Purification of IpaC, a protein involved in entry of *Shigella flexneri* into epithelial cells and characterization of its interaction with lipid membranes

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Abstract **Entry of *Shigella flexneri* into epithelial cells and lysis of the phagosome involve the secreted IpaA–D proteins. A complex containing IpaC and IpaB is able to promote uptake of inert particles by epithelial cells. This suggested that Ipa proteins, either individually or as a complex, might interact with the cell membrane. We have purified IpaC and demonstrated its interaction with lipid vesicles. This interaction is modulated by the pH, which might be relevant to the dual role of Ipa proteins, in induction of membrane ruffles upon entry and lysis of the endosome membrane thereafter.**

Key words: Invasion; Membrane interaction; pH dependence

1. Introduction

Bacteria of the genus *Shigella* are the causative agents of shigellosis, or bacillary dysentery, an infection confined to humans and primates. The early step of infection is the invasion of epithelial cells of the colon. The mechanism of entry of *Shigella* into cells has been studied extensively on cultured cell lines. Contact of bacteria with the cell surface induces massive rearrangements of the cytoskeleton and local ruffles through which bacteria are internalized. Within a few minutes after entry, *Shigella* lyse the membrane of the endosome and gain access to the cytoplasmic compartment. Actin polymerization and bundling of actin filaments at one pole of dividing bacteria allow *Shigella* to occupy the entire cytoplasm of the infected cell and to disseminate from cell to cell (for a review, see [1]).

Genes required for entry and dissemination are carried by a 200-kb virulence plasmid. A 30-kb fragment of this plasmid is necessary and sufficient for entry into cultured cells [2]. This fragment is composed of two regions: one of which encodes secreted proteins, the IpaA–D proteins, and the other their dedicated secretion apparatus, the Mxi-Spa translocon [3–6]. Although *ipa*, *mxi*, and *spa* genes are expressed by bacteria growing in laboratory media, only a small proportion of Ipa proteins is actually secreted by the wild-type strain under these growth conditions. In contrast, secretion of presynthesized Ipa proteins is activated upon contact of bacteria with epithelial cells, which probably allows *Shigella* to deliver a high concentration of effector molecules in the vicinity of the target cell [7].

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Characterization of *ipaA*, *ipaB*, *ipaC* and *ipaD* mutants indicated that each of the Ipa proteins, except IpaA, was required for entry into epithelial cells. Within the bacterial cytoplasm, IpaB and IpaC are independently bound to the same molecular chaperone, IpgC, which is encoded by the *ipa* operon [8]. In contrast, a protein complex containing IpaB, IpaC, probably IpaA, but not IpaD has been detected in the extracellular medium [8]. Latex beads coated with anti-IpaC antibodies have been used to recover this complex and were shown to be internalized by HeLa cells through formation of membrane ruffles similar to those induced upon entry of *Shigella* [9]. These results, i.e. the ability of the *ipaA* mutant to enter into epithelial cells and the absence of IpaD in the extracellular complex which was able to promote internalization of inert particles, have led to the proposal that IpaB and IpaC play a major role in entry of *Shigella* into epithelial cells [9]. However, little is known concerning the stoichiometry of Ipa proteins within the extracellular complex and their interactions with the cell surface.

Association of bacterial toxins in a complex is a feature characteristic of the so-called 'A–B' type toxins. The A and B moieties of diphtheria toxin are held together by a disulfide bridge whereas those of anthrax toxins exist as independent entities in the extracellular medium. In these toxins, the B moiety binds to cell surface receptors, interacts with a cellular membrane (either the plasma membrane or the membrane of the endosome), and mediates the interaction of the A moiety with the target membrane. Information on the molecular mechanism of interaction of these toxins with cellular membrane has been obtained by characterizing the ability of both the individual components and the complex to interact with lipid membranes. In vitro studies have shown that the A fragment of diphtheria and anthrax toxins may play an active role in protein translocation [10,11].

The *ipaB*, *ipaC*, and *ipaD* mutants are not only unable to enter into epithelial cells [12], but also to lyse the membrane of the phagocytic vacuole when they are internalized by macrophages [13] and to induce hemolysis when they are in close contact with red blood cells [12]. This suggested that the Ipa proteins, either independently or as a complex, might interact with and destabilize the cell membrane. Therefore, to investigate the possible interaction of Ipa proteins with the cell membrane, we have undertaken the purification of each of these and the study of their interaction with lipid membranes in vitro. Here, we report the purification of IpaC and the characterization of its ability to induce the release of a fluorescent probe encapsulated into large unilamel-

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lar vesicles. We present evidence that IpaC is able to interact with lipid vesicles and that this interaction is dependent on the pH of the vesicle suspension and on the presence of negatively charged lipids in the membrane of vesicles.

