Molecular Characterization of a Carboxy-Terminal Eukaryotic-Cell-Binding Domain of Intimin from Enteropathogenic *Escherichia coli*

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A eukaryotic cell-binding domain from the intimin (Int) polypeptide of enteropathogenic *Escherichia coli* O127 (EPEC) was investigated. Derivatives of the carboxy-terminal 280-amino-acid domains of Int (Int_{EPEC280}) and the Int homolog invasin (Inv) from *Yersinia pseudotuberculosis* (Inv_{YP280}) were fused to the *E. coli* maltose-binding protein (MBP), expressed, and purified. The smallest MBP-Int_{EPEC} fusion protein that efficiently mediated binding to HEp-2 cells, monitored by using purified fusion proteins in fluorescence activated cell sorter analysis or by using fluorescent Covaspheres coated with purified fusions, contained the carboxy-terminal 150 amino acids of Int. Replacement of Cys-937 with Ser (Int_{EPEC280CS}) destroyed the cell-binding activity of Int_{EPEC280}. Covaspheres coated with MBP-Int_{EPEC280} were associated with HEp-2 cell microvilli but failed to induce actin accumulation underneath bound particles or cell spreading on coated plastic surfaces. MBP-Int_{EPEC280}, but not MBP, MBP-Int_{EPEC280CS}, or MBP-Inv_{YP280}, inhibited EPEC entry into HEp-2 cells.

Enteropathogenic *Escherichia coli* (EPEC) is an important cause of diarrhea in developing countries (16). Ultrastructural studies of intestinal biopsy specimens from children with EPEC-induced diarrhea have shown that when EPEC bacteria bind to the intestinal brush border, microvilli are destroyed or effaced. The apical cell membranes of the bare enterocytes form cup-like pedestals upon which the bacteria closely adhere (17, 24). EPEC also adheres to tissue culture cells, such as HEp-2 cells, where it can be visualized in distinctive microcolonies, a phenomenon known as localized adherence (20). Studies utilizing electron microscopy have shown that shortly after the initial adherence to HEp-2 cells, microvilli are effaced and the underlying cytoskeleton is reorganized at the sites where individual EPEC cells are intimately associated with the cell membrane (5). Limited uptake of adhering bacteria by epithelial cells growing in culture has also been observed (6). An EPEC gene product that is essential for intimate attachment to epithelial cells is intimin (Int_{EPEC}), a 94-kDa outer membrane protein encoded by the chromosomal *eaeA* gene (11, 12). The amino terminus of Int_{EPEC} has a high degree of homology with the *eae* gene products of enterohemorrhagic *E. coli* (Int_{EHEC}) (25) and *Citrobacter freundii* biotype 4280 (Int_{CF}) (21) and with the amino terminus of invasin (Inv), the product of the *inv* gene of *Yersinia pseudotuberculosis* (Inv_{YP}) (10).

Although the amino-terminal regions of these proteins are highly homologous, Int and Inv share only limited homology at their carboxy termini (25). There are also important biological differences between Inv and Int. *E. coli* K-12 strains expressing Inv, but not Int, are able to invade HEp-2 and other mammalian cells (10). Isberg and colleagues have initiated a molecular characterization of the Inv_{YP} gene product (8–10, 14). They

have shown that although larger domains bind more efficiently to cellular receptors (23), the binding and invasive activity of Inv_{YP} resides predominantly in the 192 carboxy-terminal amino acids of the protein (14). The cell surface receptors of Inv are the $\alpha_3\beta_1$ integrins (9), which mediate communication between the cytoskeleton and extracellular matrix proteins in eukaryotes. Site-directed mutagenesis has been used to analyze the Inv_{YP192} binding and invasion domain. A potential loop formed by disulfide linkage between Cys residues at positions 907 and 982 was found to be essential for receptor recognition and invasion (15).

In a previous study, we showed that like that of Inv, a domain that mediates binding to eukaryotic cells resides within the 280 amino acids at the carboxy terminus of Int (7). In the present study, we further investigated the molecular basis of Int-eukaryotic cell interaction by using maltose-binding protein (MBP) fusions. We found that MBP-Int_{EPEC280}, but not MBP-Inv_{YP280}, inhibited invasion of cultured mammalian cells by EPEC. Furthermore, we demonstrated that the 150 carboxy-terminal amino acids of Int are sufficient to mediate cell binding and that a Cys residue at position 937 is essential for Int cell-binding activity.

