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A Strong Antibody Response to the Periplasmic C-Terminal Domain of the OmpA Protein of *Escherichia coli* Is Produced by Immunization with Purified OmpA or with Whole *E. coli* or *Salmonella typhimurium* Bacteria

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We produced in *Bacillus subtilis* the complete, as well as the N-terminal two-thirds, OmpA protein of *Escherichia coli* (called here Bac-OmpA and Bac-OmpA-dN, respectively). These Bac-OmpA proteins were used to examine the immunological properties of different parts of OmpA, free of lipopolysaccharide and other components of the outer membrane. The full-length Bac-OmpA was indistinguishable from the authentic protein isolated from *E. coli* (Coli-OmpA) both as immunogen and as antigen in enzyme immunoassay (EIA). The N-terminal Bac-OmpA-dN was a poor immunogen which gave rise to significantly lower titers of anti-OmpA antibody than did the full-length OmpA preparations. When used as an antigen in EIA, the Bac-OmpA-dN detected anti-OmpA antibody in serum samples from animals immunized with the full-length OmpA much less efficiently than did either Bac-OmpA or Coli-OmpA. The periplasmic C-terminal domain therefore appears to be an immunodominant epitope of the purified OmpA protein. Also, when rabbits and mice were immunized with intact, live or dead *E. coli*, the antibody response detected by EIA with the full-length protein, Bac-OmpA, was much stronger than that detected with the N-terminal two-thirds, Bac-OmpA-dN. Similar results were obtained with the OmpA of *Salmonella typhimurium*. Because the *ompA* gene of enterobacteria is highly conserved, the Bac-OmpA might be useful as a group-specific EIA antigen to diagnose diseases caused by members of the family *Enterobacteriaceae*.

OmpA is one of the major outer membrane proteins of *Escherichia coli* and other enteric bacteria, present at about 10^5 copies per cell (2, 20, 27). The *ompA* genes of several members of the family *Enterobacteriaceae* have been shown to be closely related (4, 5, 7). The N-terminal two-thirds of the 325-amino acid residue-long OmpA molecule of *E. coli* spans the outer membrane eight times, forming four short loops on the bacterial surface (Fig. 1) (15, 23, 34). These loops serve as receptors for several OmpA-specific bacteriophages (33). The conformation of the protein as it is embedded in the outer membrane is believed to be important for its phage receptor function, since denatured OmpA does not bind these phages but gains phage-binding ability when complexed with lipopolysaccharide (LPS), a major component of the outer membrane (6).

The C-terminal third of OmpA is hydrophilic and located in the periplasmic space (Fig. 1) (15, 23, 34). The C-terminal domain is not essential for the localization of OmpA in the outer membrane or for its function as phage receptor (5).

Antibodies to OmpA are produced in response to immunization with isolated OmpA or with whole bacteria, as well as during infection caused by enteric bacteria (8, 25, 31; M. Karvonen, R. Puohiniemi, V. Valtonen, P. Ruutu, H. Arvilommi, M. Sarvas, and P. H. Mäkelä, submitted for publication). Their epitope specificities have so far not been studied in detail. Theoretically, one would expect that the phage receptor loops on the bacterial surface would be important antigenic epitopes (14). The membrane-spanning regions embedded in the lipid bilayer would not be likely to be exposed for binding of antibodies or stimulation of B cells. The C-terminal domain located in the periplasmic space, on the other hand, could be expected to stimulate B cells for antibody production only after exposure by breakdown of the bacteria.

Anti-OmpA antibodies are cross-reactive between enterobacterial species (2, 10, 11), suggesting that OmpA might be useful as an antigen for diagnostic tests. For this purpose, it is necessary to have an easy method of preparing OmpA in large quantities and free of other outer membrane components. We considered production of OmpA in the gram-positive bacterium *Bacillus subtilis* to be a promising method for this purpose. The same approach could also provide a tool for studying the antigenic properties of different parts of the OmpA. We cloned two forms of the *ompA* gene of *E. coli* into *B. subtilis*, one coding for the full-length mature OmpA (this study) and the other coding for the N-terminal two-thirds of the protein (13). OmpA protein was isolated from both of these constructs (called here Bac-OmpA and Bac-OmpA-dN, respectively) and from *E. coli* (Coli-OmpA) as a control. These antigens were then used to study the immune response to OmpA of *E. coli* and *Salmonella typhimurium*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used are listed in Table 1. *B. subtilis* strains were derivatives of B. subtilis IH6140, a prototrophic derivative of *B. subtilis* Marburg strain 1A289 from the Bacillus Genetic Stock Center (Department of Biochemistry, Ohio State University, Columbus). IH6140 has a reduced exoprotease activity (29).