2. Material and methods

2.1. Bacterial strains and growth media

The *S. flexneri* strains used in this study are derivatives of the wild-type strain M90T (serotype 5). Strain SF620 carries a non-polar mutation in the *ipaB* gene [12]. The *sepA* gene of SF620 was inactivated by integration of the suicide plasmid pZK12 as previously described [14]. The *ipaB sepA* mutant thus constructed was designated SF6200. Bacteria were grown in tryptic soy broth (TCS) containing kanamycin (50 μg/ml).

2.2. Materials

Asolectin (mixed soybean phospholipids), L-α-phosphatidylcholine from egg yolk (egg PC), L-α-phosphatidylserine from bovine brain (PS) and calcein were obtained from Sigma (St. Louis, MO). Calcein was purified by gel filtration chromatography on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) as previously described [15]. Asolectin was purified according to [16]. The mono-Q column as well as the G50 resin used for IpaC purification were purchased from Pharmacia (Uppsala, Sweden). The monoclonal antibody J22 directed against IpaC was a kind gift of Dr. Armelle Phalipon (Institut Pasteur, Paris, France). Urea, Tris, and NaCl were of HPLC grade. All other reagents were of analytical grade.

2.3. IpaC purification

Strain SF6200 was grown at 37°C for 7 h with agitation and bacteria were harvested by centrifugation at 10 000 × *g* for 30 min (Sorvall RC26-PLUS). The culture supernatant was filtered through 0.22 μm filters and proteins were precipitated by addition of ammonium sulfate 45% (w/v). After incubation at 4°C for 16 h, the sample was centrifuged at 10 000 × *g* for 30 min and the pellet was resuspended in a buffer containing 4 M urea and 5 mM Tris, pH 9.0. The excess of ammonium sulfate was removed by chromatography over a G50 column (40 cm length, 2.6 cm diameter) using the same buffer. The protein sample was then loaded on a Mono-Q column (anion exchange) equilibrated with the Tris-urea buffer, pH 9.0, and was eluted by an NaCl 0–15% gradient (with buffer A containing 4 M urea, 5 mM Tris, pH 9.0, and buffer B containing 4 M urea, 1 M NaCl, 5 mM Tris, pH 9.0) using a flow of 1 ml/min. Fractions of the chromatography were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) according to [17] and either stained with Coomassie Blue or transferred onto a nitrocellulose membrane and probed with the anti-IpaC J22 mAb [18]. Purity of the protein was assessed by sequencing the band corresponding to IpaC after transfer onto a polyvinylidenefluoride (PVDF) membrane. The protein N-terminal sequence was determined on the membrane by automated Edman degradation using a Beckman LF 3400 D protein microsequencer (Dr. R. Wattiez, Université de l'Etat à Mons, Belgium).

2.4. Fluorescence measurements

Fluorescence spectra were recorded on a SLM 8000 fluorimeter operating in the ratio mode with excitation and emission slits of 4 nm. An internal correction was made for changes in lamp intensity by using a reference solution of 3 mg/ml of rhodamine B in ethylene glycol. During measurement, samples were under constant magnetic stirring and the temperature within the 1 cm path-length quartz cuvette was maintained at 37 ± 0.5°C.

2.5. Calcein release assay

Large unilamellar vesicles (LUV) containing 90 mM calcein were prepared in 10 mM Hepes, pH 7.2, according to the procedure of [19] by using an extruder (Lipex Biomembranes Inc., Vancouver, Canada). The non-encapsulated dye was removed from the liposome suspension by chromatography on a Sephadex G-50 column equilibrated with 10 mM Hepes, 150 mM NaCl, and 1 mM EDTA, pH 7.2. The liposome concentration was determined by measuring the lipid phosphorus content as previously described [20]. The osmolarity of the solution (calcein and buffer) was measured with a Roebling osmometer and fixed to 310 ± 5 mosmol/kg of water.

