INFECTION AND IMMUNITY, July 1987, p. 1564–1572
0019-9567/87/071564-09$02.00/0

Vol. 55, No. 7

Two-Dimensional Gel Electrophoresis and Immunoblotting of *Campylobacter* Outer Membrane Proteins

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Received 1 December 1986/Accepted 4 April 1987

We characterized outer membrane proteins (OMPs) from selected *Campylobacter jejuni*, *C. coli*, and *C. fetus* strains by two-dimensional gel electrophoresis (2DGE), using isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and by immunoblotting with immune rabbit serum. The flagellar band with a molecular mass of 63 kilodaltons (kDa) demonstrated previously by one-dimensional SDS-PAGE was shown by 2DGE to consist of one or several charge trains, depending upon the species, strain, and type of preparation studied; each of the individual peptides was found to be antigenic by immunoblotting. In contrast, in all of the strains studied, the major OMP (43 to 44 kDa) of *C. jejuni* and *C. coli* consisted of a single isomeric form which was weakly immunogenic. Several minor proteins (29 to 31 kDa) were found to be strongly immunogenic by immunoblotting. *C. fetus* strains possessed two major OMPs of 45 to 47 kDa, each of which consisted of either a single isomer or a major isomer comprising at least 90% of the major OMP. Serum-resistant strains of *C. fetus* possessed an acid-labile 100-kDa glycoprotein (pI, 4.1) which was markedly diminished or absent in serum-sensitive strains. These 2DGE analyses provide information that is useful in taxonomic and epidemiologic studies and for the purification of surface antigens for the development of campylobacter vaccines and may also facilitate the identification of specific virulence factors.

Although *Campylobacter jejuni* is an important cause of diarrheal illness in humans throughout the world, and *C. fetus* is a recognized cause of septicemia in immunocompromised hosts (8), the pathogenesis of campylobacter infections is incompletely understood. As with other gram-negative enteric pathogens, the outer membrane of *Campylobacter* species includes lipopolysaccharide and specific proteins, which are exposed on the surface of the bacterium and are antigenic to humans (5–7, 20, 21, 25–27, 30, 31, 37). The outer membrane components of a variety of enteric pathogens play important roles in several virulence functions including complement activation, adherence to and invasion of the gastrointestinal mucosa, and resistance to serum bactericidal activity and phagocytosis (11).

Outer membrane proteins (OMPs) of *Campylobacter* species have recently been studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a method capable of resolving proteins on the basis of differences in relative molecular weight (5–7, 20, 21, 25–27, 37). In contrast to SDS-PAGE, which can resolve no more than 100 protein bands in a given sample (29), separation of proteins by two-dimensional gel electrophoresis (2DGE) is based on differences in both relative molecular weight and isoelectric point and can resolve between 5,000 (14) and 10,000 (2) proteins in a given sample. To determine whether the individual bands seen by SDS-PAGE are composed of multiple protein components with different isoelectric points, we subjected a variety of preparations of *Campylobacter* sp. proteins to 2DGE and immunoblotting.

We found by using 2DGE that the 63-kilodalton (kDa) flagellar protein of all flagellated *Campylobacter* species studied showed multiple components, whereas the major OMP (43 kDa) consisted of a single or predominant isomeric form. That many minor OMP bands recognized by SDS-PAGE were resolved into multiple individual spots or charge trains indicates that the complexity of campylobacter OMPs and antigens is greater than previously recognized.

MATERIALS AND METHODS

Bacterial strains. The *Campylobacter* strains used in this study were from the culture collection of the Denver Veterans Administration Medical Center Campylobacter Laboratory. All *Campylobacter* strains used in this study (Table 1) were identified by using standard criteria (17), passaged, and stored as previously described (5). The hippurate hydrolysis test was used to differentiate between *C. jejuni* and *C. coli* (15). Cultures were grown statically at 37°C for *C. fetus* or 42°C for *C. jejuni* and *C. coli* and incubated in an atmosphere of 5% oxygen–10% carbon dioxide–85% nitrogen for 48 h. Low-passage (LP; serum-resistant) and high-passage (HP; serum-sensitive) variants of *C. fetus* 82-40 were prepared as described previously (10).

Preparation of *Campylobacter* protein preparations. *Campylobacter* sp. cells were fractionated to prepare outer membranes as previously described (5). Cells were sonicated on ice four times for 30 s with 20-s rests by using a Branson Sonifier (model S-75; Branson Sonic Power Co., Danbury, Conn.). Alternatively, cells were disrupted by two passages through a French pressure cell (SLM Aminco, Urbana, Ill.) at 15,000 lb/in^2. The preparation was then centrifuged two times at 5,000 × *g* for 20 min to remove whole cells, and the supernatant was centrifuged for 1 h at 100,000 × *g* at 4°C. The pellet resulting from either procedure was suspended in 3 ml of sterile distilled water, added to 20 ml of 1% sodium

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TABLE 1. *Campylobacter* sp. strains studied by 2DGE

Campylobacter strain	Source
C. jejuni	
83-85 (81116, F^+M^+)	Human feces
84-84 (F^+M^-)	Laboratory derivative of 81116
83-86 (F^-M^-)	Laboratory derivative of 81116
81-93 (PEN 1)	Human feces
81-94 (PEN 2)	Human feces
C. coli	
80-212	Hog feces
80-218	Hog feces
80-222	Hog feces
80-236	Hog feces
C. fetus subsp. *fetus*	
79-22	Human cerebrospinal fluid
80-109	Human blood
81-170 (ATCC 19438)	Bovine vagina
81-173	Human cerebrospinal fluid
81-200	Human feces
82-40 LP	Human blood
82-40 HP	Laboratory derivative of 82-40LP
83-88	Human blood
84-32 (23D)	Bovine placenta
84-54 (23B)	Laboratory derivative of 23D

lauryl sarcosinate (Sarkosyl; CIBA-GEIGY Corp., Summit, N.J.) in 7 mM EDTA for a 20-min incubation at 37°C, and then centrifuged at 100,000 × *g* for 2 h at 4°C. The pellet was suspended in Tris buffer and recentrifuged at 100,000 × *g* for 2 h, and the resultant Sarkosyl-insoluble pellet was suspended in 1.0 ml of sterile distilled water and stored at 4°C. To prepare acid-extractable surface proteins, cells were suspended in 0.2 M glycine-hydrochloride buffer (pH 2.2) for 15 min at 25°C and then centrifuged at 11,000 × *g* for 15 min. The supernatant was centrifuged at 100,000 × *g*, the pellet was suspended, and the pH was neutralized with NaOH as previously described (5, 24, 33).