The bacteria were grown in liquid culture overnight at 37°C with shaking (250 rpm); *E. coli* was grown in L-broth

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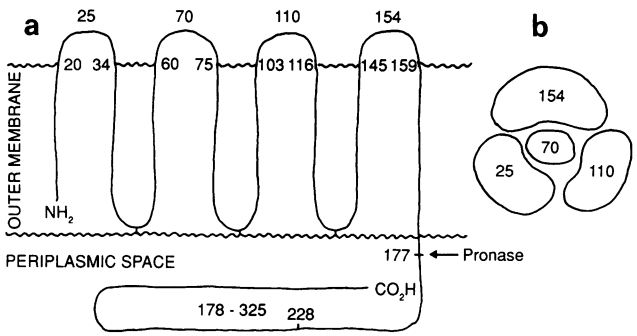

FIG. 1. Schematic model of the OmpA protein in the outer membrane of *E. coli*. In the two-dimensional view (a), the regions exposed outside are designated by amino acid residue numbers. The pronase cleavage site shown is in the stretch of the transition between the membrane embedded and the periplasmic part of the protein. The other panel (b) shows the OmpA protein viewed from outside the cell. Reprinted from the *Journal of Bacteriology* (23) with permission of the publisher.

(19), and *B. subtilis* was grown in twofold-concentrated L-broth containing 10 mg of NaCl per liter, 10 μg of kanamycin per ml, and 30 μl of potato extract per ml (12).

Recombinant DNA techniques. The recombinant DNA techniques used were essentially those of Maniatis et al. (21).

Gel electrophoresis. Proteins were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 12% acrylamide (17, 30) and then either stained with Coomassie brilliant blue or immunoblotted with rabbit anti-OmpA antiserum (Table 2) by using peroxidase-conjugated anti-rabbit immunoglobulin G (Bio-Rad Laboratories, Richmond, Calif.) as the second antibody (30).

Isolation of Bac-OmpA. The method for extraction of Bac-OmpA from *B. subtilis* was based on previous experience with the N-terminal fragment of OmpA containing amino acids 8 to 228 (13). Strain IH6543, containing pKTH217, was grown to early stationary phase, i.e., 6 to 9 h after reaching the optical density of 100 Klett units (Klett-Summerson photometer, red filter). The cells were protoplasted with lysozyme and then disrupted by osmotic shock and sonication. After removal of the unbroken cells and protoplasts by low-speed centrifugation (3,000 × *g*, 10 min, 4°C), the membranes and particulate material were pelleted by centrifugation at 30,000 × *g* for 60 min at 4°C (Fig. 2, lane 3). Part of the membrane proteins was extracted from the pellet (the particulate fraction) with 5% Triton X-100 in 50 mM Tris-hydrochloride buffer (pH 8) in the presence of 50 mM EDTA (Fig. 2, lane 4). Bac-OmpA was solubilized from the remaining pellet (Fig. 2, lane 5) with 2% Sarkosyl in the buffer described above in the presence of 1 mM EDTA (Fig. 2, lane 6), and the insoluble material (for instance, flagellin, 36 kilodaltons [kDa]) was removed by centrifugation (20,000 × *g*, 20 min, 4°C) (Fig. 2, lane 7).

The Sarkosyl supernatant, which contained the bulk of the Bac-OmpA protein, was then dialyzed extensively against 2 mM $NaHCO_3$. It still contained bacillar proteins (Fig. 2, lane 6); the ratio of OmpA to total protein was 1:6. (Total protein concentrations were determined by the method of Markwell et al. [22]. The amount of OmpA was estimated visually from SDS-PAGE by comparing the staining intensity of a series of dilutions of the preparation with known amounts of low-molecular-size standards [Pharmacia, Uppsala, Sweden].) This degree of purity was sufficient for the purpose of the following experiments. The yield of OmpA was approximately 1 mg/liter of culture.

Antigens. Bac-OmpA, OmpA protein produced in *B. subtilis* IH6543, was prepared as described above. Bac-OmpA-dN was isolated from IH6443 in a similar manner. It consists of a truncated OmpA containing the N-terminal two-thirds of the protein as a tandem duplication. The duplication was used because of its higher yield of the protein; in preliminary experiments by the enzyme immunoassay (EIA) used in this study and by studying the phage receptor activity (unpublished data), this form has given results identical to those yielded by the nonduplicated form. Bac-Mock, the control antigen, was prepared in a similar manner from strain IH6418, which contains the expression vector without *ompA*. Coli-OmpA was purified by preparative SDS-PAGE (30) from *E. coli* K-12 (strain PL 2), and Salmonella-OmpA was prepared from *S. typhimurium* his-515 (26). All antigens were stored at −70°C.

