Antigenic Properties of Porins from *Yersinia* Outer Membranes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 6, pp. 657-660, June, 1996
Original article submitted April 5, 1995

The pore-forming proteins porins isolated from *Yersinia pseudotuberculosis* and *Y. enterocolitica* outer membranes and purified were found, by means of enzyme-linked immunosorbent assays, to be genus-specific antigens. Antiporin antibodies were detected in rabbit antisera to the isolated proteins as well as to total outer membrane proteins and whole bacterial cells. Enzyme-linked immunosorbent and immunoblotting assays demonstrated an antigenic relatedness of porins from five *Yersinia* species.

Key Words: *Yersinia porins; antigenic relatedness; enzyme-linked immunosorbent assays*

The pore-forming proteins (porins) present in the outer membrane of gram-negative bacteria have pores allowing for transmembrane transfer of hydrophilic substances [8], and some also serve as receptors for bacteriophages [8]. Being among the major surface antigens of bacterial cells, porins play an important role in the pathogenesis of infection by promoting adhesion of bacteria to (and possibly their invasion into) host cells [3] and affecting the immune response [5,9].

Recent years have witnessed mounting interest in the immunochemical and immunobiological properties of outer membranes proteins, including porins, found in gram-negative bacteria. Porins from several members of the family Enterobacteriaceae have been shown to be antigenically related [6,13], and variations in biological activity among surface epitopes of outer membrane proteins have been reported [16]. However, information on the antigenic structures of porins in the genus *Yersinia* is scarce [2,10].

Identification of antigenically related surface components on the outer cell membrane within Enterobacteriaceae genera may provide a basis for the development of new diagnostic agents and vaccines with wide ranges of action that can be used in the control of intestinal infections.

The purpose of this study was to examine cross-reactions between *Yersinia* porins.

MATERIALS AND METHODS

Peptidoglycan-protein complexes were isolated from *Yersinia pseudotuberculosis* and *Y. enterocolitica* outer membranes as described by Rosenbusch [14]. Porins were obtained in an oligomeric form by dissociation of these complexes in the presence of 1% sodium dodecyl sulfate (SDS) and 0.5 M NaCl [12] at 37°C (to obtain a trimer from *Y. pseudotuberculosis*) or at 22°C (to obtain a trimer from *Y. enterocolitica*) and in a monomeric form, by their dissociation in the presence of 2% SDS at 100°C [14]. Further purification of the protein preparations was with Sephacryl C-200 gel chromatography in the presence of 0.25% SDS, and the degree of purification was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [11]. Gel-separated proteins were stained with a Coomassie Brilliant Blue G 250 solution in 3.5% perchloric acid [1]. Monosaccharides were determined by the standard procedure [4] and protein, by a modification of Lowry's method in the presence of 2% SDS [7].

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0007-4888/96/0006-0593$15.00

Rabbit antisera to the isolated and purified proteins and to total outer membrane proteins and whole bacterial cells were obtained as previously described (2-3 rabbits were immunized with each antigen) [2]. Enzyme-linked immunosorbent assays (ELISA) were carried out using the standard procedure [2]. Immune serum titers were determined in three replicate assays (the mean absorbance of test samples exceeded threefold the absorbance of control samples).

The *Yersinia* porins obtained were compared in immunoblotting assays [15]. Protein electrotransfer from the gel to nitrocellulose was effected at 100 V and 4°C for 3 h in a Tris-HCl buffer (pH 10.3) containing 20% methanol and 0.1% SDS. Blots were incubated for 2 h with rabbit sera diluted 50- to 100-fold and with 1:1000 dilutions of peroxidase-conjugated rabbit immunoglobulins (produced at the N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow) in a Tris-saline buffer (pH 10.3) supplemented with 0.05% egg albumin and 0.05% Tween-20, using as substrate 0.05% 4-chloro-1-naphthol (Sigma) in 1 M acetate buffer (pH 6.0) containing 17% methanol and 0.03% H_2O_2. The reaction was continued for 10-30 min in the presence of diphenylenediamine (1 mg/ml) and sodium metabisulfate (0.5 mg/ml) to increase the staining intensity. All procedures were carried out at 37°C with agitation.

RESULTS

The homogeneity of the *Y. pseudotuberculosis* and *Y. enterocolitica* porin preparations was verified with SDS-PAGE. The oligomeric porin forms (trimers) from both *Yersinia* species had similar molecular weights (105-110 kD). Heating at 100°C for 5 min converted the *Y. pseudotuberculosis* and *Y. enterocolitica* trimers to denatured monomers with apparent molecular weights of 40.0 and 39.4 kD, respectively. The porin preparations purified with Sephacryl gel chromatography contained 75-80% protein and not more than 5% monosaccharides.

