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The StcE Protease Contributes to Intimate Adherence of Enterohemorrhagic Escherichia coli O157:H7 to Host Cells

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Enterohemorrhagic Escherichia coli (EHEC) O157:H7 is a diarrheal pathogen that causes attaching and effacing (A/E) lesions on intestinal epithelial cells. Strains of the O157 serogroup carry the large virulence plasmid pO157, which encodes the etp type II secretion system that secretes the genetically linked zinc metalloprotease StcE. The Ler regulator controls expression of many genes involved in A/E lesion formation, as well as StcE, suggesting StcE may be important at a similar time during colonization. Our laboratory has previously demonstrated that StcE cleaves C1-esterase inhibitor, a regulator of multiple inflammation pathways. Here we report two new substrates for StcE, mucin 7 and glycoprotein 340, and that purified StcE reduces the viscosity of human saliva. We tested the hypothesis that StcE contributes to intimate adherence of EHEC to host cells by cleavage of glycoproteins from the cell surface. The fluorescent actin stain (FAS) test was used to observe the intimate adherence represented by fluorescently stained bacteria colocalized with regions of bundled actin formed on HEp-2 cells. An E. coli O157:H7 strain with a stcE gene deletion was not affected in its ability to generally adhere to HEp-2 cells, but it did score threefold lower on the FAS test than wild-type or complemented strains. Addition of exogenous recombinant StcE increased intimate adherence of the mutant to wild-type levels. Thus, StcE may help block host clearance of E. coli O157:H7 by destruction of some classes of glycoproteins, and it contributes to intimate adherence of E. coli O157:H7 to the HEp-2 cell surface.
up-regulated by the global regulator Lcr, which is encoded on the chromosomal LEE element and regulates four of the five LEE operons (45, 51).

Our laboratory has demonstrated that StcE is not a general probe but has only one identified substrate, C1-esterase inhibitor (C1-INH). C1-INH is a serine protease inhibitor (serpin) and regulator of the complement system and other mechanisms of inflammation (14). Interestingly, StcE cleaved C1-INH retains functional serpin activity to inhibit the classical complement cascade (43). Furthermore, StcE binds and localizes functional C1-INH to cell membranes, effectively enhancing the ability of C1-INH to regulate complement effectors at sites of potential lytic complex formation. Thus, our model of the StcE–C1-INH interaction is that StcE potentiates C1-INH activity to reduce inflammation and complement-mediated lysis at the site of infection (43). This activity would be especially important when Shiga-like toxins and other EHEC effectors compromise the intestinal barrier, resulting in the entry of blood and complement proteins to the intestinal lumen.

Here we identify two additional substrates for StcE activity, glycoprotein 340 (gp340) and mucin 7 (MUC7), and report that StcE reduces the viscosity of human saliva. The substrates are heavily glycosylated proteins with defensive roles found in saliva and other tissues (10, 35, 53). The mucinase activity of StcE and the fact that its expression is coregulated with the sphingomyelinase activity for saliva proteins of similar size did suggest probable identities. The identities were confirmed by immunoblotting with a monoclonal antibody against gp340 (mouse anti-human; 1:10,000; MBL, Woburn, Mass.) and polyclonal MUC7 antisera (mouse anti-human; 1:1000; kind gift of J. G. M. Bolscher). Other substrates tested included mucin, heparan sulfate proteoglycan, heparin, fibronectin, vitronectin, fibrinogen, laminin, fibrinopeptides, chymotrypsinogen, plasminogen, delayed-type hypersensitivity antigens, bovine submaxillary mucin, and ovabulin, all purchased from Sigma.

Viscosity determinations. Whole human saliva was collected from an investigator, cleared by centrifuging for 30 min at 12,000 × g at 4°C, and split into 10-ml samples. The viscometer and samples were equilibrated at 37°C prior to treatment and measurement. The viscosity of the samples was measured before and after a 3-h treatment with StcE (70 μg) or buffer (20 mM Tris, pH 7.5). Both the treatment and measurements took place at 37°C. Relative viscosity was assessed by measuring elution time in a Cannon–Fenske routine viscometer (range, 0.5 to 4 cp; Cannon Instrument Company, State College, Pa.). The elution time for water was considered 0% viscosity, while buffer-treated saliva was considered 100%. Two to four measurements were taken per time point, and the experiment was repeated three times.