Hyperimmune sera. Table 2 shows the hyperimmune sera used. New Zealand White rabbits were immunized with 20 to

TABLE 1. Bacterial strains and plasmids used

Strain (properties)	Plasmid carried	Amino acid residues of OmpA encoded by the plasmid	Reference or source
B. subtilis			
IH6418	pKTH132	None	13, 29
IH6186	pKTH41	229 to 325	P. Kallio[a]
IH6443	pKTH160	Tandem duplication of 8 to 228	This paper
IH6539	pKTH215	8 to 228	Fig. 2
IH6543	pKTH217	8 to 325	Fig. 2
E. coli			
PL 2 (K-12)			CGSC 4498[b]
EH814 (K-12)	pKTH98	8 to 228	P. Kallio[a]
IH3080 (O18:K1, mouse virulent)			37
S. typhimurium			
SH2183 (smooth)			32
SL3261 (smooth, nonvirulent, *aroA*)			12
his-515 (rough)			26

[a] P. Kallio, Ph.D. thesis, University of Helsinki, Helsinki, Finland, 1987.
[b] CGSC, *E. coli* Genetic Stock Center, School of Medicine, New Haven, CT.

TABLE 2. Hyperimmune sera used

Animal	Serum number(s)	Immunogen	Dose	Reference or source
Rabbit	KH590, KH591	Coli-OmpA	20 μg[a]	This paper
	KH721, KH722	Bac-OmpA-dN	40 μg[a]	This paper
	KH1018, KH1019	Bac-OmpA	20 μg[a]	This paper
Mouse	HH1	Live *E. coli* IH3080	10^5 CFU[b]	36, 37
	HH2	Heat-killed *E. coli* IH3080	5×10^7 CFU[b]	This paper
	HH8	Live *S. typhimurium* SL3261	10^6 CFU[c]	24
	HH53	*Salmonella* OmpA	10 μg[d]	This paper
	HH65	Heat-killed *S. typhimurium* SH2183	10^8 CFU[d]	24

[a] Three injections in Freund complete adjuvant into popliteal lymph nodes over a period of 6 weeks (18).
[b] One intraperitoneal injection without adjuvant.
[c] One intravenous injection without adjuvant.
[d] Two intravenous injections without adjuvant.

40 μg of protein in Freund complete adjuvant per injection. Three injections over a period of 6 weeks were given into the popliteal lymph nodes, and the rabbits were bled 10 days after the last injection (18). $(CBA \times C57Bl/6)F_1$ hybrid mice were immunized with live or heat-killed bacteria or with 10 μg of protein per injection, and sera from 10 mice were pooled (24). All sera were stored at −20°C until use.

EIA. The EIA was performed by the method of Voller et al. (35), with some modifications. Flat-bottomed microdilution plates (Immunolon; Dynatech, London, United Kingdom) were coated with 100 μl of the antigen diluted in Tris hydrochloride buffer, pH 8.5, to a concentration of 5 μg/ml. The plates were incubated overnight at 22°C and then washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween and once with PBS alone. The sera to be assayed were diluted 1:100, 1:1,000, 1:10,000 (and further when necessary) in PBS containing 10% fetal calf serum (PBS-FCS) (Difco Laboratories, Detroit, Mich.). To each well, 100 μl of each dilution was added. After 2 h of incubation at 37°C, the plates were washed as described above. Peroxidase-conjugated anti-rabbit immunoglobulin G or anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) was diluted in PBS-FCS, and 100 μl was added to each well. The plates were incubated for 1 h at 22°C. After being washed as described above, 100 μl of the substrate, containing 0.4 mg of 1,2-phenylenediaminedihydrochloride (Fluka AG, Buchs, Switzerland), and 0.4 μl of 30% H_2O_2 in 1 ml of 0.1 M phosphate-citrate buffer at pH 5.5 were added to the wells. The reaction was stopped after 15 min at 22°C by adding H_2SO_4. The A_{492} was measured by using a Titertek Multiskan spectrophotometer (Labsystems, Helsinki, Finland), and the endpoint titer (16) was calculated at the cutoff value of 0.300.

RESULTS

Subcloning and expression of the *ompA* gene in *B. subtilis*. The *ompA* gene was subcloned in two steps into the plasmid pKTH132, which allows a good expression of many foreign proteins in *B. subtilis* (13, 29), as shown in Fig. 3. In the first step, the 5′ part of the *ompA* gene (coding for amino acids 8 to 228, previously cloned in pKTH98 [Table 1]) was inserted in the vector between its *Hin*dIII and *Bam*HI sites. The plasmid pKTH215 expressed an approximately 31-kDa peptide reacting with anti-OmpA antiserum (data not shown). In the second step, the 3′ part (amino acids 229 to 325, plasmid pKTH41 [Table 1]) of *ompA* was inserted into the single *Bam*HI site of pKTH215. The correct orientation of the inserted fragment in pKTH217 was confirmed by the presence of the expected fragments after digestion with *Hin*dIII and *Bgl*II. The *ompA* gene of plasmid pKTH217 was expressed in *B. subtilis* (Fig. 2) in a manner similar to that of the N-terminal two-thirds of OmpA which was found in the particulate fraction with uncleaved signal peptide, as described earlier (13). Thus, the size of the protein that reacted with anti-OmpA antiserum was approximately 38 kDa, indicating the presence of the signal sequence. The protein, Bac-OmpA, was found in the particulate fraction, from which it was solubilized as described in Materials and Methods and the legend to Fig. 2.