SDS-PAGE. Preparations of total and outer membrane *C. jejuni* proteins for SDS-PAGE were carried out as previously described (5). Stacking and separating gels consisted of 4.5 and 12% acrylamide (Bio-Rad Laboratories, Richmond, Calif.), respectively, and protein concentrations of preparations were standardized (22). Electrophoresis was carried out with a Protean Dual 16-cm slab cell apparatus (Bio-Rad) with a constant current of 35 mA per gel. Gels were resolved with the modified silver stain of Oakley et al. (28).

2DGE. Samples of known protein content were lyophilized overnight and then solubilized at 100°C for 5 min in 50 μl of 0.05 M Tris buffer (pH 6.5) containing 2% SDS, 1% dithiothreitol, and 10% glycerol. In initial experiments, 2DGE was performed with an ISO-DALT apparatus (Electro-Nucleonics Corp., Oak Ridge, Tenn.); a MegaIso isoelectric-focusing (IEF) apparatus (Integrated Separation Systems, Newton, Mass.) and a Protean II Multi-Cell (Bio-Rad) were used in later experiments. Resolution capability was similar for the two systems. In both systems, 20 IEF tube gels and 10 molecular weight separation slab gels were run simultaneously. IEF gels 16.5 cm in length were poured in tubes 20 cm long by 1.5 mm (inside diameter) and allowed to polymerize for 2 h before use. IEF gel composition was 34 g of acrylamide per liter containing (per liter): 1.8 g of *N,N*-methylenebisacrylamide as a cross-linker, 8 mol of urea, 20 g of total ampholytes (16 g of Bio-Rad Biolyte, pH 5 to 7, and 4 g of Bio-Rad Biolyte, pH 3.5 to 10), and 19 g of Nonidet P-40 detergent. After unpolymerized acrylamide was removed, IEF gels were prefocused at 200 V for 1 h with 2 liters of anolyte (10 mM H_3PO_4) and 200 ml of degassed catholyte (20 mM NaOH), and then 2- to 15-μl samples containing 2 or 40 μg of protein were added to the basic end of the gel. Focusing of the first-dimension IEF gels was done at 700 V for 20 h, and then the IEF gels were unloaded into vials containing 5 ml of Tris-buffered equilibrating solution (pH 6.8) containing 2% SDS, 0.1% dithiothreitol, and 10% glycerol and maintained at room temperature for 10 min on a rocker. Gels were then placed on ice until the last IEF gel was equilibrated. For uniformity, all IEF gels were then frozen at −70°C until the second dimension was run (3 h to 7 days later).

For the second-dimension separation, we used slab gels consisting of an exponential polyacrylamide gradient (90 to

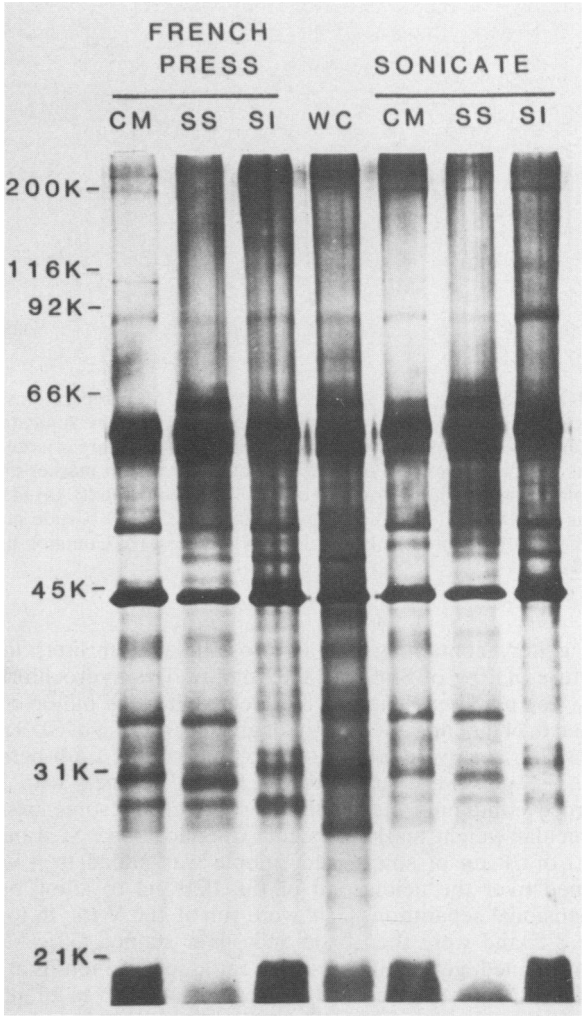

FIG. 1. Silver-stained (28) SDS-PAGE of several preparations of the F^+M^+ strain of *C. jejuni*. Samples were obtained after 24 h of growth on brucella agar incubated at 42°C and represent whole-cell (WC), crude membrane (CM), Sarkosyl-soluble (SS), and Sarkosyl-insoluble (SI) preparations prepared by using a French pressure cell or by sonication. See the text for details. Each lane contained 1 μg of protein, and the gel contained 12% acrylamide. In each preparation, major bands at 44 kDa (Omp 1) and 63 kDa (flagellar protein) were seen with minor bands at 200, 90, 52, and 29 to 31 kDa.