Material and methods. E. coli was maintained in Eagle’s modified minimal essential medium (Mediartech, Herndon, Va.) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, Ga.), 10 mM sodium pyruvate, penicillin, streptomycin, and amphotericin B. After achieving confluence, cells were passaged by lifting with 0.25% trypsin–EDTA (Mediatech) and diluted to 1:5 or 1:10.

Biotinylated experiments. Proteins on HEP-2 cell surfaces were biotinylated on carboxyl and aldehyde groups by using biotin hydrazide with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) according to the manufacturer’s protocol (Pierce, Rockford, Ill.). Cells were then incubated in phosphate-buffered saline (PBS) or PBS plus StcE (5 μg/ml) at 37°C. After 2 h the samples were separated into supernatant and two detergent-soluble cell fractions (2% saponin–PBS and 0.5% digitonin–saponin–PBS). The biotinylated proteins were captured using NeutrAvidin immobilized on beads (Pierce). Beads from each fraction were then washed, boiled in sample buffer, and separated on both an 8% acrylamide gel and a 1% agarose gel. Proteins were blotted to nitrocel-

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AF074613. The medium used for infection was Dulbecco’s modified minimum essential medium supplemented as above but without antibiotics. The overnight bacterial culture was diluted 1:25 in this medium for an average inoculation of $8 \times 10^6$ CFU/ml. The medium-bacteria mixture (0.25 ml) was placed on the cells and incubated at 37°C for a total of 6 to 7 h. The medium was removed at midpoint of incubation and replaced with fresh medium with no additional bacteria.

After infection, cells were washed thoroughly (five to six times) with PBS and fixed with paraformaldehyde (3% for 10 min). Cells were washed with PBS (three times for 3 min) after fixation. A 15-min blocking step with antibody dilution solution (2% bovine serum albumin, 0.1% Triton X-100 in PBS) was used to inhibit nonspecific staining. Bacteria were treated with a monoclonal antibody (1:200; 30 min) specific for the O157 O-antigen (U.S. Biologicals, Swampscott, Mass.). After washing in PBS, cells were stained with the secondary antibody solution containing goat anti-rabbit antibody conjugated to Alexa 488 (1:1,000; Molecular Probes) and the actin-staining fungal toxin phalloidin, conjugated to Alexa 594 (1:400, Molecular Probes).

Samples were analyzed using a Zeiss fluorescence microscope and a 40× plan apochromat numerical aperture 1.3 objective (Carl Zeiss MicroImaging Inc., Thornwood, N.Y.). Images were acquired with an AxioCam monochrome charge-coupled device camera (Zeiss) and OpenLab software (Improvision, Lexington, Mass.). Samples were blindly coded to prevent potential bias. Random fields from sample wells were selected out of focus on the phalloidin channel such that no actin bundles were discernible that might bias field selection. Approximately 10 fields of each sample were imaged, and the number of foci that had bundled actin were counted. Foci were defined as either a single bacterium or a cluster of bacteria separated from other foci by more than the length of a bacterium. The experiment was repeated five times with similar results.

**Statistical methods.** The means of the viscosity determinations ($n = 4$ measurements per sample) were compared using the Student $t$ test. FAS data from the five experiments were combined and analyzed using a one-way analysis of variance test to determine if all the means were equal. A Dunnett’s posttest was then used to compare the means of different samples to that of the control (EDL933) and to determine $P$ values. Numbers of fields observed for each sample were as follows: EDL933 ($n = 54$), EDL933 plus rStcE* ($n = 34$), WAM2815 ($n = 68$), WAM2815 plus rStcE* ($n = 58$), WAM2997 ($n = 33$).

**Nucleotide sequence accession numbers.** The sequence for the secreted form of StcE has been deposited in the GenBank database under accession number AF074613.