Immunization with OmpA proteins. Rabbits were immunized with the three OmpA preparations: the full-length Coli-OmpA isolated from *E. coli* and the full-length Bac-OmpA and the N-terminal two-thirds of OmpA (Bac-OmpA-dN) produced in *B. subtilis*. The results are shown for one rabbit in each category in Fig. 4; the other sera gave similar results. Before immunization (normal rabbit serum), no anti-OmpA antibodies were detectable. Both full-length preparations elicited a high titer of antibodies ($>10^6$) detected by EIA with either Coli-OmpA or Bac-OmpA as the antigen (Fig. 4). These two OmpA preparations were equally good antigens in the EIA. In contrast, the antibody titers of both hyperimmune sera were 1,000-fold less when the N-terminal Bac-OmpA-dN was used as the EIA antigen, demonstrating that the main antibody response to purified OmpA was directed to the C-terminal part of the protein.

Immunization of rabbits with the N-terminal Bac-OmpA-dN yielded an antibody titer 1,000-fold lower (10^4) than those obtained by immunizing rabbits with Coli-OmpA or Bac-OmpA (compare serum KH722 to the sera KH591 and KH1018 in Fig. 4). As expected, the titer of serum KH722 was practically the same with any of the three OmpA preparations as the antigen. Thus, the N-terminal two-thirds of purified OmpA seems to be of poor immunogenicity.

Similar results were obtained when mice were immunized with isolated Salmonella-OmpA (serum HH53); a high titer (10^6) was found by EIA with the full-length Bac-OmpA antigens, and a much lower titer (10^2) was found with the N-terminal Bac-OmpA-dN. These data also confirm the high immunological cross-reactivity between the OmpA proteins of *E. coli* and *S. typhimurium* (7).

Immunization with whole bacteria. In the experiments described above, the OmpA proteins were initially solubilized in a strong detergent and thus denatured. To study the antigenicity of OmpA in its native, membrane-associated conformation, mice were immunized with whole, heat-killed

