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Determining a Sub-nanometer Resolution Structure of a *Helicobacter pylori* VacA Toxin Oligomer

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Helicobacter pylori is a gram negative bacterium that colonizes the human stomach. The presence of this bacterium can lead to the development of peptic ulcers, and in more severe cases, the development of gastric adenocarcinoma or lymphoma. One of the major virulence factors secreted by *H. pylori* is vacuolating cytotoxin A (VacA). VacA is secreted as an 88 kDa soluble monomer but transitions into a membrane protein when in contact with cells. VacA s1/i1/m1 causes the formation of large intracellular vacuoles and has been shown to induce various cellular effects, including T cell inhibition, mitochondrial dysfunction, and cell death.

VacA is comprised of a 33kDa (p33) domain located at the N-terminus required for oligomerization and internalization and a C-terminal 55kDa (p55) domain required for receptor binding and oligomerization. Currently, the only high resolution structural information is a crystal structure of the p55 domain determined at 3.4 Å resolution which showed that the p55 domain is comprised of three beta helices [1]. Negative stain electron microscopy (EM) analysis revealed that at a neutral pH, s1/i1/m1 VacA forms multiple oligomers including single-layer hexamers and heptamers, as well as double layer dodecamers and tetradecamers [2]. Unfortunately, these structures are at a ~15Å resolution, too low to distinguish any secondary structural features. Also, no discernible information was obtained on the structure of the p33 pore-forming domain. However, the structures were able to provide a model for VacA oligomerization and the location of the p55 and p33 domains.

Since s1/i1/m1 VacA forms multiple types of oligomers, it makes structural analyses difficult. In order to pursue sub-nanometer structural determination, it is necessary to obtain a more homogeneous sample. The laboratory of Dr. Timothy Cover provided a rung deletion mutant, Δ511-536, which importantly, retains its ability to vacuolate cells [3]. Figure 1A shows the region of the p55 domain deleted in this mutant. To determine whether the homogeneity of double-layer VacA oligomers could be improved by removing regions of the p55 domain involved in double-layer formation, VacA Δ511-536 was subjected to negative stain analysis. Single-particle electron microscopy was performed and determined that VacA Δ511-536 oligomerizes into one major dodecameric conformation (Fig. 1B and C, ~66%), with the remaining oligomers being hexamers, heptamers, or one conformation of tetradecamer (Fig. 1C).

Using this mutant that forms more homogenous oligomers, we have found vitrified ice conditions that produce high contrast images (Fig. 1D). Over 600 images were taken using a FEI Tecnai F30 at 200kV using low-dose conditions and defocus values ranging from -1 to -5 µm on a Gatan 4Kx4K Ultrascan CCD. Class averages generated in SPIDER revealed that the particles were randomly oriented within the ice (Fig. 1E).

VacA forms anion-selective channels in planar lipid bilayers and in membranes of cells, including the plasma membrane and most likely also in endosomal and mitochondrial membranes [4-6]. Single channel analysis predicts VacA channels are composed of six subunits and mimic the action of endogenous sodium-chloride channels [5,6]. The p33 domain is postulated to form the anion-selective channel; however, atomic force microscopy (AFM) studies of VacA bound to mica-supported lipid-bilayers were not able to definitively determine either the type(s) of oligomers bound to the lipid or the conformation of the central pore-forming region of VacA in this lipid environment [4-14]. Using single particle EM and cryo-electron tomography we have characterized the oligomerization state of VacA bound to lipid.

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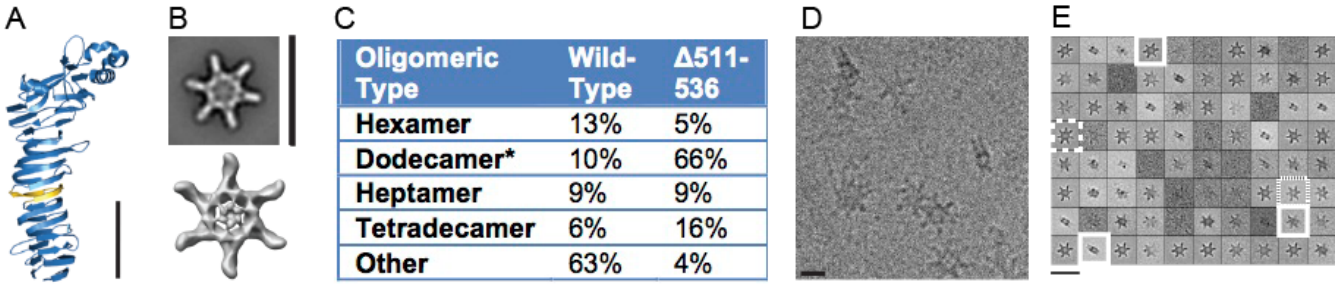

Oligomeric Type	Wild-Type	Δ511-536
Hexamer	13%	5%
Dodecamer*	10%	66%
Heptamer	9%	9%
Tetradecamer	6%	16%
Other	63%	4%

Figure 1. Analysis of VacA Δ511-536 reveals a shift in oligomeric type. A) 2.4 Å crystal structure of s1/i1/m1 VacA p55. The rung deletion is highlighted *yellow*. *scale bar*=2.5nm B) Mutant forms one major dodecamer conformation. *top*-negative stain average of oligomer, *scale bar*=42nm, *bottom*-3D reconstruction of oligomer. '*' represents dodecamer type seen in panel B. C) Table showing percentages of each type of oligomer in both s1/i1/m1 and Δ511-536 VacA. *Other*=other dodecamer types not including type seen in panel B. D) Representative vitrified ice image of of VacA Δ511-536 collected on an FEI F30 electron microscope at 200kV. E) 80 class averages generated from 6478 particles. *dash box*=tetradecamer*; solid box*=dodecamer *(different views); small dash box*=hexamer. *Side length of panels*=36nm.

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