MATERIALS AND METHODS

Construction and purification of MBP-Int_{EPEC} and MBP-Inv_{YP} fusion proteins. MBP-Int_{EPEC} and MBP-Inv_{YP} fusion proteins were constructed and purified as previously described (7). MBP-Int_{EPEC680} (amino acids 260 to 939), MBP-Int_{EPEC280} (amino acids 660 to 939), MBP-Int_{EPEC150} (amino acids 790 to 939), MBP-Int_{EPEC120C} (amino acids 820 to 939), MBP-Int_{EPEC120N} (amino acids 790 to 909), MBP-Int_{EPEC70} (amino acids 870 to 939), MBP-Int_{EPEC40} (amino acids 820 to 859), and MBP-Int_{EPEC280CS} (same as Int_{EPEC280} but with a Cys-to-Ser substitution at position 937) were generated by cloning PCR products downstream from the *E. coli* *malE* gene (encoding MBP) carried on plasmid pMALc2 (New England Biolabs). Figure 1 shows the relative locations of the primers (listed in Table 1) and a schematic presentation of the different fusion proteins generated by PCR (18) with EPEC strain O127ab:H6 DNA as the template (7). MBP-Inv_{YP740} (amino acids 247 to 986), MBP-Inv_{YP280} (amino acids 707 to 986), and MBP-Inv_{YP280CS} (same as Inv_{YP280} but with a Cys-to-Ser substitution at position 982) were generated in a similar way (Table 1) with plasmid pRI203, encoding the *Y. pseudotuberculosis* *inv* gene (10), as the template.

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FIG. 1. (A) Schematic representation of *inv* and *eaeA* gene products. The conserved N-terminal domains are presented as open boxes. The Inv binding domain is represented by the hatched box, and the Int binding domain is represented by the black box. The grey hatched box of Inv represents an amino acid sequence that has no homology within Int. C-982 of *inv* and C-937 of *eaeA* are the Cys residues subjected to site-directed mutagenesis. (B) Schematic representation of the Int_{EPEC} domains cloned downstream from MBP. The solid horizontal bars represent the region of the *eaeA* binding domain incorporated into each fusion protein. The numbers on the left represent amino acids of the domains. The relative locations and orientations of the different primers used to generate the MBP fusion proteins are shown. The DNA sequences of the primers are listed in Table 1. The ability of each of the MBP fusion proteins to mediate the binding of coated Covaspheres to HEp-2 cells is indicated at the right. ++, strong binding of coated particles; +, moderate binding; –, no binding.

Detection of binding of MBP-Int_{EPEC} fusion proteins to HEp-2 cells by fluorescence-activated cell sorter (FACS) analysis. HEp-2 cells (1.5×10^5) were grown overnight in 24-well tissue culture plates in 1 ml of Dulbecco's modified Eagle's medium with 10% fetal calf serum and 2 mM glutamine (complete medium). Binding assays with the different fusion proteins (5 μ g/ml) and a FACS were performed as previously described (7), except that complete medium was used throughout the binding procedure.

Coating of Covaspheres with MBP-Int_{EPEC} and MBP-Inv_{YP} fusion proteins, HEp-2 adherence assay, and actin staining. Fluorescent polymer microspheres (Covaspheres MX; Duke Scientific) were coated with MBP or with the MBP fusion proteins in accordance with the manufacturer's instructions. Briefly, coating was performed as follows. A 500- μ l volume of Covaspheres was sonicated for 1 min, and 100- μ l aliquots were each mixed with 100 μ l of 1 mg of a fusion protein solution per ml. The Covaspheres were incubated for 75 min at room temperature, washed, and resuspended in 1 ml of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.2, by repeating the sonication procedure. To determine the Covasphere-coating efficiencies of the different fusion proteins, 100- μ l samples from the coated beads were transferred to new tubes. A 900- μ l volume of 1% BSA-PBS containing either 1 μ l of polyclonal anti-MBP serum or normal rabbit serum (as a negative control) was added, and the Covaspheres were incubated at room temperature for 1 h. The beads were washed three times with PBS and incubated for 1 h with 1 ml of 1% BSA-PBS containing 1 μ l of a goat anti-rabbit horseradish peroxidase conjugate. The Covaspheres were washed three times with PBS and resuspended in 0.2 ml of PBS, and samples of 1, 5, and 10 μ l were transferred to Costar 96-well enzyme immunoassay-radioimmunoassay plates. A 50- μ l volume of horseradish peroxidase substrate solution (25 mM citric acid, 60 mM NaH₂PO₄, 0.4 mg of *o*-phenylenediamine per ml, 0.012% H₂O₂) was added to each well, and the intensity of the color reaction was determined at 490 nm. Polyclonal anti-MBP antibody staining of coated Covaspheres revealed that the different fusion proteins coated the fluorescent beads with similar efficiencies. No signal was obtained when the Covaspheres were incubated with normal rabbit serum, indicating that all of the reactive sites on the beads were saturated.