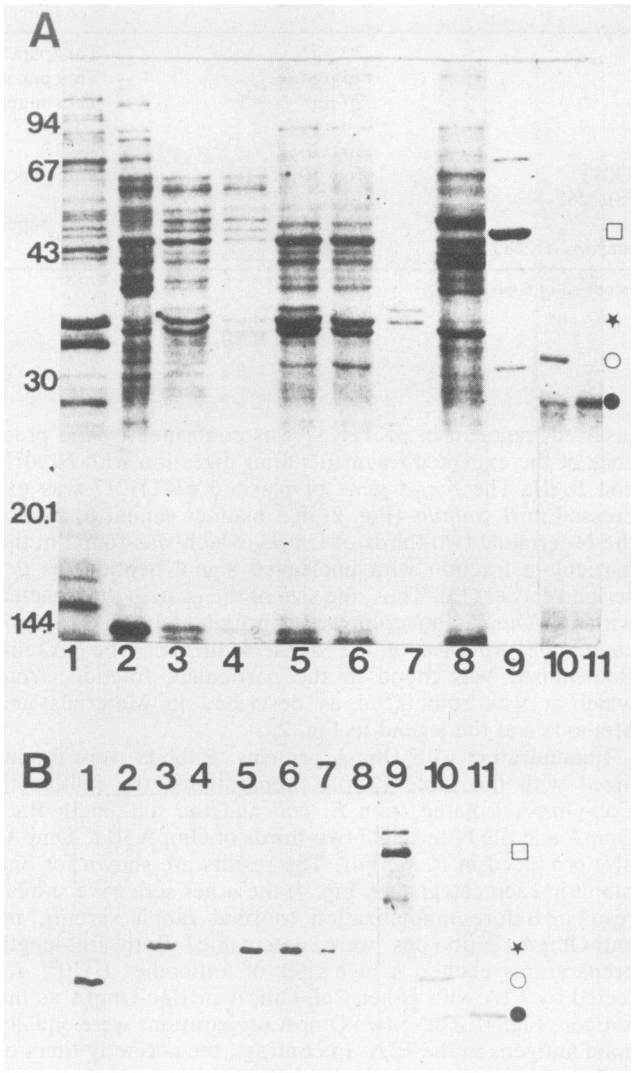

FIG. 2. Expression and extraction of Bac-OmpA. Lane 1, Crude outer membrane preparation (prepared by the method of Palva [28]) of *E. coli* containing the 35-kDa OmpA protein. Lane 2, The particulate fraction of *B. subtilis* IH6418 containing the expression vector pKTH132 (without the *ompA* gene). Lane 3, The particulate fraction of *B. subtilis* IH6543 with pKTH217 coding for full-length OmpA. Lane 4, The supernatant, and lane 5, the pellet, after Triton X-100 extraction and centrifugation of the particulate fraction of IH6543. Lane 6, The supernatant (Bac-OmpA), and lane 7, the pellet, after Sarkosyl extraction and centrifugation of the Triton X-100 pellet of IH6543. Lane 8, Bac-Mock, prepared in a manner similar to that for Bac-OmpA (lane 6) from IH6418. Lane 9, Bac-OmpA-dN (molecular size of approximately 54 kDa; see Materials and Methods), prepared in a manner similar to that for Bac-OmpA from IH6443. Lanes 10 and 11, The purified OmpA protein of *E. coli*; the sample was boiled in 4% SDS (lane 10) or was not boiled (lane 11) before application to the gel. (A) Coomassie blue staining. The numbers to the left show the position and size (in kiladaltons) of molecular size markers. (B) Immunoblotting with anti-OmpA antiserum KH591 (Table 2). Symbols on the right indicate positions of heated (100°C, 5 min) (○) and unheated (●) Coli-OmpA and of Bac-OmpA (★) and Bac-OmpA-dN (□). Preparation of the fractions is described in Materials and Methods.

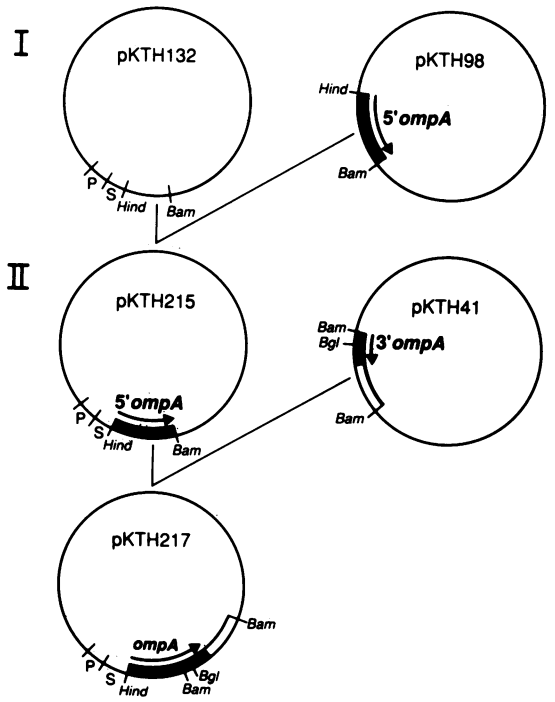

FIG. 3. Cloning of the *ompA* gene (amino acids 8 to 325) into a bacillar expression vector downstream of the bacillar α-amylase promoter and signal sequence present in the plasmid (see Results). P and S represent the promoter and signal sequences, respectively, of the α-amylase gene of *Bacillus amyloliquefaciens*. 5′ *ompA* is the 661-base-pair (bp) fragment coding for the N-terminal amino acids 8 to 228 of OmpA; 3′ *ompA* is the 1,830-bp fragment, the 5′ end of which (288 bp) codes for amino acid residues 229 to 325, followed by the stop signals of OmpA. The rest of the insert is chromosomal DNA of *E. coli* (1).

E. coli or *S. typhimurium* cells (Fig. 4). The heat-killed bacteria elicited a high anti-OmpA antibody titer (10^4) when measured by EIA with Bac-OmpA and elicited a 100-fold-lower titer (10^2) when measured with the N-terminal Bac-OmpA-dN. The OmpA was thus a good immunogen even in intact bacteria, and again a strong antibody response was directed to its C-terminal part—a remarkable finding considering the periplasmic location of the C terminus.

In further experiments, we examined the antibody response to OmpA in live bacteria. Ten mice were injected intraperitoneally with a sublethal dose of live *E. coli* 018:K1 that produces peritonitis and septicemia (36, 37). They recovered in a few days; the serum was collected 3 weeks later and pooled (serum HH1). Another group of 10 mice was injected with 10^6 cells of a nonvirulent *aroA* mutant of *S. typhimurium* (12); the bacteria survived in the liver and spleen of the animals for approximately 2 weeks (24). The serum, HH8, was collected 3 weeks after infection. The antibody titers were high (10^4) in both sera when Bac-OmpA was used as the EIA antigen, and again they were 100-fold lower with the N-terminal Bac-OmpA-dN antigen. Serum from uninfected mice did not react with either antigen (Fig. 4), and the control antigen Bac-Mock did not react with either serum (titer less than 100; data not shown). Thus, there was a substantial immune response to OmpA during infection, and much of it was again directed to the C-terminal part of the molecule. Furthermore, OmpA of *E. coli* produced in *B. subtilis* could be used to measure the OmpA antibody response to *Salmonella* infection.