**RESULTS**

**StcE cleaves gp340 and MUC7.** The only previously known substrate for the StcE protease was C1-INH (45). We hypothesized that saliva would provide a potential source of substrates, as it is the first host tissue that EHEC encounters. The rStcE* protease was incubated with whole human saliva, and the proteins were separated by SDS-PAGE. The results (Fig. 1A) demonstrated that the migration of two Coomassie-stained, large-molecular-weight protein bands changed as a result of rStcE* treatment. Treatment with a proteolytically inactive rStcE* mutant (45) did not cause this migration change (data not shown). The larger protein (~300 kDa) was identified by mass spectrometry as gp340, a common salivary protein that is encoded by an alternately spliced variant of the gene **DMBT1** (deleted in malignant brain tumors 1). The smaller protein did not yield useful mass spectrometry data but, based on the literature and its size (72), was surmised to be MUC7. Both proteins were positively identified as substrates in immunoblot assays with antiserum to MUC7 (Fig. 1B) and antibody to gp340 (Fig. 1C).

**StcE reduces the viscosity of saliva.** Heavily glycosylated proteins such as gp340, MUC7, and other mucins and glycoproteins are largely responsible for the viscosity of mucous material such as saliva. We hypothesized that StcE cleavage of these proteins may result in reduced viscosity of saliva. This hypothesis was tested using a Cannon-Fenske viscometer, where flow rate through a capillary tube measures relative viscosity. Addition of rStcE* caused a 66% reduction in viscosity of saliva compared to that of water (data not shown). This difference was statistically significant ($P < 0.0001$), and the experiment was repeated three times with similar results.

**StcE does not cleave all glycosylated proteins tested.** StcE activity against lactoferrin, ovalbumin, bovine submaxillary mucin, bovine fetuin, and porcine gastric mucin was tested (data not shown). The results demonstrated that StcE did not cleave these proteins.

**StcE alters HEp-2 cell surface proteins.** The finding that StcE could cleave two known mucin-like proteins and reduce viscosity of a glycoprotein-containing material led to the hypothesis that StcE may cleave mucin-like proteins from the surfaces of host cells. To test this hypothesis, we biotinylated HEp-2 cells with biotin-hydrazide and EDC. This treatment labels surface proteins via cross-linking to carboxyl and aldehyde groups. Supernatants (S1) of untreated or StcE-treated cells contained secreted proteins or proteins cleaved from cells by StcE. The detergent-soluble fractions (S2 and S3) contained cell-associated proteins. Cell surface-associated proteins were biotinylated and were captured using NeutrAvidin beads. Analysis of samples separated by SDS-PAGE and blotted showed that supernatants from rStcE* treated cells contained additional bands not present in the untreated sample, and the cell-associated fractions contained two bands that were reduced or missing in the rStcE* treated samples (Fig. 2A). The biotinylated rStcE* treated samples were separated on an agarose gel in order to observe larger-molecular-weight proteins, such as mucins. The blot of the agarose gel showed similar results (Fig. 2B) to those seen in Fig. 2A, where bands in the
S2 and S3 fractions disappeared after rStcE treatment. Attempts to identify the proteins in these bands by mass spectrometry and immunoblotting with antiserum to MUC7 or antibody to gp340 were unsuccessful (data not shown).

**StcE contributes to intimate adherence.** The demonstration that StcE cleaves large proteins from host cell surfaces suggested that StcE might play a role in adherence of _E. coli_ O157:H7 to host cells. We tested this by measuring viable CFU adhering to HEp-2 cells after 3 or 6 h of infection. The results indicated that general adherence of wild-type EDL933 or an isogenic _stcE_ deletion mutant (WAM2815) was the same (data not shown). We then tested if StcE contributed to intimate adherence of the bacterium to host cells. We used the FAS test (23, 41) to measure the number of bacterial foci that formed actin bundles on HEp-2 cells, the prototypic cell line for such experiments. We found that the _stcE_ deletion strain WAM2815 scored threefold lower on the FAS test than the isogenic wild-type strain or the _stcE_-complemented strain, WAM2997, after a 6-h infection (Fig. 3). In addition to genetic complementation, addition of rStcE protein at the beginning of the infection could abolish the _stcE_ deficiency of the WAM2815 strain. The FAS experiments also concurred with previous experiments suggesting that StcE did not contribute to general adherence. The important finding was the difference in intimate adherence, a step thought to be important in establishing colonization.