To assay cell binding, 25 μ l of the coated Covasphere suspension was added to subconfluent HEp-2 cells, which were grown overnight on coverslips and incubated for 3 h at 37°C, unless specified otherwise. The monolayers were washed three times with PBS and fixed with 3% glutaraldehyde, and adherence of beads to the cells was determined by fluorescence microscopy as previously described (7). For actin staining, cells were incubated with fluorescent or nonfluorescent coated Covaspheres, fixed, permeabilized with 0.1% Triton X-100 for 5 min at room temperature, and stained with fluorescent isothiocyanate-phalloidin (Sigma) as previously described (13).

Adherence of eukaryotic cells to wells coated with MBP-Int_{EPEC} and MBP-Inv_{YP} fusion proteins. Costar 96-well enzyme immunoassay-radioimmunoassay plates were coated with either MBP-Inv_{YP} or MBP-Int_{EPEC} fusion protein as described previously (7). The efficiencies with which the different fusion proteins coated the well plastic were determined as described above. Coated wells were washed with PBS and blocked with 1% BSA in PBS for 1 h at 37°C. A 50- μ l volume of 10^6 HEp-2 cells per ml in complete medium was added to each well and incubated for 40 to 60 min at 37°C in 5% CO₂. Adherence of cells to the coated surfaces was determined as previously described (7). Briefly, the well were washed with PBS, fixed with 70% methanol for 10 min, incubated with 0.2% crystal violet for 5 min, and washed again with PBS. The cells were then lysed with 1% sodium dodecyl sulfate at 37°C, and the optical density at 562 nm was determined.

Inhibition of EPEC invasion by MBP fusion proteins. Inhibition assays were performed as described by Leong et al. (14). Purified MBP fusion proteins (50 μ g) were incubated with 2×10^5 HEp-2 cells in 1 ml of RPMI 1640–0.4% BSA–20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7) in 24-well tissue culture plates for 1 h at room temperature. A 10- μ l volume of bacteria (EPEC strain O127ab:H6) from an overnight culture grown statically at 37°C was added, and incubation proceeded for an additional 3 h at 37°C in 5% CO₂. Bacterial invasion was quantitated by gentamicin-resistant viable counts as previously described (6, 14). Relative invasion in the presence of fusion proteins was expressed as a percentage of the invasion level obtained from cells preincubated with column buffer only (i.e., 100%).

Statistics. Data are presented as means $\pm$ the standard errors of the means. Means were compared by using Student's *t* test, and *P* values of 0.05 or less were considered statistically significant.

Scanning electron microscopy. HEp-2 cell were grown on coverslips and incubated with coated Covaspheres as described above. Cell were fixed with glutaraldehyde, postfixed in osmium tetroxide, dehydrated with 2,2-dimethoxypropane, and transferred to 100% ethanol. The specimens were then critical point dried with liquid carbon dioxide in a Polaron E3000 critical point drying apparatus, sputter coated with gold-palladium with a Polaron E5100 series II coater, and viewed at 30 kV in a JEOL 5300 scanning electron microscope.

RESULTS

Molecular characterization of the Int_{EPEC} carboxy-terminal cell-binding domain. To define the region within Int_{EPEC}280 that is required for cell binding, derivatives containing both amino- and carboxy-terminal deletions (Fig. 1 and 2) were expressed as MBP fusion proteins. HEp-2 cell binding was assessed quantitatively by FACS analysis with purified fusion proteins and by fluorescence microscopy with coated Cova-