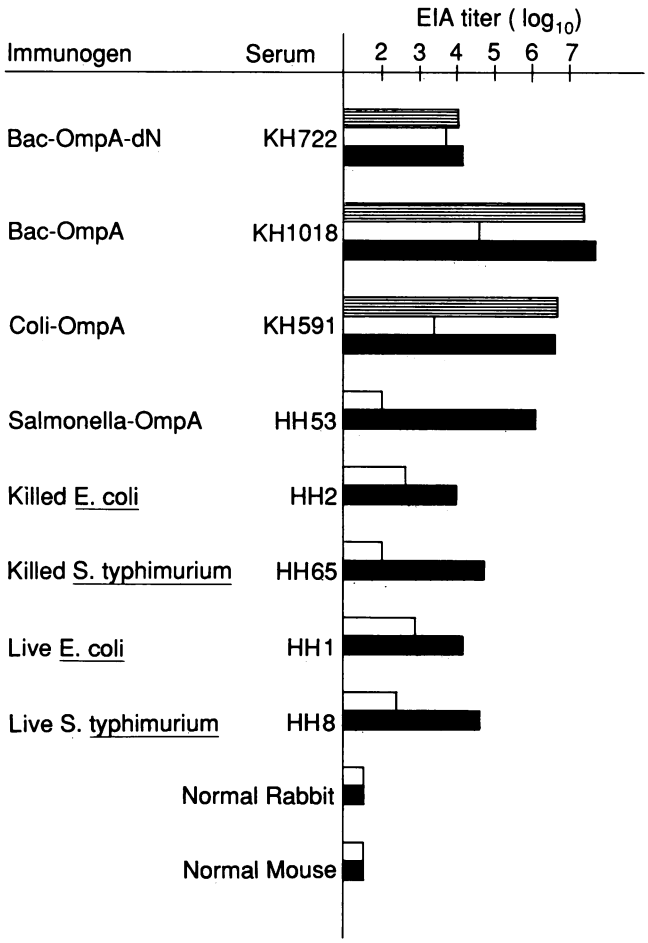

FIG. 4. Comparison of EIA titers measured with different OmpA preparations: Bac-OmpA (■), Bac-OmpA-dN (□), and Coli-OmpA (▤). Rabbits (KH sera) or mice (HH sera) were immunized with different OmpA preparations or with live or killed bacteria as indicated (for details, see Table 2). Because the two KH sera for each immunogen were similar, only one of each is shown. The HH titers represent pooled sera from 10 mice. The titer of sera of mice immunized with killed or live bacteria was lower because of the lower dose of the antigen. Negative results, titers below 100, were assigned the arbitrary value of 30.

DISCUSSION

Two main conclusions can be drawn from the data presented. First, OmpA protein produced in a heterologous host can be used to detect antibodies formed in response to infection or immunization with bacteria possessing OmpA. Second, the antibodies detected with purified OmpA preparations are mainly directed to the C-terminal, periplasmic part of the protein. These conclusions are based on comparisons of the N-terminal (Bac-OmpA-dN) and full-length (Bac-OmpA) OmpA protein produced in *B. subtilis* with OmpA isolated from *E. coli* (Coli-OmpA). All of these preparations were free of LPS and other outer membrane components; the gram-positive organism *B. subtilis* does not have these, and the Coli-OmpA was isolated by SDS-PAGE from *E. coli* cell envelopes treated with SDS to dissociate the tight molecular associations between outer membrane components (30). Because of the isolation procedures, the proteins were at least partially denatured; the consequences of this denaturation will be discussed shortly.

Both full-length OmpA proteins, Coli-OmpA and Bac-OmpA, behaved similarly as antigens in the EIA, detecting equal levels of anti-OmpA antibodies in several hyperimmune sera. This strongly suggests that Bac-OmpA, which is much easier to prepare in large quantities, could be used as a diagnostic antigen instead of OmpA isolated from *E. coli*. The illustration, by this EIA, of similar titers of antibodies after immunization with *E. coli* or *Salmonella typhimurium* is also a promising indicator of the broad specificity of such assays.

A high titer of antibodies to the C-terminal domain of OmpA was produced during the immune response to whole bacteria, i.e., when mice were immunized with whole, killed bacteria or infected with live bacteria. We have found this to be true also in the anti-OmpA response during enterobacterial infections in humans (Karvonen et al., submitted). At the same time, there was little antibody response to linear epitopes in other parts of the OmpA molecule as detected by EIA using the denatured truncated Bac-OmpA-dN antigen. This set-up did not allow us to evaluate the possible presence of antibodies to conformational epitopes on the surface of the outer membrane. Such an approach would have necessitated the use of whole bacteria or outer membranes as antigens, and then antibodies to other outer membrane components could have obscured the results. We have tried to recreate conformational epitopes by complexing the denatured OmpA with LPS; this has indeed been successful with both Coli-OmpA and Bac-OmpA-dN, as shown by the appearance of the ability to bind OmpA-specific bacteriophages, but the anti-OmpA titer did not increase when such complexes were used as EIA antigens (unpublished data). Recently, Henriksen et al. (9) have provided data pertinent to this question. They isolated a number of monoclonal antibodies after immunization of mice with whole formalinized bacteria. All 10 antibodies that reacted with an outer membrane preparation also reacted in Western blots, suggesting that the main antigenic epitopes were linear. Furthermore, the five OmpA-specific antibodies did not bind to intact bacteria as tested by bacterial agglutination, EIA, and fluorescent-antibody binding. These data thus confirm and extend our findings suggesting that the antibody response to OmpA, either in the denatured form or as part of intact bacteria, is directed to the C-terminal domain of the protein.