Fluorescence of calcein, which is entrapped in unilamellar vesicles at a self-quenching concentration, is increased after release of calcein from liposomes. The IpaC-induced release of calcein was recorded by measuring the fluorescence of the liposome suspension at excitation and emission wavelengths of 490 and 520 nm, respectively. Complete release of the dye was obtained by lysing the LUV with Triton X-100 at a final concentration of 0.1%. The percentage of fluorescence $F(t)$ at time t is defined as:

$$\%F(t) = \frac{I(t) - I_0}{I_f - I_0} \times 100$$

where I_0 is the initial fluorescence obtained after dilution of the vesicles in the appropriate buffer, I_f is the total fluorescence observed after addition of Triton X-100 and $I(t)$ is the fluorescence at time t corrected for the dilution. An aliquot of the LUV suspension (between 5 and 12.5 μl) containing the dye (pH 7.2) was added to the fluorescent cuvette containing the buffer at the chosen pH (total volume of 1 ml). After pH and temperature equilibration, the protein was added to the suspension and the fluorescence was immediately measured. Buffers used for the different pH were as follows: 10 mM Hepes, 150 mM NaCl, 1 mM EDTA, pH 7.2; 10 mM Mes, 150 mM NaCl, 1 mM EDTA, pH 6.5–6.0; and 10 mM acetate, 150 mM NaCl, 1 mM EDTA, pH 5.0–5.4.

3. Results

3.1. Purification of IpaC

IpaC was purified from the culture medium of the *S. flexneri* strain SF6200 in which both the *ipaB* and *sepA* genes have been inactivated. This strain was chosen for the following reasons: (1) during growth in laboratory media, an *ipaB* mutant secretes IpaC in greater amounts as compared to the wild-type strain, in which Ipa secretion is regulated by contact with the target cell; (2) part of IpaB and IpaC are present in a complex in the extracellular medium of the wild-type strain. Purification of IpaC from a strain devoid of IpaB eliminated the possibility of contamination with IpaB; and (3) in preliminary attempts to purify IpaC from the culture medium of the *ipaB* mutant SF620, we observed that SepA, a major extracellular protein of *S. flexneri* [14], was eluted from the anion exchange chromatography column at the same salt concentration as IpaC and was the only contaminating protein found in fractions containing IpaC (data not shown). This led us to construct strain SF6200 by inactivating the *sepA* gene in the *iapB* mutant SF620 (see Section 2).

Starting from the culture medium of strain SF6200, the main steps in purification of IpaC were an ammonium sulfate precipitation followed by an anion exchange chromatography using a Tris buffer, pH 9.0, containing 4 M urea. The pH of the buffer was chosen by taking into account the high isoelectric point of IpaC (pI = 8.96). In the absence of urea, IpaC was found in many fractions (data not shown), probably because of non-specific interactions with other secreted proteins, which greatly reduced the yield of purification of IpaC. In the presence of 4 M urea, IpaC was eluted from the anion exchange chromatography at a salt concentration of 140 mM NaCl (Fig. 1), as indicated by SDS-PAGE analysis of fractions of the chromatogram (see inset in Fig. 1). The 40-kDa protein present in fraction 22 was identified as the IpaC protein (41 kDa) on the following criteria: after electrophoresis and transfer onto a membrane, this protein was strongly recognized by the J22 monoclonal antibody directed against IpaC [18] (data not shown), and its N-terminal sequence was determined as Met–Glu–Ile–Gln–Asn–Thr–Lys–Pro–