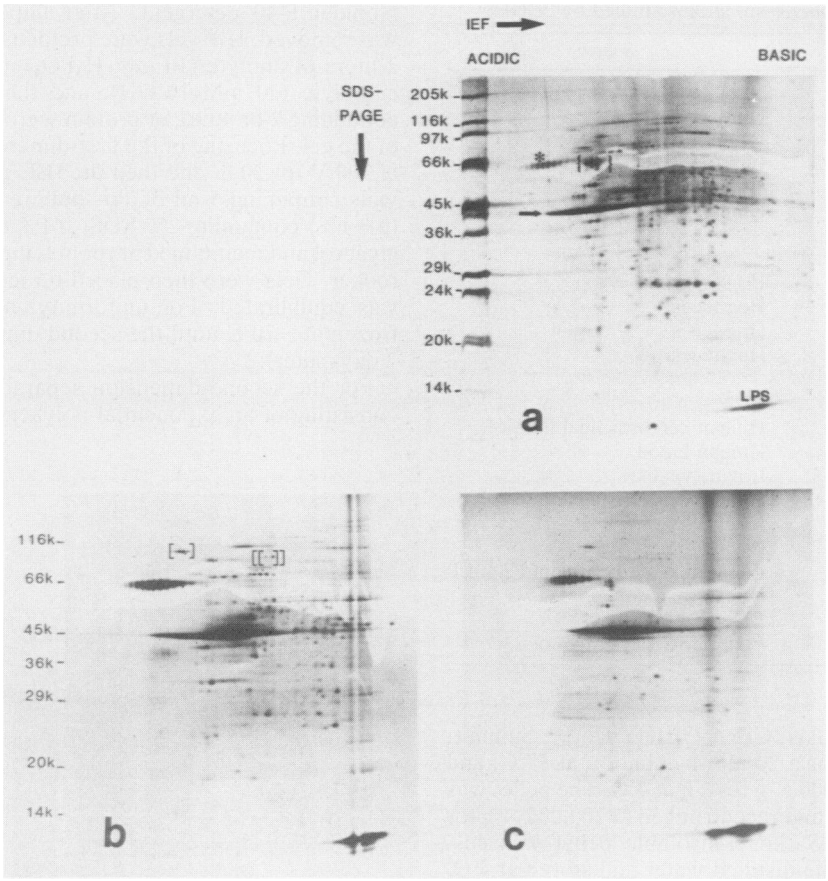

FIG. 2. Silver-stained 2DGE of *C. jejuni* F^+M^+ preparations. Samples were obtained after 24 h of growth on brucella agar at 42°C and represent whole-cell (a), crude membrane (prepared by sonication) (b), and Sarkosyl-insoluble membrane (c) preparations. All 2DGE gels contained 40 μg of protein unless otherwise noted and are oriented with the acidic side of the gel to the left and high-molecular-weight proteins at the top of the figure. Positions of molecular weight marker proteins in kilodaltons (k) are shown at the left. All 2DGE gels contained an exponential acrylamide gradient of 9 to 18% (see the text). (a) Major spots observed in all three preparations included flagellar protein charge trains (*), Omp 1 (→), and lipopolysaccharide (LPS). Crude membranes and Sarkosyl-insoluble membrane proteins lacked three densely staining proteins present in whole cells (braces). (b) Common minor spots included charge trains at 90 kDa (brackets) and 83 kDa (double brackets).

180 g/liter, containing 8 g of cross-linker per liter) in a mixture of 10 g of SDS and 0.37 mol of Tris hydrochloride (pH 8.5) per liter. The pH 8.6 electrophoresis buffer contained 0.24 mol of Tris, 0.2 mol of glycine, and 1 g of SDS per liter. Slab gels were allowed to polymerize for 1.5 h before use. IEF gels were sealed to the top of slab gels with 1% agarose containing electrophoresis buffer. In some cases, molecular weight markers (Sigma Chemical Co., St. Louis, Mo.) or 20 μg of solubilized sample was added to a well formed over the acidic end of the IEF gel to allow one-dimensional separation. Gels were run at 160 V for 16 to 18 h at 8°C and were then fixed and silver stained (33). After being stained, gels could be stored for at least 6 months at an ambient temperature in Seal-A-Meal bags with 5 to 10 ml of 3% acetic acid as preservative.

The pH gradient was measured by the method of Marshall et al. (23). Briefly, IEF gels focused with or without sample were sliced into 1.0-cm segments which were equilibrated overnight at 4°C in sealed polypropylene tubes containing 0.5 ml of boiled deionized water. The tubes were incubated at room temperature for 1 h before the pH was measured with a calibrated Beckman Phi 71 pH meter with a microelectrode (Beckman Instruments, Inc., Fullerton, Calif.).

Immunoblotting. Immunoblotting with immune rabbit serum was performed to assess the antigenicity of proteins observed in silver-stained two-dimensional gels. After the second dimension was completed, cellular components were transferred to sheets of BA-85 nitrocellulose paper (17 by 17 cm; Schleicher & Schuell, Keene, N.H.) either in the DALT tank (35) or in a Transphor apparatus (Hoefer Scientific, San Francisco, Calif.). Transfers were performed at 100 mA for 16 h in 20% methanol containing 25 mM Trizma base and 192 mM glycine; subsequently, gels were allowed to air dry for 48 to 72 h before immunostaining, which was performed as previously described (7). Briefly, after nonspecific binding was blocked with bovine serum albumin (Sigma) in borate buffer, the nitrocellulose paper was incubated at 25°C for 4 h in a 1:100 dilution of the test serum sample in bovine serum albumin-borate buffer. After an additional washing, the nitrocellulose paper was incubated at 25°C for 2 h with a 1:2,000 dilution of horseradish peroxidase-conjugated *Staphylococcus aureus* protein A (Amersham Corp., Arlington Heights, Ill.). The nitrocellulose paper was washed again and then placed in DAB solution (50 mM Tris and 0.025% diaminobenzidine with 2 drops of 3% hydrogen peroxide for optimal development). Antisera had been prepared by using

formalinized *Campylobacter* sp. cells as described previously (7).