The C-terminal part of OmpA is highly conserved in the enteric bacteria (3, 4, 7); since it is the epitope efficiently recognized by antibodies formed during infection, it could be expected to be an ideal, broadly cross-reactive antigen to be used in immunodiagnosis of diseases caused by enteric bacteria. We have actually shown that Bac-OmpA can be used to diagnose both *E. coli* and *Salmonella* infections in humans (Karvonen et al., submitted); the data of Henriksen et al. (9) suggest that anti-OmpA antibodies are broadly cross-reactive within the family *Enterobacteriaceae* but not outside it. On the other hand, the immunodominance of the C-terminal domain means that anti-OmpA antibodies would not be expected to be protective since their target is not exposed on the bacterial surface. This is consistent with the inability of anti-OmpA antibodies to protect against experimental *E. coli* and *Salmonella* infections (37), although the presence of smooth LPS and capsule in the infective bacteria could have led to the same result. Actually, it is tempting to speculate that the lack of accessibility to possibly protective antibodies may have been an important factor in allowing the C-terminal part of OmpA to remain so conserved.

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LITERATURE CITED

1. **Beck, E., and E. Bremer.** 1980. Nucleotide sequence of the gene ompA coding the outer membrane protein II* of *Escherichia coli* K-12. Nucleic Acids Res. **13**:3011–3027.
2. **Beher, M. G., C. A. Schnaitman, and A. P. Pugsley.** 1980. Major heat-modifiable outer membrane protein in gram-negative bacteria: comparison with the OmpA protein of *Escherichia coli*. J. Bacteriol. **143**:906–913.
3. **Braun, G., and S. T. Cole.** 1982. The nucleotide sequence coding for major outer membrane protein OmpA of *Shigella dysenteriae*. Nucleic Acids Res. **10**:2367–2378.
4. **Braun, G., and S. T. Cole.** 1984. DNA sequence analysis of the *Serratia marcescens ompA* gene: implications for the organization of an enterobacterial outer membrane protein. Mol. Gen. Genet. **195**:321–328.
5. **Bremer, E., S. T. Cole, I. Hindenach, U. Henning, E. Beck, C. Kurz, and H. Schaller.** 1982. Export of a protein into the outer membrane of *Escherichia coli* K-12. Stable incorporation of the OmpA protein requires less than 193 amino-terminal amino-acid residues. Eur. J. Biochem. **122**:223–231.
6. **Datta, D. B., B. Arden, and U. Henning.** 1977. Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. J. Bacteriol. **131**:821–829.
7. **Freundl, R., and S. T. Cole.** 1983. Cloning and molecular characterization of the *ompA* gene from *Salmonella typhimurium*. Eur. J. Biochem. **134**:497–502.
8. **Henriksen, A. Z., and J. A. Maeland.** 1987. Serum antibodies to outer membrane proteins of *Escherichia coli* in healthy persons and patients with bacteremia. J. Clin. Microbiol. **25**:2181–2188.
9. **Henriksen, A. Z., J. A. Maeland, and O. D. Brakstad.** 1989. Monoclonal antibodies against three different enterobacterial outer membrane proteins. Acta Pathol. Microbiol. Immunol. Scand. **97**:559–568.
10. **Hofstra, H., and J. Dankert.** 1980. Major outer membrane proteins: common antigen in *Enterobacteriaceae* species. J. Gen. Microbiol. **119**:123–131.
11. **Hofstra, H., M. J. D. Van Tol, and J. Dankert.** 1980. Cross-reactivity of major outer membrane proteins in *Enterobacteriaceae* studied by crossed immunoelectrophoresis. J. Bacteriol. **143**:328–337.
12. **Hoiseth, S. K., and B. A. D. Stocker.** 1981. Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. Nature (London) **291**:238–239.
13. **Kallio, P., M. Simonen, I. Palva, and M. Sarvas.** 1986. Synthesis of OmpA protein of *Escherichia coli* K12 in *Bacillus subtilis*. J. Gen. Microbiol. **132**:677–678.
14. **Käyhty, H.** 1985. Antibody response to bacterial surface components, p. 93–108. *In* T. K. Korhonen, E. A. Dawes, and P. H. Mäkelä (ed.), Enterobacterial surface antigens: methods for molecular characterization. Elsevier/North-Holland Publishing Co., Amsterdam.