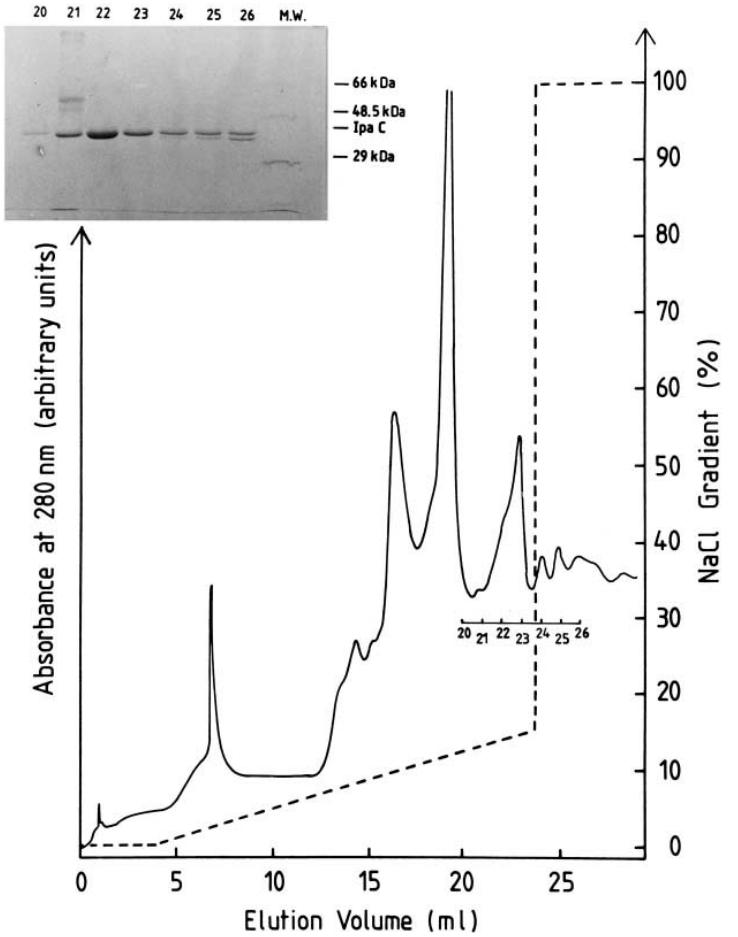

Fig. 1. Purification of IpaC. The elution profile of a representative run on the Mono-Q column is shown, with the absorbance at 280 nm indicated by a continuous line and the salt gradient profile indicated by a dashed line (100% = 1 M NaCl). The SDS-PAGE analysis of fractions of the chromatogram containing IpaC is shown in the inset. Numbers of fractions are indicated on the chromatogram.

Thr–Gln–Thr–Leu–Tyr–Thr–Asp, which corresponds to that of IpaC. No other sequence was detectable in that band, which, in addition to the analysis of that fraction by SDS-PAGE and Coomassie Blue staining, indicated that IpaC has been purified with a degree of purity to over 90%. Moreover, no degradation seemed to occur during purification.

3.2. *Interaction of IpaC with the lipid membrane as a function of pH*

To investigate the possible interaction of IpaC with a lipid membrane, we studied the ability of the purified protein to induce the release of a fluorescent probe encapsulated into large unilamellar vesicles. Since interaction of some bacterial toxins with a lipid membrane is strongly pH-dependent, we have investigated the effect of pH on the release of calcein induced by IpaC.

The kinetics of calcein release after addition of IpaC (19.5 nM) to a suspension of asolectin vesicles (5 μM) at pH values ranging from 7.2 to 5.0 are shown in Fig. 2. Under these assay conditions, the lipid/protein molar ratio was 256 and the protein/vesicle ratio was 324. The concentration of urea in the assay never exceeded 64 mM, a concentration which had no effect on stability of vesicles, regardless of the pH of the suspension (data not shown). For all the pH values tested, IpaC induced a rapid release of calcein. In addition, the efficiency of release was modulated by the pH, a maximal release being obtained at pH 5.4 (see inset in Fig. 2).

A characteristic of some bacterial toxins, such as diphtheria toxin, is that they aggregate upon incubation in conditions which promote their interaction with the lipid membrane. This aggregation leads to a decreased ability of the protein to destabilize the lipid vesicles [15]. To test this property, IpaC was diluted in order to reach a final concentration of 19.5 nM in buffers which did not contain urea and incubated for 1 h at 37°C prior to addition of calcein-loaded vesicles and monitoring of calcein release. As shown in Fig. 2 (curves A and E), preincubation of IpaC at a low concentration of urea at either pH 5.0 or pH 7.2 decreased the ability of the protein to induce calcein release, although the effect was less marked at pH 7.2 than at pH 5.0. This suggested that, in the absence of urea, IpaC was partially aggregated and could no longer interact with the lipid membrane.