TABLE 1. DNA sequences of primers used in PCRs for MBP fusions

Primer ^a	Sequence ^b
F-Int ₂₈₀5'	CTGAATTCGCCAGCATTACTGAGATTAAAG
F-Int ₁₅₀5'	CTGAATTCACGGAATAATATACATGGC
F-Int _{120C}5'	CTGAATTCACAACATATTTCCGTTATCTC
F-Int ₇₀5'	CTGAATTCCTCAGAACTCAACTGGAAAAT
F-Int ₆₈₀5'	CTGAATTCGGTCCGGGTGAGCGTTTTC
R-Int ₄₀5'	CTTCTAGATGTTATTCAGCATCATATAG
R-Int _{120N}5'	CTTCTAGATTCGCATCTTGAGCTGTTTGT
R-Int ₉₃₉5'	CTTCTAGATTATTTACACAAGTGGCAT
R-Int _{939CS}5'	CTTCTAGATTATTTACAGAAGTGGCAT
F-Inv ₂₈₀5'	CTGAATTCACCGTCTCCACACCGGATATC
F-Inv ₇₄₀5'	CTGAATTCGAGGCCTGGACCGATTATTAC
R-Inv ₉₈₆5'	CTTCTAGATTATTTGACAGCGCACAGAG
R-Inv _{986CS}5'	CTTCTAGATTATTTGACAGCGCACAGAG

^a F, forward primer; R, reverse primer.

^b Underlined sequences represent nucleotides specifying the restriction enzyme sites used for cloning.

FIG. 2. Polyacrylamide gel electrophoresis of MBP-Int_{EPEC} fusion proteins MBP-Int_{EPEC280} (lane 2), MBP-Int_{EPEC280CS} (lane 3), MBP-Int_{EPEC150} (lane 4), MBP-Int_{EPEC120C} (lane 5), MBP-Int_{EPEC120N} (lane 6), and MBP-Int_{EPEC70} (lane 7). Both MBP-Int_{EPEC280} and MBP-Int_{EPEC280CS} preparations consistently resulted in doublet bands; this appears to represent proteolytic sensitivity introduced into the MBP part of the fusions, as demonstrated with specific antisera and factor X cleavages (data not shown), and therefore affected neither binding nor detection. Sizes of molecular weight markers (lane 1) are indicated in thousands on the left.

spheres. MBP-Int_{EPEC150}, containing 150 amino acids from the carboxy terminus of Int, was the smallest protein that showed HEp-2 cell-binding activity by FACS analysis (Fig. 3). No significant quantitative differences in cell-binding activity above that detected for MBP was revealed by FACS analysis for fusion protein. MBP-Int_{EPEC120C}, MBP-Int_{EPEC120N}, MBP-Int_{EPEC70}, or MBP-Int_{EPEC40}, which contains the 40 amino acids of Int upstream from Cys-860 (Fig. 3).

In studies with fluorescence microscopy and Covaspheres, examination of many independent monolayers exposed to the coated particles revealed that the binding pattern obtained with Covaspheres coated with MBP-Int_{EPEC150} (Fig. 4A) was similar to that obtained with both MBP-Int_{EPEC280} (Fig. 4B) and MBP-Inv_{YP280} (Fig. 4C). No cell binding was seen with Covaspheres coated with MBP or fusion protein MBP-Int_{EPEC120C}, MBP-Int_{EPEC120N}, MBP-Int_{EPEC70}, or MBP-Int_{EPEC40} (Fig. 1). HEp-2 cell binding of Covaspheres coated with MBP-Int_{EPEC280} and MBP-Inv_{YP280}, but not that of purified fusion proteins, was inhibited at 4°C, and no effect on the binding pattern was seen when the beads were resonicated prior to the assay (data not shown). No evidence of actin condensation was detected in fluorescein isothiocyanate-phalloidin preparations of HEp-2 cells incubated with nonfluorescent MBP-Inv_{YP280} or MBP-Int_{EPEC280}-coated Covaspheres (data not shown). However, phalloidin staining (data not shown) and scanning electron microscopy (Fig. 5B) revealed that coated spheres were attached predominantly to cell microvilli.

HEp-2 cell spreading on plastic surfaces coated with MBP-Int_{EPEC} fusions. Since there were similarities in the binding to eukaryotic cells of Covaspheres coated with MBP-Int_{EPEC280} and that of those coated with MBP-Inv_{YP280}, the ability of MBP-Int_{EPEC} fusion proteins to cause cell spreading on coated plastic wells was assessed. The well-coating efficiencies of all of the fusion proteins tested were found to be similar by enzyme-linked immunosorbent assay (data not shown). MBP-Inv_{YP280} has been shown previously to mediate attachment and spreading of HEp-2 cells on coated surfaces (7, 22), whereas MBP-Int_{EPEC280} lacked this activity (7). MBP fusion proteins containing longer Inv carboxy-terminal sequences bind with greater affinity to integrin receptors (23) and induce efficient cell spreading. To assess if this was the case for Int, MBP-

Int_{EPEC680} was constructed and the purified fusion protein was used to coat Covaspheres. MBP-Inv_{YP740} was prepared as a control. Covaspheres coated with either of these fusions showed only moderate binding to HEp-2 cells compared with MBP-Int_{EPEC280} and MBP-Inv_{YP280} (Fig. 1). However, in contrast to MBP-Inv_{YP280} and MBP-Inv_{YP740}, neither MBP-Int_{EPEC280} nor MBP-Int_{EPEC680} mediated HEp-2 cell spreading (data not shown).