**DISCUSSION**

Our laboratory previously showed that _E. coli_ O157:H7 strain EDL933 secretes StcE, an extracellular zinc metalloprotease. In our initial studies (45), StcE did not behave as a general protease active against many common protein substrates but appeared to only cleave the complement regulator, C1-INH. In further studies, proteins from cell lysates (HEp-2, Caco-2, and LS174T cells) treated with StcE that were separated by SDS-PAGE and stained with Coomassie blue showed no bands that shifted or decreased in intensity. In this study, however, we identified two salivary glycoproteins, gp340 and MUC7, as substrates for StcE proteolysis. We additionally showed that purified rStcE reduces the viscosity of human saliva by 66%, probably the result of this StcE mucinase activity. We propose that StcE may contribute to the low infectious dose of _E. coli_ O157:H7 by preventing the formation of mucin-bacteria aggregates that could lead to bacterial elimination in mucosal environments such as the oral cavity. Furthermore, we demonstrate that StcE degrades several large cell surface proteins on HEp-2 cells. Coincident with or because of this activity, _E. coli_ O157:H7 expressing StcE has an increased ability to carry out intimate adherence with HEp-2 cells as measured by FAS. Our model (Fig. 4) suggests that StcE mediates increased intimate adherence by reducing the mucous glycocalyx cell surface barrier, allowing a closer interaction between the bacterium and host cell. In the colon, StcE cleavage of mucins and glycocalyx matrix could also lead to a reduction of commensal bacteria in the immediate area, thereby enhancing the ability of _E. coli_ O157:H7 to compete for space and nutrients.

We recently demonstrated that StcE interaction with C1-INH surprisingly potentiates rather than destroys C1-INH regulation of classical complement activation. StcE binds to bacterial or host cell surfaces and then localizes active C1-INH, which subsequently reduces complement-mediated lysis of both bacteria and host cells (43). Therefore, we believe the mucinase activity of StcE is important in early stages of colonization, while the StcE-mediated potentiation of C1-INH activity may be important during the bloody colitis phase of _E. coli_ O157:H7 disease. At this stage its hypothesized that Shiga-like toxins and other effectors compromise the intestinal epithelial barrier and damage the intestinal endothelium, which results in entry of blood and serum effectors into the intestinal lumen (56). As a corollary to the bifunctional model of StcE activity, we hypothesize that the kinetics and binding of...
StcE to the different substrates (e.g., C1-INH versus mucins) are different. These studies are currently under way in our lab.

Both saliva and intestinal mucus are composed of heavily glycosylated proteins, including the proteins of the mucin family. The structure of the mucins is characterized by a central region composed of tandem repeats that are serine/threonine rich and heavily glycosylated (55). The result is a protein with a "bottle-brush" conformation that can be 50 to 200 nm long and have molecular masses of hundreds to thousands of kilodaltons (68). Mucins can be cell bound or secreted and provide lubrication in the gastrointestinal tract. A firm, tightly adherent mucus layer over mucosal cells is 100 μm thick, while the loosely adherent mucus layer can be several times thicker (18, 66). Secreted mucins are gel forming, in part because they can form chains of disulfide-linked monomers. They also function to protect the single cell layer of the intestinal epithelium from the "outside world" of food and microorganisms constantly flowing through the intestine. The mucus layer is impermeable to large molecules but permeable to ions, sugars, nutrients, and other small molecules (68).

