic bend of the wild type strain at the linker region (between subdomains SII and SIII). An apparent difference between the *waaL* mutant and wild type adhesins is the overall reduced density in the structure, specifically in subdomain SI that appears to have the same diameter as subdomain SII. In several cases (members of Groups I and II, Fig. 1) the adhesins appear slightly more elongated. The observed structural differences indicate that the lack of glycans reduces the stability of EmaA and prevents it from adopting the proper fold necessary for correctly expressing a functional structure capable of binding collagen.

References

[1] Yu, C., Mintz, K.P., Ruiz, T., J. Bacteriol. **191** (2009), p.6253-6261.
[2] Tang, G., Ruiz, T., Mintz, K.P., Infect. Immun. **80** (2012), p.2868-2877.
[3] Azari, F., Radermacher, M., Mintz, K.P., Ruiz, T., J. Struct. Biol. **177** (2011), p.439-446.
[4] Frank J., Radermacher, M., Penczeck, P., Zhu, *et al.*, J. Struct. Biol. **116** (1996), p.190-199
[5] Radermacher, M., Micoscopy & Microanalysis, **19** (2013), Supplement S2 p.762-763.
[6] Petterson, E.F., Goddard, T.D., et al., J. Comput. Chem. 25 (2004), p.1605-1612.
[7] Yu, L., Snapp, R.R., Ruiz, T., and Radermacher, M. (2010). J Struct Biol 171, 18.
[8] This work was supported by NIH grant DE024554, (T.R. & K.P.M) and benefitted from developments from NIH grant GM078202 (M.R.).

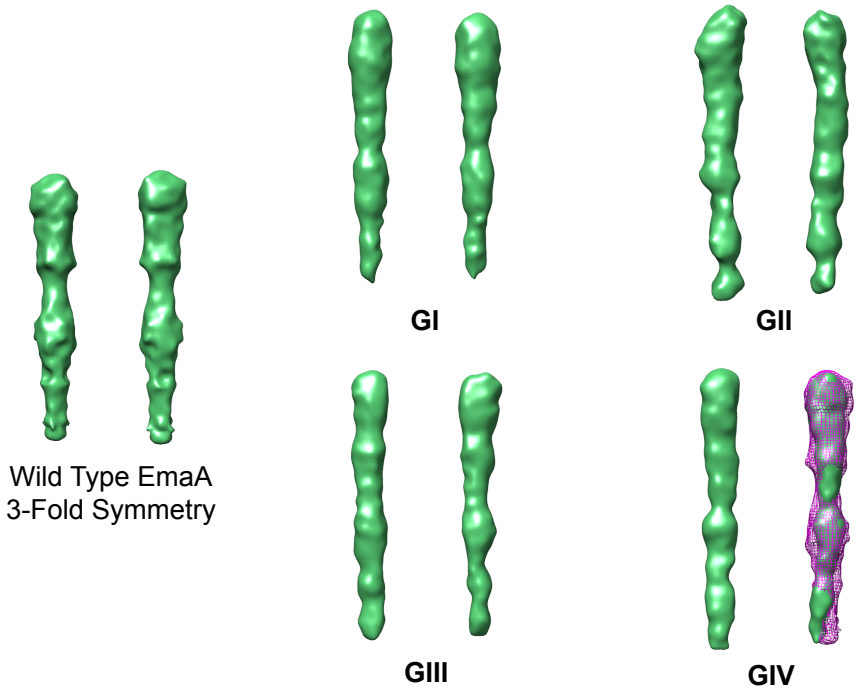

Figure 1. Surface representation of EmaA subvolume averages. Left: Wild type EmaA; average [1]. Right: Averages of four groups of EmaA from the *waaL* mutant strain obtained by PPCAEM followed by visual inspection. All surfaces represent the 30 nm functional domain. Surface pairs are related by a 90° rotation. Purple mesh shows the wild type EmaA.

https://doi.org/10.1017/S1431927616006504 Published online by Cambridge University Press