RESULTS

SDS-PAGE profiles of French-pressed and sonicated samples. By one-dimensional SDS-PAGE, profiles of whole-cell, crude membrane, Sarkosyl-soluble, and Sarkosyl-insoluble proteins from *C. jejuni* F^+M^+ (flagellated, motile [6]) were not significantly different, regardless of whether the cells were lysed in a French pressure cell or by sonication (Fig. 1). The major band (Omp 1, using the nomenclature of Trust and Logan [36]) migrated at 44 kDa in all preparations. In the Sarkosyl-insoluble (outer-membrane-enriched) preparations, other significant bands were seen at 63 kDa (flagellar protein [6]), 52 kDa, and 29 to 31 kDa. Minor bands in all preparations were seen at 200 and 90 kDa. Variable bands were present at 65, 60, 48, and 35 kDa. The lipopolysaccharide band (30) at the bottom of the gel (below 21 kDa) was enriched in the crude membrane and Sarkosyl-insoluble membrane preparations.

2DGE profiles of sonicated samples. The pH gradient measured in representative IEF gels with the ampholyte composition described above was essentially linear and extended from pH 4.0 to 6.8. The presence of protein in an IEF gel did not significantly affect the measured range or slope of the gradient.

The 2DGE profiles of whole-cell, sonicated crude membrane, and sonicated Sarkosyl-insoluble membrane preparations of *C. jejuni* F^+M^+ (analyzed by SDS-PAGE in Fig. 1) are compared in Fig. 2. Because no significant differences in 2DGE profile were observed for comparable protein samples obtained by either French pressing or sonication, only sonicated samples are shown. The major proteins resolved in all samples were Omp 1 (broad band at 43 kDa) and flagellar protein (charge train at 63 kDa). Lipopolysaccharide appeared in the basic side of the dye front below the 14-kDa marker in all three profiles. Common minor components included charge trains at 90 and 83 kDa. Crude membranes and Sarkosyl-insoluble membrane proteins lacked three densely staining proteins at 63 kDa which were present in whole cells at the basic side of the flagellar protein charge train (braces, Fig. 2a). A large number of proteins, chiefly migrating below 45 kDa, were resolved in the crude membrane preparation (Fig. 2b), that were not resolved in the outer membrane preparation (Fig. 2c). The Sarkosyl-insoluble membrane preparation appeared enriched for Omp 1, flagellar protein, and lipopolysaccharide, as seen in the relative paucity of minor proteins (Fig. 2c).

Comparison of Sarkosyl-insoluble membrane preparations from *C. jejuni* and *C. coli*. The patterns of Sarkosyl-insoluble membrane preparations of three strains of *C. jejuni* and one strain of *C. coli* are shown in Fig. 3. In all four strains Omp 1 was the major component. The related *C. jejuni* strains F^+M^+ (Fig. 3a) and F^+M^- (Fig. 3b) possessed a cluster of relatively acidic (pI, 4.3 to 4.6) flagellar protein charge trains at 57 to 62 kDa which were not seen in *C. jejuni* F^-M^- (Fig. 3c). In contrast, a single prominent, more basic (pI, 4.5 to 5.0) flagellar protein charge train was observed at 63 kDa in *C. coli* 80-212 (Fig. 3d). *C. jejuni* F^+M^+ and F^+M^- and *C. coli* 80-212 all showed charge trains at 90 kDa that were similar in appearance (double arrows, Fig. 3b), but such a charge train was not observed in the aflagellate *C. jejuni* F^-M^- (Fig. 3c). In addition, relatively basic charge trains were observed at 75 and 80 kDa in *C. coli* 80-212 (Fig. 3d), which were not observed in the three related *C. jejuni* strains.

Immunoblot analysis of *C. jejuni* strains. Serum from rabbits immunized against whole-cell preparations of *C. jejuni* PEN 2 (Fig. 4) and F^+M^+ (data not shown) showed similar patterns of antigenic recognition against the respective 2DGE preparations of whole cells; numerous individual components were antigenic. Major immunoreactivity was observed at 70, 63 (flagellar protein), 47, 31, 29, 18, and <14 (LPS) kDa. Little, if any, reactivity was seen over the main band of Omp 1, although staining of a basic streak at 43 kDa was present (arrow, Fig. 4b). Immunoblotting of 2DGE transfers resolved multiple immunogenic proteins at 29 to 31 kDa (Fig. 4b), which are minor constituents among whole-cell components as determined by silver staining (Fig. 4a).