3.3. *Interaction of IpaC with the lipid membrane as a function of lipid composition*

Membranes of eucaryotic cells contain about 20% of negatively charged lipids [21], which are also present in the same proportion in asolectin [15]. To investigate the role of charged lipids in interaction of IpaC with the lipid membrane, we used vesicles containing various amounts of egg phosphatidylcho-

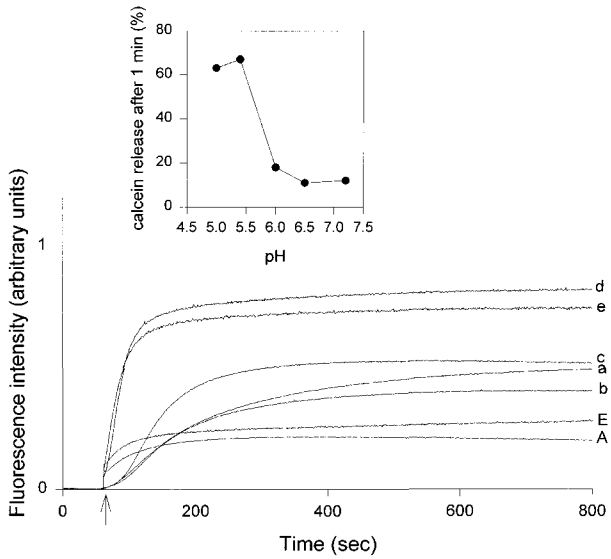

Fig. 2. Effect of pH on the release of calcein induced by IpaC. Curves a–e show kinetics of calcein release from asolectin LUV (5 μM) induced by IpaC (19.5 nM) at different pH: (a) pH 7.2; (b) pH 6.5; (c) pH 6.0; (d) pH 5.4; (e) pH 5.0. The arrow indicates the addition of the protein. For each pH value, the percentage of calcein release obtained after 1 min, calculated as described in Section 2, is shown in the inset. Curves A and E show kinetics of calcein release from asolectin LUV (5 μM) induced by IpaC (19.5 nM) which had been preincubated for 1 h at a low concentration of urea at pH 7.2 (A) and 5.0 (E) prior to the addition of calcein-loaded vesicles. On these two curves, the arrow indicates the addition of vesicles. The fluorescence due to vesicles was subtracted from the spectra. In all cases, the kinetics were rescaled between 0 (F_0) and 1 (F_{100}).

line (egg PC), a neutral lipid, and phosphatidylserine (PS), a negatively charged lipid. Fig. 3 shows that, at pH 5.0, the release of calcein is dependent on the concentration of negatively charged lipid in the membrane, virtually no calcein release being observed with the neutral vesicles (Fig. 3) even at concentrations of IpaC up to 146 nM (data not shown). A similar effect was observed at pH 7.2 (data not shown), suggesting that, at both pH, an electrostatic component was involved in the interaction of IpaC with a lipid membrane.

3.4. Determination of an apparent binding constant of IpaC to asolectin vesicles

To determine an apparent binding constant of IpaC to asolectin vesicles, calcein release was studied as a function of IpaC concentration, for four different lipid concentrations (5, 12.5, 25 and 37.5 μM) at both pH 7.2 (Fig. 4A) and pH 5.0 (Fig. 4B). As the lipid concentration was increased, a higher concentration of IpaC was required to release a determined amount of calcein, which suggested that the leakage rate was determined by the amount of membrane-bound protein per lipid molecule. This quantity, r, is related to such experimental parameters as the total protein concentration, C_t, the free protein concentration, C_f, and the lipid concentration, L, through the equation: $C_t = C_f + rL$ (for a more detailed description of this formalism, see [22]). Plots of C_t versus L for different extents of release (between 15 and 50%) were obtained (not shown) from a plot of the percentage of calcein release versus C_t (Fig. 4A–B) at different L values. According to the mass equation, the corresponding r and C_f values were calculated from the slope and the intercept, respectively. The relationships between r and C_f (binding isotherm) are shown in Fig. 5. An apparent binding constant was evaluated by linearly extrapolating the curve to the zero concentration of free protein [23], which gave a K_{app} of 34×10^3 M^{-1} at pH 7.2 and of 410×10^3 M^{-1} at pH 5.0.

4. Discussion

Analysis of strains carrying non-polar mutations in *ipaA*, *ipaB*, *ipaC*, and *ipaD* has indicated that each of the Ipa proteins, but IpaA, was required to induce entry into epithelial [12], and also to lyse the membrane of the phagocytic vacuole when these strains were internalized by macrophages [13]. Moreover, a complex containing IpaB, IpaC, and IpaA, but not IpaD, has been detected in the culture medium of *Shigella* [8] and it has been demonstrated recently that this complex was able to promote uptake of inert particles by epithelial cells [9]. These data suggested that IpaB and IpaC proteins, either individually or as a complex, might interact with the cell membrane. Here, we have reported the purification to homogeneity of IpaC and the characterization of the interaction of the purified protein with lipid membranes as a function of pH and lipid composition of the membrane.