Characterization of the Cys-937-to-Ser mutation of Int_{EPEC280} with coated Covaspheres. In a previous report, Leong et al. (15) have shown that a 76-amino-acid disulfide loop within the binding domain of Inv is required for Inv-mediated cell binding. As two Cys residues are present in a location similar to that in Inv in the carboxy termini of the different intimins (7, 25), an MBP-Int_{EPEC280CS} fusion protein in which the Cys residue at position 937 was replaced with Ser was constructed and tested for the ability to mediate cell binding. An MBP-Inv_{YP280CS} fusion similar to that described by Leong et al. (15) was prepared and employed as a control. The Cys-to-Ser changes destroyed the cell-binding abilities of both the MBP-Int_{EPEC280} (Fig. 4D) and the control MBP-Inv_{YP280} (data not shown) fusion proteins.

Inhibition of EPEC cell invasion by MBP fusion proteins. Since intimate attachment and cell invasion by EPEC is dependent on Int (19), the ability of the MBP-Int_{EPEC280} fusion protein to inhibit EPEC invasion of epithelial cells was tested. Monolayers of HEp-2 cells were incubated for 1 h at room temperature with different MBP fusion proteins. The cells were then infected with EPEC and incubated at 37°C for 3 h, and the number of intracellular CFU was determined by direct counting. As can be seen in Fig. 6, preincubation of cells with MBP-Int_{EPEC280}, but not preincubation with MBP, MBP-Int_{EPEC280CS}, or MBP-Inv_{YP280}, significantly reduced EPEC recovery (to about 17%) from infected monolayers. These results demonstrate the importance of Cys-937 and the specificity of Int_{EPEC280} binding to eukaryotic cells.

DISCUSSION

The Int and Inv polypeptides play central roles in the interaction of enteropathogenic bacteria with eukaryotic cells. Int is essential for the attachment-effacement lesion induced by

FIG. 3. Binding of different MBP-Int_{EPEC} fusion proteins to HEp-2 cells as determined by FACS analysis. Cells were incubated with 5 µg of each fusion protein in 1 ml of complete medium at 37°C for 45 min. Each bar represents the mean of five measurements of fluorescence intensity, and the thin line represents the standard error of the mean.

FIG. 4. Covaspheres coated with either MBP-Int_{EPEC150} (A), MBP-Int_{EPEC280} (B), or MBP-Inv_{YP280} (C) were incubated with HEp-2 monolayers for 3 h and visualized by fluorescence microscopy. Similar patterns of bound particles were observed for these fusion proteins. No binding was observed when the cells were incubated with MBP-Int_{EPEC280CS} (D). Magnification, $\times 100$.

EPEC in vitro and in vivo, for cell invasion in vitro, and for the full expression of EPEC virulence in volunteers (1, 2). An EHEC *eae* mutant strain was found to be deficient in the induction of actin accumulation in HEp-2 cells and was incapable of attaching intimately to colonic epithelial cells in a newborn piglet model of infection (3). *Yersinia* Inv is one of the best-characterized bacterial proteins that mediate the interaction of microbial pathogens with mammalian cells. A unique feature of Inv is its ability to confer a high-level invasion phenotype on noninvasive *E. coli* HB101 without the need for cooperation from other specialized bacterial accessory proteins (10). An extensive investigation of Inv led to the identification of integrins as a cellular receptor (9).