2DGE profiles of *C. fetus* strains. The 2DGE profiles of a variety of *C. fetus* whole-cell and acid-extracted preparations were studied. All preparations showed poorly focused double bands at 45 to 47 kDa, which represented the major OMPs. Similar doublets at 45 to 47 kDa have previously been observed in Sarkosyl-insoluble membrane proteins of *C. fetus* strains analyzed by SDS-PAGE (5, 20). All five known serum-resistant *C. fetus* strains and the one intermediate strain examined (9) showed prominent relatively acidic (pI, 4.1 to 4.3) proteins at 100 kDa with variable minor acidic components present between 60 and 125 kDa. Resolution of this 100-kDa band with periodic acid-Schiff stain indicated that it was glycosylated (data not shown), confirming an earlier observation (24). In contrast, serum-sensitive strains demonstrated very little or no acidic glycoprotein at 100 to 125 kDa. Isogenic *C. fetus* strains 82-40 LP (serum resistant) and 82-40 HP (serum sensitive) (9) differed primarily in the acidic high-molecular-weight region, which was markedly diminished in strain 82-40 HP (Fig. 5a and b). Acid extraction of serum-resistant strains of *C. fetus*, including strain 82-40 LP, resulted in relative enhancement of the 100-kDa protein (Fig. 5c). However, numerous acidic proteins were observed directly beneath the 100-kDa glycoprotein, presumably representing its degradation products (Fig. 5c). Such degradation products were not observed in whole-cell preparations of strain 82-40 LP (Fig. 5a). In addition, acid extraction of *C. fetus* strains resulted in the appearance of three to five charge trains of flagellar protein at 57 to 63 kDa (best shown in Fig. 5d) in contrast to the single flagellar protein charge train at 63 kDa observed in whole-cell preparations (Fig. 5b).

Immunoblot analysis of *C. fetus* 82-40 LP and HP. Serum from rabbits immunized against whole-cell preparations of serum-resistant *C. fetus* 23D (essentially identical to *C. fetus* 82-40 LP [9]) showed major immunoreactivity against both flagellar protein (63 kDa) and a diffuse band at approximately 20 kDa, probably representing LPS constituents, in whole-cell preparations of strains 82-40 LP (Fig. 6a) and 82-40 HP (Fig. 6b). Multiple isomeric forms of flagellar protein were observed in both strains. In addition, significant immunoreactivity was observed over the acidic 100-kDa glycoprotein in strain 82-40 LP (Fig. 6a), but not in strain 82-40 HP (Fig. 6b). In acid extracts of the two strains, multiple immunoreactive charge trains were observed between 63 and 50 kDa, presumably representing degradation products of flagella (Fig. 6c and d). Acid extracts of strain 82-40 LP showed numerous immunoreactive degradation products of the 100-kDa protein as well (Fig. 6c).

Analysis of major OMPs from various *Campylobacter* species. To determine whether the major OMPs of various *Campylobacter* species possess a multimeric composition similar to that of flagellar protein, 2-μg samples of Sarkosyl-insoluble membrane preparations from five strains each of *C.*

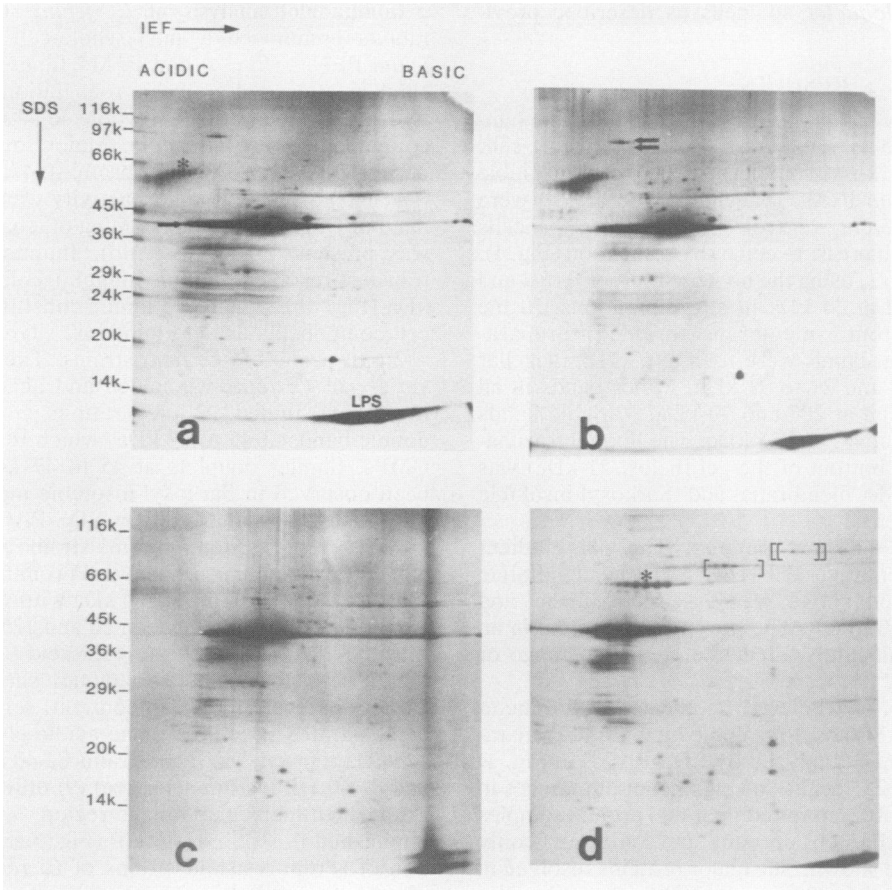

FIG. 3. Silver-stained 2DGE of Sarkosyl-insoluble membrane proteins from four *Campylobacter* strains. Strains: *C. jejuni* F^+M^+ (a), *C. jejuni* F^+M^- (b), *C. jejuni* F^-M^- (c), and *C. coli* 80-212 (d). In all strains the broad Omp 1 spot is present at about 44 kDa (→, panel a). Flagellar protein is present as an acidic charge train (*, panels a and d) in all strains except *C. jejuni* F^-M^- (panel c). Lipopolysaccharide (LPS, panel a) was resolved near the bottom of the gel in all strains. An additional charge train at 90 kDa (⇇, panel b) was seen in all strains except *C. jejuni* F^-M^- (panel c). Relatively basic charge trains at 75 kDa (brackets, panel d) and 80 kDa (double brackets, panel d) in *C. coli* 80-212 were not observed in the *C. jejuni* strains examined (panels a to c).

jejuni, *C. coli*, and *C. fetus* were subjected to 2DGE. All strains of *C. jejuni* and *C. coli* showed only a single isomeric form of Omp 1 at 43 kDa (data not shown). All five strains of *C. fetus* showed doublets at 45 to 47 kDa, which in four cases showed only single isomeric forms for each. In contrast, the heavier component (47 kDa) of the doublet of *C. fetus* 82-40 LP was shown to contain at least two minor isomeric forms by 2DGE (data not shown).

