

A Cellular Metalloproteinase Activates *Vibrio cholerae* Pro-cytolysin*

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Many strains of *Vibrio cholerae* produce a cytolysin (VCC) that forms oligomeric transmembrane pores in animal cells. The molecule is secreted as a procytolysin (pro-VCC) of 79 kDa that must be cleaved at the N terminus to generate the active 65-kDa toxin. Processing can occur in solution, and previous studies have described the action of mature VCC thus generated. However, little is known about the properties of pro-VCC itself. In this study, it is shown that pro-VCC exist as a monomer in solution and binds as a monomer to eukaryotic cells. Bound pro-VCC can then be activated either by exogenous, extracellular, or by endogenous, cell-bound proteases. In both cases, cleavage generates the 65-kDa VCC that oligomerizes to form transmembrane pores. A wide variety of exogenous proteinases can mediate activation. In contrast, the activating cellular protease is selectively inhibited by the hydroxamate inhibitor TAPI, and thus probable candidates are members of the ADAM-metalloproteinase family. Furin, MMP-2, MMP-9, and serine proteinases were excluded. Cells over-expressing ADAM-17, also known as tumor necrosis factor α converting enzyme, displayed increased activation of VCC, and knockout cells lacking ADAM-17 had a markedly decreased capacity to cleave the protoxin. The possibility is raised that pro-VCC is targeted to membrane sites that selectively contain or are accessible to cellular ADAM-metalloproteinases. Although many microbial toxins are activated by furin, this is the first evidence for processing by a cellular metalloproteinase. We identified ADAM-17 as a potent activator of pro-VCC.

Vibrio cholerae El Tor-O1 and non-O1 secrete a membrane-damaging cytotoxin, here designated the *V. cholerae* cytolysin (VCC)¹ that permeabilizes animal cell membranes (1, 2). The toxin induces lysis of enterocytes and exhibits enterotoxicity in experimental diarrhea models (2, 3). VCC thus contributes to the pathogenesis of gastroenteritis caused by *V. cholerae* strains, especially with strains not producing cholera toxin (4). Such strains also occasionally cause septicemia (5, 6), and VCC

may also be pathogenetically relevant in this setting. Genetic characterization of VCC identified the structural gene *hlyA* encoding a protein which results, after removal of a signal peptide during secretion, in a protoxin (pro-VCC) of 79 kDa (7, 8). This is the sole form present in supernatants after 8 h of bacterial culture. With time, the protein can undergo N-terminal proteolytic cleavage, and mature 65-kDa VCC is detectable in aged (48 h) culture supernatants (9). Artificial activation of pro-VCC can be achieved in solution by a wide variety of proteinases; cleavage then occurs at one of multiple sites between Leu-146 and Asn-158, depending upon the processing agent. In all cases, functionally active 65-kDa toxin is obtained (10). It was shown that the 65-kDa mature VCC binds to lipid membranes in a nonspecific, reversible fashion (11) and assembles into an SDS-resistant oligomer that creates discrete transmembrane pores. First biochemical evidence suggested a pentameric structure (12), but electron microscopic data are in favor of a heptamer (13), as is known to be the case with staphylococcal α -toxin (14, 15) and aerolysin (16). The VCC pore differs from the former pore-forming toxins in being highly anion-specific (17). It is blocked by inhibitors of anion channels and causes formation of vacuoles when it comes to reside in vesicular membranes following endocytosis (18).

Although mature VCC is irreversibly denatured by urea, pro-VCC refolds correctly after the removal of urea, and active VCC is obtained upon trypsinization. This result indicates that the N-terminal pro-peptide acts as an intramolecular chaperone (19). It was also reported that pro-VCC was weakly hemolytic, a finding that was interpreted to indicate that the N-terminal pro-peptide interferes with pore formation (19). As will be detailed in this communication, our data led us to another conclusion.

A theoretical analysis has led to the recognition that VCC shares structural and, probably, functional characteristics with pore-forming toxins of *Staphylococcus aureus* (α -toxin and leukocidin) and with aerolysin (20). VCC is most closely related to staphylococcal cytolysins, and all have a common cytolytic core with a similar overall fold and a similar putative membrane spanning region. In addition, VCC contains a lectin domain that is absent in the other cytolysins.

The properties of pro-VCC have not been described in any detail. Processing may occur in solution, as is also known for aerolysin (21). However, protoxin is the secreted form in both cases, and it is not *ad hoc* evident that fluid-phase processing should occur in a biological setting. Enteric pathogens generally adhere to target cells, and this intimate contact may shield secreted toxins from the action of soluble host proteases. In septicemia or tissue infections, there would be yet less reason to assume that pro-VCC should mainly be processed prior to contact with target cells. It has remained unclear whether

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¹ The abbreviations used are: VCC, *Vibrio cholerae* cytolysin; HBSS, Hanks' balanced salt solution; TAPI, TNF- α protease inhibitor; DSS, disuccinimidyl-suberate-suberate.