IpaC has been purified from the culture medium of an *ipaB sepA* mutant, which rules out any possible contamination by IpaB. The purification scheme involves only an ammonium sulfate precipitation and a single chromatography step (anion exchange). The purification had to be carried out in the presence of urea, since, in the absence of urea, IpaC was most probably aggregated. Indeed, preincubation of IpaC in a buffer containing only a low concentration of urea reduced the ability of the protein to induce calcein release. Some virulence factors known for their ability to interact with a lipid membrane, such as the *Escherichia coli* hemolysin, require a concentration of up to 6 M urea to be maintained in solution [24].

The ability of diphtheria (DT) and anthrax (PA, LF, and EF) toxins to interact with the cell membrane and to reach the cell cytoplasm is pH-dependent [25–27], a phenomenon which has also been observed for interaction of these toxins with artificial lipid membranes [11,15]. The amount of IpaC required to induce 50% release of calcein from asolectin large

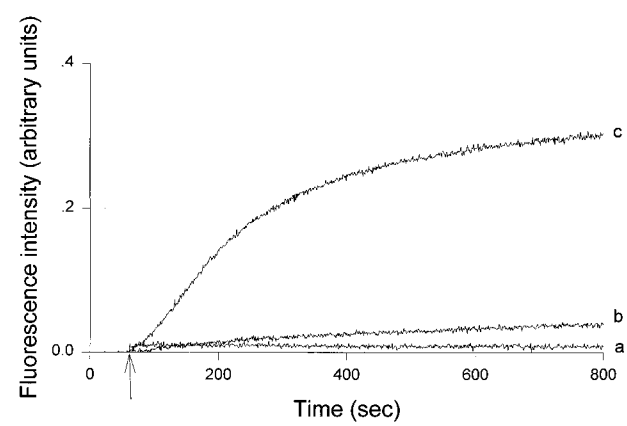

Fig. 3. Effect of negatively charged lipids on the release of calcein induced by IpaC. Kinetics of calcein release from LUV made of egg PC and of mixtures of egg PC and PS are shown: (a) egg PC; (b) egg PC/PS (9:1, w/w); (c) egg PC/PS (8:2, w/w). The arrow indicates the addition of the protein. Kinetics were rescaled between 0 (F_0) and 1 (F_{100}).

unilamellar vesicles at low pH is in the same range as that of these toxins (LF and EF: 3.5 nM [11] and DT B fragment: 250 nM [15]) which have been demonstrated to insert into and to translocate across a cell membrane. These comparisons strongly suggest that IpaC might interact with the cell membrane.

We have determined apparent binding constants of IpaC to lipid vesicles at neutral and low pH. However, these values were obtained by measuring the efficiency of IpaC to destabilize a lipid membrane. Therefore, the 10 times difference observed between the two pH values could result from two different and non-exclusive mechanisms, either an increase of the amount of protein bound to the lipids or an increase of the efficiency of the bound protein in destabilizing the membrane. A pH-driven multimerization process, for example, would be a way to increase the efficiency of calcein release without any increase of the amount of bound protein. Nonetheless, the different behaviours we are observing at neutral and low pH might be relevant to the dual role of Ipa proteins in inducing membrane ruffles upon entry and lysis of the endosome membrane thereafter. When secretion is activated close to the eukaryotic cell membrane, the resulting high local concentration of Ipa proteins might be sufficient to allow a certain proportion of IpaC, or of a complex containing IpaC, to interact with the membrane. This would be sufficient to induce entry.