DISCUSSION

2DGE has been used to analyze the protein composition of a variety of clinically important gram-negative microbes such as *Chlamydia* and *Neisseria* spp. (3, 18). In this study we have shown that many of the characteristic OMPs of *C. jejuni*, *C. coli*, and *C. fetus* strains which are observed as bands by one-dimensional SDS-PAGE can be further resolved by using 2DGE into individual components based on differences in isoelectric point. We were able to determine which spots on 2DGE represented characteristic bands on SDS-PAGE (e.g., Omp 1, flagellar protein, and lipopolysaccharide) by running both one- and two-dimensional separations in the same gel.

The major 63-kDa band seen on SDS-PAGE, shown previously to represent flagellar protein (6, 20, 27, 36), consisted of numerous isomers forming distinctive charge trains in all flagellated *Campylobacter* strains examined by 2DGE. In general, the presence of protein charge trains in 2DGE is thought to represent either multiply charged forms of a given protein moiety (14, 15, 29) or individual amino acid substitutions in the primary sequence of a protein due to tRNA recognition errors during translation (32). The appearance of charge trains can be an artifact resulting from protein insolubility due to inadequate concentrations of urea or detergent in IEF gels caused by protein carbamylation in the presence of isocyanate formed by decomposition of urea or by subjecting samples to lyophilization (1, 14, 29). However, we believe the flagellar protein charge trains, as well as other higher-molecular-weight charge trains seen in these gels, are not artifactual but represent true protein heterogeneity, since essentially identical charge trains were observed in 2DGE studies of whole-cell samples that were not frozen or lyophilized before being solubilized (35) in urea mix by established methods without heating (data not shown). In addition, flagellar proteins of some strains of at least one other microorganism, *Caulobacter crescentus*, form charge trains when subjected to 2DGE (16), suggesting that the charge heterogeneity of flagellar proteins observed in *Campylobacter* species is not unique among flagellated microorganisms. Although the significance of multiply charged

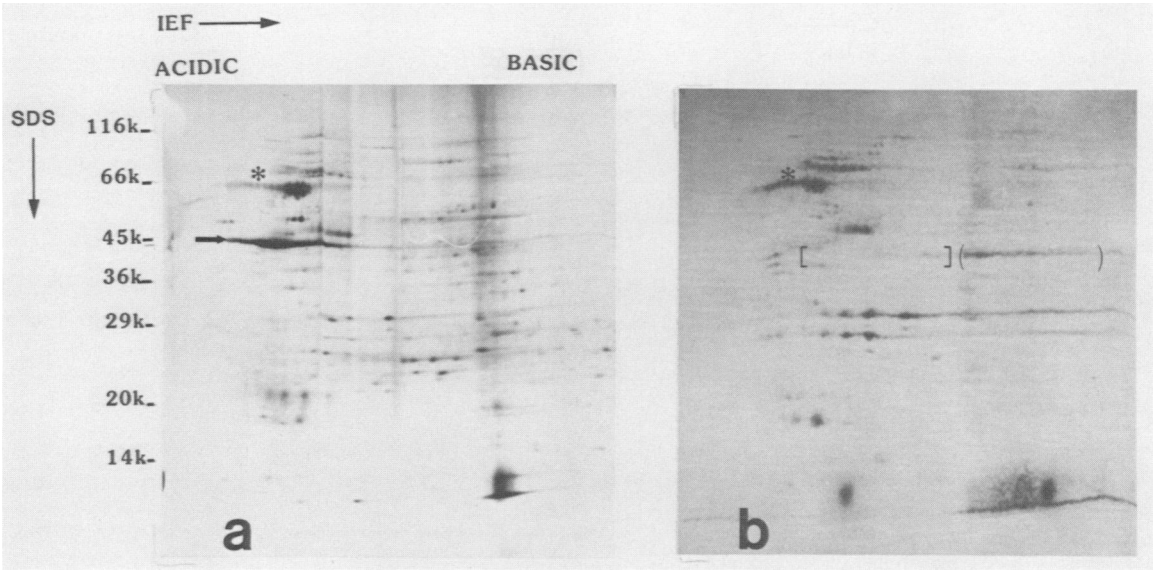

FIG. 4. Comparison of silver-stained 2DGE gel (a) with an immunoblot prepared simultaneously (b) of *C. jejuni* PEN 2 whole-cell preparation. Major antigenic proteins were observed at 70, 63, (flagellar protein [*]), 56, 45, 31, and 29 kDa. Note that the broad bandlike spot of Omp 1 seen in silver-stained gels (→, panel a) is not recognized by homologous immune rabbit serum under these conditions (brackets, panel b). Instead, a diffuse linear pattern of immunostaining is observed at the same horizontal level (i.e., 43 kDa) as Omp 1 (parentheses, panel b). Numerous individual antigenic components at 31, 29, 18, and 16 kDa are also present.