eaeA and *inv* are known to be members of a family of related genes in pathogenic bacteria. The genes products, which are outer membrane proteins similar in size, share a high degree of homology in their amino-terminal domains and contain two Cys residues in similar locations in their more diverse carboxy-terminal binding domains (7, 25). In this study, we investigated the molecular basis of binding mediated by the carboxy-terminal domain of Int_{EPEC}. We found that the smallest MBP-Int_{EPEC} fusion protein that displayed cell-binding activity consisted of 150 amino acids of the carboxy terminus of Int. Deletion of 30 amino acids from either side of the 150-amino-acid domain rendered the molecule inactive. In contrast, the smallest Inv fragment that was able to mediate cell binding consisted of 192 amino acids of the carboxy terminus (14). However, since the shortest reported fragment of the Inv car-

boxy-terminal domain tested for invasive activity contained 136 amino acids (14), it is possible that a fragment longer than this, but smaller than Inv_{YP192}, could mediate cell binding. We also demonstrated that Cys-937 is required for the biological activity of Int, as MBP-Int_{EPEC280CS} was no longer capable of mediating HEp-2 cell binding by coated Covaspheres and inhibiting EPEC invasion of HEp-2 cells. It has been suggested that the 76-amino-acid disulfide loop of Inv presents a putative recognition peptide in a particular conformation and that destruction of the disulfide bridge changes the strict conformation required for binding (15). It is still not clear whether a disulfide loop is formed between the two Cys residues at the carboxy terminus of Int_{EPEC} and whether such a loop has a structural function similar to that of the loop in Inv. Further studies are planned to investigate this possibility.

The kinetics of Int_{EPEC280}- and Inv_{YP280}-mediated Covasphere cell binding were similar, showing a lag of about 1 h before clusters of bound particles were observed. The lag in the binding of coated particles, compared with binding of free fusion proteins, which reached saturation within 15 min, may reflect the need for occupation of multiple binding sites and recruitment of additional receptors to stabilize the association of the Covaspheres with the cell membrane. This hypothesis is supported by the fact that binding to fixed cells and binding at 4°C were markedly inhibited (results not shown). No accumulation of actin around bound Covaspheres was observed. This result is in agreement with previous reports that showed that intimate attachment of EPEC to eukaryotic cells and induction

FIG. 5. Scanning electron microscopy of HEp-2 cells grown on coverslips and incubated for 3 h with MBP (A) or MBP-Int_{EPEC280} (B). Covaspheres coated with MBP-Int_{EPEC280} bound predominantly to cell microvilli. Covaspheres coated with MBP-Int_{EPEC280CS} generated a cell-binding pattern similar to that of MBP (not shown). Bars, 5 μ m.

FIG. 6. Invasion of HEp-2 cells by EPEC in the presence of MBP fusion proteins. One hour prior to the addition of bacteria to the monolayers, the epithelial cells were incubated with either column buffer (C) or different MBP fusion proteins, as indicated on the left. Relative percent invasion was determined as recovery in the presence of an inhibitor divided by recovery in the presence of column buffer (i.e., 100% invasion). Results are presented as the mean (% relative invasion) of three different experiments, each conducted in duplicate, $\pm$ the standard error of the mean. MBP-Int_{EPEC280} is the only fusion protein that significantly reduced recovery from infected cells.

of actin reorganization are parts of a process involving, in addition to Int, other bacterial gene products (i.e., EaeB; 4).

The amino acid sequence homology, the apparent structural similarities between Int and Inv, and the similar kinetics and patterns of binding (including involvement of cell microvilli) of Int- and Inv-coated Covaspheres to HEp-2 cells suggest that like Inv, Int may recognize an integrin-like receptor. However, MBP-Inv_{YP280} facilitates HEp-2 cell spreading on coated surfaces, a phenomenon characteristic of integrin ligands, while MBP-Int_{EPEC280} does not, under the assay conditions employed (7). Larger Inv carboxy-terminal fragments have greater affinity for integrins than does Inv_{YP192} (23). In this study, we generated an MBP-Int_{EPEC680} fusion protein to test the possibility that further upstream sequences might be required to reveal any cell-spreading activity associated with Int. However, MBP-Int_{EPEC680}-coated surfaces did not induce HEp-2 cells to spread. The reason for the less efficient Covasphere binding to HEp-2 cells mediated by both MBP-Inv_{YP740} and MBP-Int_{EPEC680} is not clear, although steric hindrance caused by the larger fusion proteins is a possibility. Previously, we showed that Inv, but not Int, inhibits cell spreading on Inv-coated surfaces (7). Here we show that Int, but not Inv, is capable of inhibiting EPEC invasion of HEp-2 cells. These results do not support the hypothesis that intimate attachment of EPEC to cells is mediated by interaction with the same receptor as Inv (i.e., integrins). Nevertheless, since the cellular receptors for Int have not been identified, the possibility of involvement of integrins in Int-mediated cell binding cannot be excluded.

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