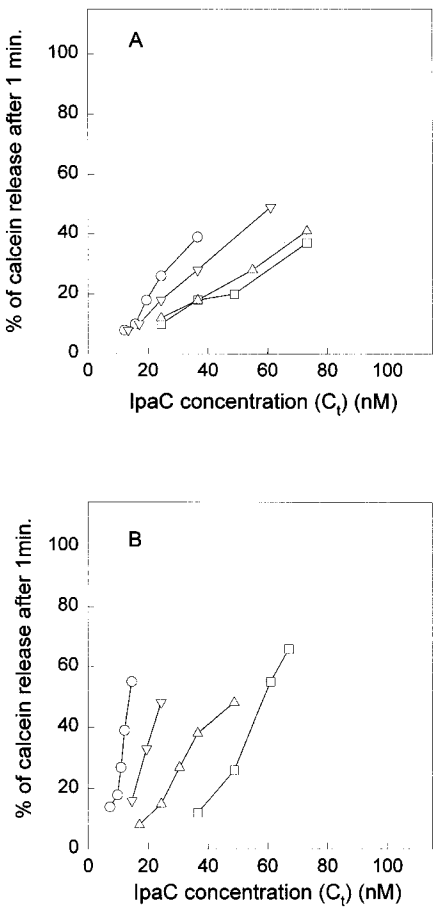

Fig. 4. Effect of IpaC and lipid concentrations on the release of calcein. Percentages of calcein released from asolectin LUV were determined at various concentrations of IpaC and lipids at pH 7.2 (A) and pH 5.0 (B). Lipid concentration: ○, 5 μM; ▽, 12.5 μM; △, 25.0 μM; □, 37.5 μM.

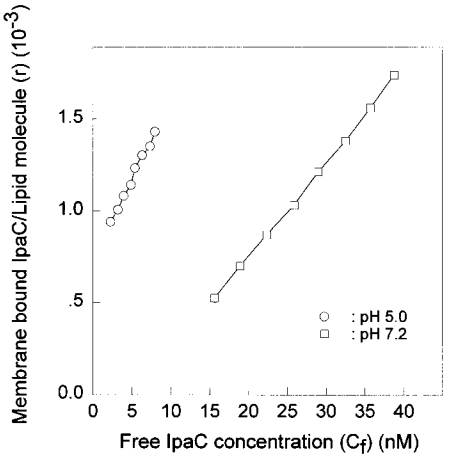

Fig. 5. Evaluation of the apparent binding constant of IpaC to asolectin LUV. Binding isotherms, calculated from Fig. 4 as described in the text, are shown for two values: □, pH 7.2; ○, pH 5.0.

Once the bacterium has been internalized within a vacuole, an increase in the amount of membrane-bound Ipa proteins or an increased efficiency of the interaction of these proteins with the membrane might be responsible for the disruption of the endosome membrane. The pH of the *Shigella*-induced vacuole is not known, but it has been proposed recently that the pH of the vacuole in which *Salmonella* reside after entry into epithelial cells is acidic [28]. This suggests that the pH effect we observed for binding of IpaC to lipids might be a physiologically related process.

Interaction of IpaC with the lipid membrane requires the presence of negatively charged lipids in the membrane. Indeed, calcein release was not observed from egg PC vesicles even at a high concentration of IpaC (146 nM), and addition of negatively charged lipids in the membrane of egg PC vesicles allowed us to show that the IpaC-induced calcein release was dependent on the concentration of negatively charged lipids in the membrane. The effect of acidic lipids on the activity of IpaC could be due to a direct interaction of IpaC with the negative charges at the surface of the vesicles or to a lower pH at the surface of a membrane containing negatively charged lipids. We can rule out the latter hypothesis since asolectin vesicles contain about 20% of negatively charged lipids which generate a surface pH that would be about 0.5 unit lower than the pH of the suspension [15]. If solely the surface pH was involved in the interaction of IpaC with the lipid membrane, a similar efficiency would be observed for binding of the protein to asolectin and egg PC vesicles, except that the two curves would be shifted of a value of 0.5–1 pH unit, as previously observed with diphtheria toxin [15]. Therefore, the dependence of IpaC-induced calcein release on the presence of negatively charged lipids suggests that negative charges are essential for the protein either to bind at the vesicle surface or to induce a conformational change which would allow insertion of the protein in the lipid membrane. Characterization of secondary and tertiary structures of IpaC as a function of the pH and the presence of negatively charged vesicles is under investigation using Fourier transform infrared spectroscopy.

Interaction of several virulence factors with the cell membrane results in the formation of a channel in the membrane. In the case of diphtheria toxin, this channel is involved in

translocation of the enzymatic moiety of the toxin (see [10], for a review), whereas, in the case of the *E. coli* hemolysin, the channel allows the transport of ions [29], which leads to an interference with the cell metabolism and/or to lysis of the cell (see [30], for a review). Although we have shown that IpaC was able to interact with a lipid membrane, preliminary experiments failed to demonstrate that IpaC was forming a channel in a planar lipid bilayer (Dr. F. Homblé, personal communication), which suggests that interaction of IpaC with the lipid membrane of LUV does not result in channel formation. This does not exclude the possibility that a channel is formed upon interaction of *Shigella* with the cell surface, since other Ipa proteins, such as IpaB, might participate in the formation of such a structure. Understanding the molecular mechanism of action of the *Shigella* effectors of entry will require purification of each of the other Ipa proteins, as well as that of a well defined IpaC–IpaB complex, and characterization of their interaction with the lipid membrane.