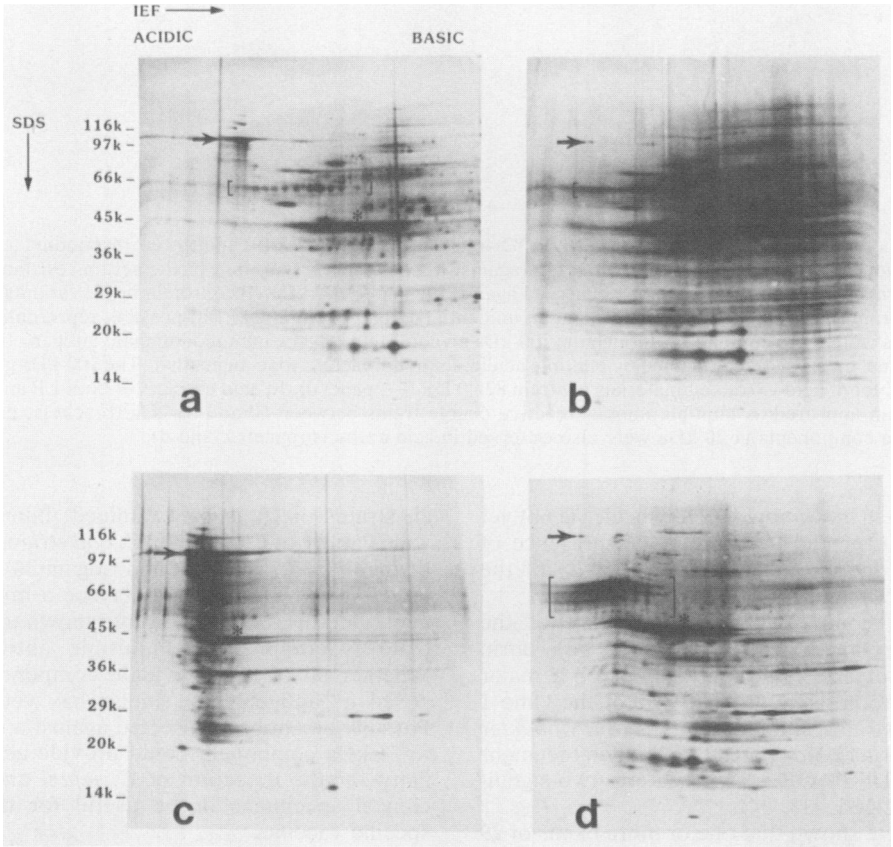

FIG. 5. Silver-stained 2DGE of whole-cell preparations of *C. fetus* 82-40 LP (a) and 82-40 HP (b) and the corresponding acid-extracted proteins (c and d, respectively). The acidic 100-kDa glycoprotein is prominent in the LP variant (→, panels a and c) but is barely detectable in the HP variant (→, panels b and d). Flagellar protein is present as a single charge train in both whole-cell preparations of both LP and HP variants (brackets, panels a and b), whereas multiple-charge trains between 50 and 63 kDa were observed in acid extracts of the HP variant (brackets, panel d). The major OMP is present as a poorly focused doublet at 45 to 47 kDa in all four preparations (*).