The emerging picture of StcE protein substrates is that they are heavily glycosylated, mostly via O-linkages, and that these

**FIG. 3.** Immunofluorescent images of HEp-2 cells infected by strains of *E. coli* O157:H7. HEp-2 cells were infected for 6 h with strains EDL933, WAM2815, and WAM2997, either with or without 2 μg of StcE. (A) Samples were stained for actin (red) and bacteria (green). Images are representative of data shown in panel B. (B) Actin-bundling foci were enumerated in sample fields for each infection. EDL933, wild-type EHEC; WAM2815, *stcE:cat*; WAM2997, WAM2815 complemented with the *stcE* gene. Data shown are the means and standard errors of the means of the combined results from five independent experiments. *, only the mean of sample WAM2815 was significantly different from the mean of the control, EDL933, based on the Dunnett posttest ($P < 0.001$).
regions contain repeated amino acid sequences. The previously identified SteE substrate, C1-INH, shares these features with MUC7 and gp340. C1-INH has a highly conserved C-terminal serpin domain, but in contrast to other serpins, it has a unique 120-amino-acid N terminus that is heavily glycosylated. One region of the N terminus (residues 67 to 97) contains a series of imperfect repeats of the sequence Glx-Pro-Thr-Thr (11, 19). Recent results in our laboratory indicate that the C1-INH cleavage site for SteE lies between the N terminus and serpin domain, probably just C terminal to the glycosylated region and repeats (43). This suggests that other SteE substrates are likely to also contain repeated sequences and multiple O-glycosylations. It should be noted, however, that SteE is not a general mucinase because it does not cleave all glycosylated proteins, as evidenced by our inability to detect cleavage of gelatin (45), porcine gastric mucin, bovine submaxillary mucin, bovine fetuin, lactoferrin, or ovalbumin (data not shown).

Other enteropathogens produce proteins with mucinase activity. For instance, the HA/P zinc metalloprotease of Vibrio cholerae and the Pic protease of Shigella flexneri and enteroinvasive E. coli have both been shown to have mucinase activity. In both cases, however, the proteases are active against a wide variety of proteins. HA/P degrades fibronectin, ovomucin, and lactoferrin and is 61% identical to Pseudomonas aeruginosa elastase, another broad-spectrum protease (27, 32). In infections, HA/P has been suggested to be a factor mediating adherence as well as detachment (4, 5, 26). Pic is an auto-transported serine protease that cleaves mucins from murine and bovine sources, including gelatin (33). Thus, in the class of enteropathogen mucinases, SteE seems unique in that it seems to have a more limited set of substrates.

MUC7 (also called MG2), one of the SteE substrates identified here, is found at high levels in saliva (100 to 980 μg/ml) as well as in lung secretions and tears. It has six repeats of 23 residues, 9 of which are possible glycosylation sites (10, 67). It binds a variety of bacteria, complexes with soluble immunoglobulin A and lactoferrin, and has antifungal activity (47, 63, 64). Roughly 70% of its molecular weight is composed of posttranslational modifications in the form of glycosylations (62).

The other substrate identified here, gp340, is one of at least two proteins resulting from alternative splicing of transcripts from the DMBT1 gene (34, 53, 65). Known DMBT1 proteins include DMBT1 and gp340 (previously identified as salivary agglutinin). They are members of the scavenger receptor cysteine-rich (SRCR) family and are primarily composed of 14 nearly identical SRCR domains. These are separated by SRCR-interspersed domains, which contain many potential O-glycosylation sites. On either side of the 13th SRCR domain lie two CUB (C1r/C1s Uegf Bmp1) domains. After the 14th SRCR domain is a ZP (zona pellucida) domain (9, 54). Each of these domain types is thought to mediate protein-protein interactions. gp340/DMBT1 have characteristics of a pattern-recognition receptor and bind the collectins SP-D and SP-A, which are also known to aggregate bacteria and are involved in mucosal inflammation responses (35, 65, 69). A peptide derived from one of the gp340/DMBT1 SRCR domains binds a wide variety of bacteria, including E. coli (9). This activity is thought to mediate clearance of pathogens from mucosal environments through creation of protein-bacteria aggregates and interactions with phagocytic cells (38).

Together, MUC7 and gp340 have been described to have important roles in innate oral defense mechanisms. Although the bacterium may not reside in the oral cavity for long periods of time, mastication mixes food with saliva proteins, which are all swallowed together and interact during initial digestion. MUC7 and gp340, as well as unknown SteE substrates, may be present throughout the mucus layers of the gastrointestinal tract.