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References

[1] Parsot, C. and Sansonetti, P.J. (1995) Curr. Topics Microbiol. Immunol. 209, 25–42.
[2] Maurelli, A.T., Baudry, B., d'Hauteville, H., Hale, T.L. and Sansonetti, P.J. (1985) Infect. Immun. 49, 164–171.
[3] Andrews, G.P., Hromockyj, A.E., Coker, C. and Maurelli, A.T. (1991) Infect. Immun. 59, 1997–2005.
[4] Allaoui, A., Sansonetti, P.J. and Parsot, C. (1992) J. Bacteriol. 174, 7661–7669.
[5] Allaoui, A., Sansonetti, P.J. and Parsot, C. (1993) Mol. Microbiol. 7, 59–68.
[6] Sasakawa, C., Komatsu, K., Tobe, T., Suzuki, T. and Yoshikawa, M. (1993) J. Bacteriol. 175, 2334–2346.
[7] Ménard, R., Sansonetti, P.J. and Parsot, C. (1994a) EMBO J. 13, 5293–5302.
[8] Ménard, R., Sansonetti, P.J., Parsot, C. and Vasselon, T. (1994b) Cell 79, 515–525.
[9] Ménard, R., Prévost, M.-C., Gounon, P., Sansonetti, P. and Dehio, C. (1996) Proc. Natl. Acad. Sci. USA 93, 1254–1258.
[10] London, E. (1992) Biochim. Biophys. Acta 1113, 25–51.
[11] Kochi, S.K., Martin, I., Schiavo, G., Mock, M. and Cabiaux, V. (1994) Biochemistry 33, 2604–2609.
[12] Ménard, R., Sansonetti, P.J. and Parsot, C. (1993) J. Bacteriol. 175, 5899–5906.
[13] Zychlinksky, A., Kenny, B., Ménard, R., Prévost, M.-C., Holland, B. and Sansonetti, P.J. (1994) Mol. Microbiol. 11, 619–627.
[14] Benjelloun-Touimi, Z., Sansonetti, P. and Parsot, C. (1995) Mol. Microbiol. 17, 123–135.
[15] Defrise-Quertain, F., Cabiaux, V., Vandenbranden, M., Wattiez, R., Falmagne, P. and Ruysschaert, J.M. (1989) Biochemistry 28, 3406–3412.
[16] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Phalipon, A., Arondel, J., Nato, F., Rouyre, S., Mazie, J.C. and Sansonetti, P.J. (1992) Infect. Immun. 60, 1919–1926.
[19] Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
[20] Mrsny, R.J., Volwerk, J.J. and Griffith, O.H. (1986) Chem. Phys. Lipids 39, 185–191.
[21] Urade, R., Hayashi, Y. and Kito, M. (1988) Biochim. Biophys. Acta 946, 151–163.
[22] Matsuzaki, K., Harada, M., Funakoshi, S., Fujii, N. and Miyajima, K. (1991) Biochim. Biophys. Acta 1063, 162–170.
[23] Matsuzaki, K., Nakai, S., Handa, T., Takaishi, Y., Fujita, T. and Miyajima, K. (1989) Biochemistry 28, 9392–9398.
[24] Ostolaza, H., Bartolomé, B., Serra, J.L., de la Cruz, F. and Goni, F.M. (1991) FEBS Lett. 280, 195–198.
[25] Sandvig, K. and Olsnes, S. (1980) J. Cell. Biol. 87, 828–832.
[26] Friedlander, A.M. (1986) J. Biol. Chem. 261, 7123–7126.
[27] Gordon, V.M., Leppla, S. and Hewlett, E. (1988) Infect. Immun. 56, 1066–1069.
[28] Rathman, M., Sjaastad, M.D. and Falkow, S. (1996) Infect. Immun. in press.
[29] Bhakdi, S.N., Mackman, N., Nicaud, J.M. and Holland, I.B. (1986) Infect. Immun. 52, 63–69.
[30] Welch, R.A. (1991) Mol. Microbiol. 5, 521–528.