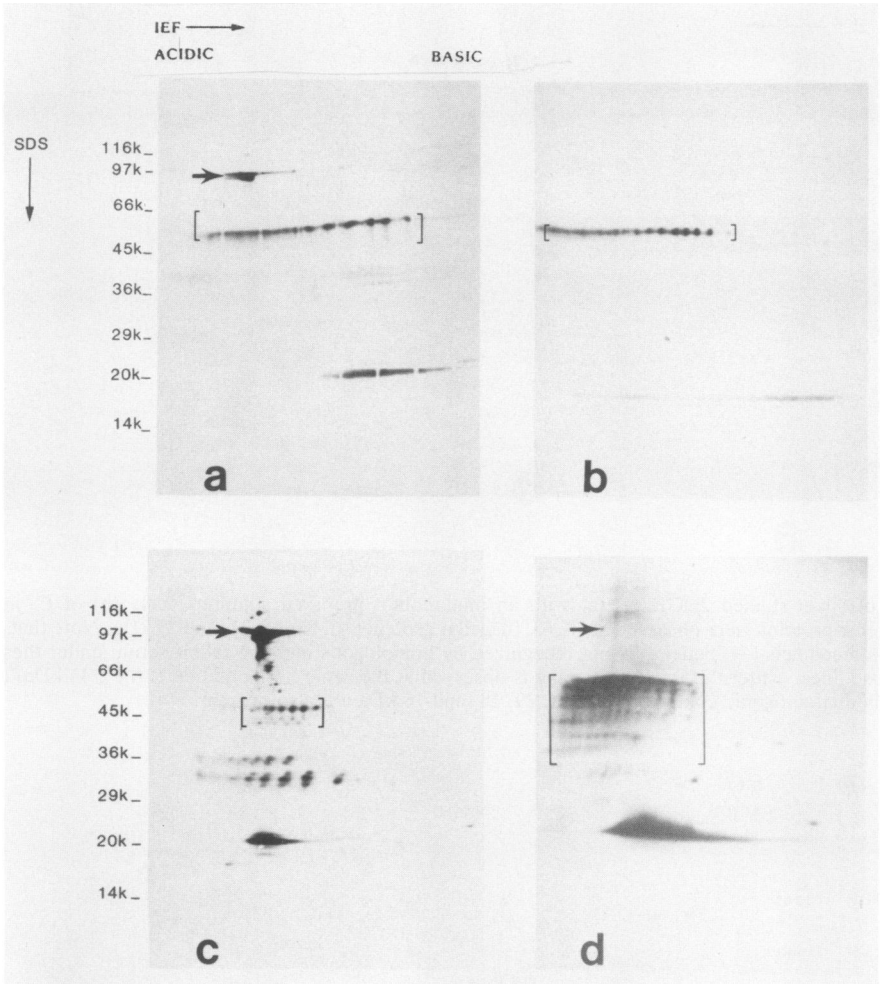

FIG. 6. Immunoblots of whole-cell preparations of *C. fetus* 82-40 LP (a) and 82-40 HP (b) and the corresponding acid-extracted materials (c and d, respectively) developed using immune rabbit serum against a whole-cell preparation of the serum-resistant *C. fetus* 23D. In the whole-cell preparations, major immunoreactivity was observed against the acidic 100-kDa glycoprotein in LP variants only (→, panel a), the flagellar protein charge train (brackets, panels a and b) and an unidentified component at 20 kDa, possibly representing LPS. Immunoblots of acid-extracted proteins were more complex. Although the 100-kDa glycoprotein was the immunodominant antigen observed in acid extracts of strain 82-40 LP (→, panel c), it was accompanied by multiple acidic, immunoreactive spots beneath it. The 100-kDa glycoprotein was barely detectable in an immunoblot of acid-extracted materials of strain 82-40 HP (→, panel d). In acid extracts of both LP and HP variants of strain 82-40, the flagellar protein appeared as multiple immunoreactive charge trains between 50 and 63 kDa (brackets, panels c and d). Poorly focused, immunoreactive components at 20 kDa were also observed in acid extracts (panels c and d).

forms of flagellar protein in *Campylobacter* species is not yet known, it has been suggested (6, 12) that the presence of flagellar proteins may contribute to the complexity of the heat-labile serotyping system of Lior et al. (19, 37).

In contrast to the complex isomeric structure of the flagellar proteins, the Omp 1, which appears to be a porin (20), was composed of either a single isomer or a major isomeric component comprising at least 90% of the Omp 1 protein in all of the strains studied. Thus, *Campylobacter* species differ from *Escherichia coli* K-12, in which the major OMP band seen by SDS-PAGE is resolved into two significant components by 2DGE (13, 34).

Previous studies have shown that one or more bands of 29 to 31 kDa are present in all strains of *C. jejuni* studied (7, 21, 27). The 29- to 31-kDa bands are surface exposed and antigenic, as determined by [^{125}I]lactoperoxidase labeling and Western blotting with hyperimmune rabbit serum, are extractable by a low-pH buffer, and are present in essentially all strains of *C. jejuni* examined; humans infected with a wide variety of *C. jejuni* or *C. coli* strains make antibodies to them (4, 7, 33, 36). Trust and Logan (36) have suggested that the 29- to 31-kDa protein may be a microcapsular component. Our 2DGE analyses have shown that the 29- to 31-kDa band is composed of multiple antigenic components. Whether all of the individual components are surface exposed or antigenically similar has yet to be established. Possibly an antibody directed against a specific, isolated 29- to 31-kDa component could provide adequate group specificity for the detection of *C. jejuni* and *C. coli* in human clinical specimens or be useful for the production of a specific vaccine.

McCoy et al. (24, 38) have observed a superficial glycoprotein which can be extracted at a low pH from *C. fetus* 23D and which appeared to inhibit phagocytosis by bovine neutrophils. Blaser et al. (9, 10) have shown that this glycoprotein migrates at 100 kDa and is associated with the

serum resistance of isolates from humans. Five serum-resistant strains and one intermediate-resistance strain of *C. fetus* that we studied by 2DGE showed distinct, relatively acidic glycoproteins at about 100 kDa, which appeared labile when extracted in acid. In some cases, additional high-molecular-weight acidic molecules of up to 125 kDa were also observed. None of the three serum-sensitive strains of *C. fetus* that we examined showed similar patterns of 125 or 100 kDa or lower-molecular-weight acidic proteins, a finding which supports earlier observations (9). Knowledge of its isoelectric point and tendency to degrade under acidic conditions should aid in attempts to purify this class of glycoproteins.

Immunoblotting of two-dimensional gels revealed multiple antigenic proteins at several different molecular weights, indicating that the antigenic composition of both *C. jejuni* and *C. fetus* proteins is more complex than was previously recognized. However, the bulk of Omp 1 seen in whole-cell preparations of both PEN 2 and F^+M^+ strains of *C. jejuni* was not stained during the immunoblot procedure; rather, only the basic tail at 43 kDa was antigenic under these conditions. Amido black staining of transfer blots performed under identical conditions has demonstrated that the major elongated spot of Omp 1 seen on silver-stained 2DGE gels binds to the nitrocellulose paper in both strains; thus, the failure to transfer this material is not an explanation for the lack of antigenicity seen. As seen from our 2DGE observations, the staining at 43 to 45 kDa seen in one-dimensional immunoblots may not represent the major portion of Omp 1 itself, but may represent another component, that which by 2DGE appears as a basic streak (Fig. 4). Further work is needed to clarify this matter. In addition, immunoblot analysis of whole-cell and acid extracts of *C. fetus* 82-40 LP and 82-40 HP confirmed the acid extractability of both the 100-kDa glycoprotein and the flagellar protein noted previously (24) and convincingly demonstrated the acid-labile nature of both of the latter components.

Although more labor-intensive than one-dimensional SDS-PAGE, 2DGE analysis has demonstrated a greater complexity of campylobacter OMPs and antigens than was previously observed. Because 2DGE can resolve distinct proteins of the same molecular weight, determined by differences in isoelectric point, the method should prove useful in further characterization of *Campylobacter* sp. strains and in analysis during purification of specific OMPs for the production of specific antibodies and vaccines. Such studies are currently under way in this laboratory.

ACKNOWLEDGMENTS

We thank Alex Winter and Diane Newell for sending related strains to us, Paula Napychank and Janet Hopkins for their excellent technical assistance, and Inez Curiel for typing the manuscript.

This work was supported in part by Public Health Service grant BRSG-05357 from the Biomedical Research Grant Program, Division of Research Resources, National Institute of Health, and by the Medical Research Service of the Veterans Administration and an Interagency Agreement with the U.S. Army Medical Research and Development Command.

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