Both mass spectrometry and immunoblotting with antiserum against MUC7 and a monoclonal antibody against gp340 failed to identify any proteins in samples of untreated or SteE-treated
HEp-2 cells (data not shown). Their identity may not be particularly important in terms of E. coli O157:H7 pathogenesis, given that HEp-2 cells are cervical cells (16, 57, 58). However, HEp-2 cells are the predominant model for studies of A/E formation, and results from this model system have been correlated with in vivo results (8, 24, 50). Interestingly, DMBT1 gene products are produced and secreted by cells in the colon (52, 53). Therefore, in vivo, DMBT1 gene products are likely to be colonic mucosal proteins that SteC acts upon to promote E. coli O157:H7 colonization and disease. In addition, DMBT1 gene products are likely to have roles in innate defense in the colon similar to their role in the oral cavity. The cleavage of DMBT1 gene products may also interfere with normal host signaling or cell differentiation, since the gene encodes a variety of domains as discussed above.

Previous studies have demonstrated that the common E. coli O157:H7 virulence plasmid, pO157, and therefore stcE, is not required for intimate adherence (30, 39, 70). Therefore, it is not surprising that the role of SteC in enhancing this activity has gone unrecognized. Many studies investigating the details of intimate adherence use enteropathogenic E. coli (EPEC) rather than EHEC strains. In vitro, EHEC does not adhere intimately as readily as EPEC (22). Growth under conditions unfavorable to stcE expression may partially explain this difference.

Our laboratory has recently shown that only a subset of EPEC strains contain the stcE gene, and the strain typically used in A/E studies, E2348/69, does not have stcE (44). EPEC disease is usually considered an event in the small intestine (56), while EHEC infects the large intestine. These two environments differ in many respects. The large intestine contains different mucins and 2- to 3-log-higher levels of normal flora than the small intestine (7, 18). The propensity to colonize different sites could be due to a number of factors influenced by characteristics of the host, the intestinal flora, and bacterial strain. For example, an EHEC strain that expressed an EPEC-derived intimin gene allowed EHEC to colonize tissue from a human small intestine (28).

A recent report suggested that the three main EPEC proteins involved in general adherence to differentiated Caco-2 cells were BFP, EspA, and intimin (17). BFP mediates rapid adherence (within 10 min), EspA is responsible for a low level of adherence under similar conditions, and intimin imparts no adherence unless the host cells have been preinfected with a Tir-producing strain. Among these, BFP seems to be most important for adherence. Indeed, strains with inactivated BFP are deficient for microcolony formation, show reduced adherence to host cells, and cause less diarrhea in volunteers (8, 17, 31). However, there is a class of “atypical” EPEC strains that lack the plasmid that encodes BFP and cause disease in adults in industrialized countries, as opposed to infants in underdeveloped countries, which is the typical population affected by EPEC (2, 25, 60, 71). Interestingly, the stcE-positive EPEC strains seem to be restricted to a group of atypical EPEC of the O55:H7 serotype (44). Since some atypical EPEC strains lack BFP but have stcE, it is possible that SteC helps compensate for the deficiency of BFP-mediated adherence, possibly by increasing the efficiency of EspA-mediated adherence. Interestingly, Mack et al. recently reported that addition of mucin-
enriched medium can inhibit adherence of EPEC strain E2348/69 to HEp-2 cells (48).

Our multifunctional model of SteC activity has preceded in EHEC pathogenesis. For example, the Tir protein is an EHEC receptor as well as a cytoplasmic effector that participates in host cell signaling and actin organization (15, 29). The EspA-composed filaments of the T3SS serve as adhesins as well as channels for protein transport from bacterium to host cell (1, 17, 42, 59). Characterization of the functional consequences of SteC activity on its substrates and further identification of additional substrates will lead to a better understanding of its role in E. coli O157:H7 pathogenesis. Furthermore, the association of stcE with an atypical EPEC lineage leads to a potential use of stcE as an epidemiological and evolutionary marker to describe atypical EPEC strains, which are an important emerging class of pathogenic E. coli (2, 25, 60, 71).

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REFERENCES

of which is influenced by the EAF plasmid. Infect. Immun. 60:471–478.


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