15. **Klose, M., S. MacIntyre, H. Schwarz, and U. Henning.** 1988. The influence of amino substitutions within mature part of an *Escherichia coli* outer membrane protein (OmpA) on assembly of the polypeptide into its membrane. J. Biol. Chem. **263**:13297–13302.
16. **Koskela, M., and M. Leinonen.** 1981. Comparison of ELISA and RIA for measurement of pneumococcal antibodies before and after vaccination with 14-valent pneumococcal capsular polysaccharide vaccine. J. Clin. Pathol. **34**:93–98.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Leinonen, M.** 1985. Serological methods for study of bacterial surface antigens, p. 179–206. *In* T. K. Korhonen, E. A. Dawes, and P. H. Mäkelä (ed.), Enterobacterial surface antigens: methods for molecular characterization. Elsevier/North-Holland Publishing Co., Amsterdam.
19. **Lennox, E. S.** 1955. Transduction of linked characters of the host by bacteriophage P1. Virology **1**:190–206.
20. **Lugtenberg, B., and L. van Alphen.** 1983. Molecular architecture and functioning of the outer membrane of *Escherichia coli* and other gram-negative bacteria. Biochim. Biophys. Acta **737**:51–115.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Markwell, M. A. K., S. M. Haas, L. L. Beider, and N. E. Tolbert.** 1978. Modification of Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
23. **Morona, R., M. Klose, and U. Henning.** 1984. *Escherichia coli* K-12 outer membrane protein (OmpA) as a bacteriophage receptor: analysis of mutant genes expressing altered proteins. J. Bacteriol. **159**:570–578.
24. **Muotiala, A., M. Hovi, and P. H. Mäkelä.** 1989. Protective immunity in mouse salmonellosis induced by both smooth and rough, live and killed vaccines. Microb. Pathog. **6**:51–60.
25. **Nicolle, L. E., E. Ujak, J. Brunka, and L. E. Bryan.** 1988. Immunoblot analysis of serologic response to outer membrane proteins of *Escherichia coli* in elderly individuals with urinary tract infections. J. Clin. Microbiol. **26**:2087–2091.
26. **Nikaido, H., M. Levinthal, K. Nikaido, and K. Nakane.** 1967. Extended deletions in the histidine-rough-b region of the Salmonella chromosome. Proc. Natl. Acad. Sci. USA **57**:1825–1832.
27. **Nikaido, H., and M. Vaara.** 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev. **49**:1–32.
28. **Palva, E. T.** 1978. Major outer membrane protein in *Salmonella typhimurium* induced by maltose. J. Bacteriol. **136**:286–294.
29. **Palva, I., M. Sarvas, P. Lehtovaara, M. Sibakov, and L. Kääriäinen.** 1982. Secretion of *Escherichia coli* β-lactamase from *Bacillus subtilis* by the aid of α-amylase signal sequence. Proc. Natl. Acad. Sci. USA **79**:5582–5586.
30. **Sarvas, M., and M. Nurminen.** 1985. Polyacrylamide gel electrophoretic analysis of cell envelope proteins, p. 123–137. *In* T. K. Korhonen, E. A. Dawes, and P. H. Mäkelä (ed.), Enterobacterial surface antigens: methods for molecular characterization. Elsevier/North Holland Publishing Co., Amsterdam.
31. **Sippel, J. E., H. K. Mamay, E. Weiss, S. W. Joseph, and W. J. Beasley.** 1978. Outer membrane protein antigens in an enzyme-linked immunosorbent assay for *Salmonella* enteric fever and meningococcal meningitis. J. Clin. Microbiol. **7**:372–378.
32. **Valtonen, V. V.** 1970. Mouse virulence of *Salmonella* strains: the effect of different smooth-type O-side chains. J. Gen. Microbiol. **64**:255–268.
33. **van Alphen, L., L. Havekes, and B. Lugtenberg.** 1977. OmpA major outer membrane protein *d* of *Escherichia coli* K12. FEBS Lett. **75**:285–290.
34. **Vogel, H., and F. Jähnig.** 1986. Models for the structure of outer-membrane proteins of *Escherichia coli* derived from Raman spectroscopy and prediction methods. J. Mol. Biol. **190**:191–199.
35. **Voller, A., D. E. Bidwell, and A. Bartlett.** 1979. The enzyme linked immunosorbent assay. Dynatech Europe, Borough House, Guernsey, England.
36. **Vuopio-Varkila, J.** 1987. Experimental *Escherichia coli* peritonitis in immunosuppressed mice: the role of specific and nonspecific immunity. J. Med. Microbiol. **24**:33–39.
37. **Vuopio-Varkila, J., M. Karvonen, and H. Saxén.** 1988. Protective capacity of antibodies to outer-membrane components of *Escherichia coli* in a systemic mouse peritonitis model. J. Med. Microbiol. **25**:77–84.