Both molecular forms (trimers and monomers) of *Y. pseudotuberculosis* and *Y. enterocolitica* porins were immunogenic for rabbits. As shown by ELISA, immunization of rabbits with the porin oligomers gave rise to antisera with a titer of 1:51,200, while their immunization with the porin monomers yielded antisera with a titer of 1:102,000. The lipopolysaccharides from *Y. pseudotuberculosis* and *Y. enterocolitica* bacteria of the corresponding serovars reacted with the indicated antisera at background levels. Antiporin antibodies were also produced when rabbits were immunized with total outer membrane proteins or with whole bacterial cells (Fig. 1, *a* and *b*, and Fig. 2, *b* and *c*). Porins from any given *Yersinia* species proved to be antigens reactive with antisera to different bacterial serovars of the same species. For example, the porin from *Y. pseudotuberculosis* serovar 1 reacted with antisera to *Y. pseudotuberculosis* serovars 1 through 6 with a titer of 1:12,800, while the porin from *Y. enterocolitica* 0:3 reacted with antisera to 23 *Y. enterocolitica* serovars with titers of 12,800 and 25,600 (the titers of both proteins in ELISA with antisera to the isolated homologous porins were equal, 51,200).

Moreover, the *Yersinia* species examined were all found to be antigenically related. ELISA indicated

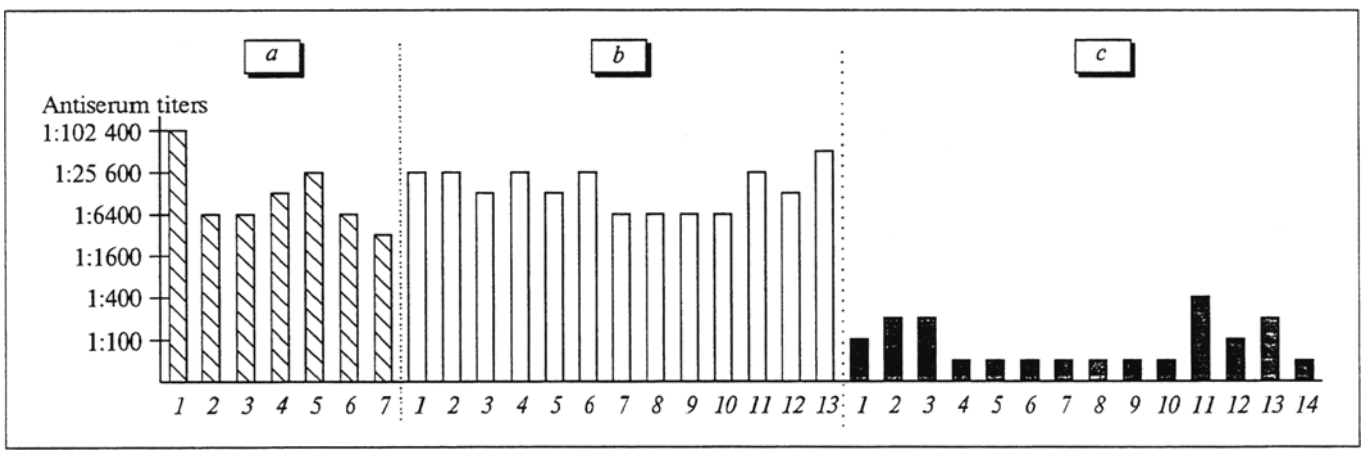

Fig. 1. ELISA of rabbit antisera to Enterobacteriaceae and Brucellaceae bacteria using *Y. pseudotuberculosis* and *Y. enterocolitica* porins. *a*) *1*: antiserum to *Y. pseudotuberculosis porin*; *2–7*: antisera to *Y. pseudotuberculosis* serovars 1–6, respectively. *b*) *1–6*: antisera to *Y. enterocolitica* serovars 3, 5, 8, 9, 6.30, and 6.31, respectively; *7–10*: antisera to *Y. kristensenii* serovars 16.f, 28.d, 11.246.c, and 12.25.e; *11*: antiserum to *Y. intermedia* serovar 17; *12*: antiserum to *Y. frederiksenii* serovar 5; *13*: antiserum to *Y. pseudotuberculosis* porin (the antigen was *Y. pseudotuberculosis* porin). c) antisera to *Shigella dysenteriae* (*1*), *S. sonnei* (*2*), *S. flexneri* (*3*); *Salmonella ABCDE* (*4*), *S. typhimurium* (*5*), *S. paratyphi B* (*6*), *S. paratyphi A* (*7*), *S. anatum* (*8*), *S. heidelberg* (*9*), *S. enteritidis* (*10*), *S. choleraesuis* (*11*); *Escherichia coli* (*12*); *Brucella* sp. (*13*); *14*: normal rabbit serum (the antigen was *Y. enterocolitica* porin).

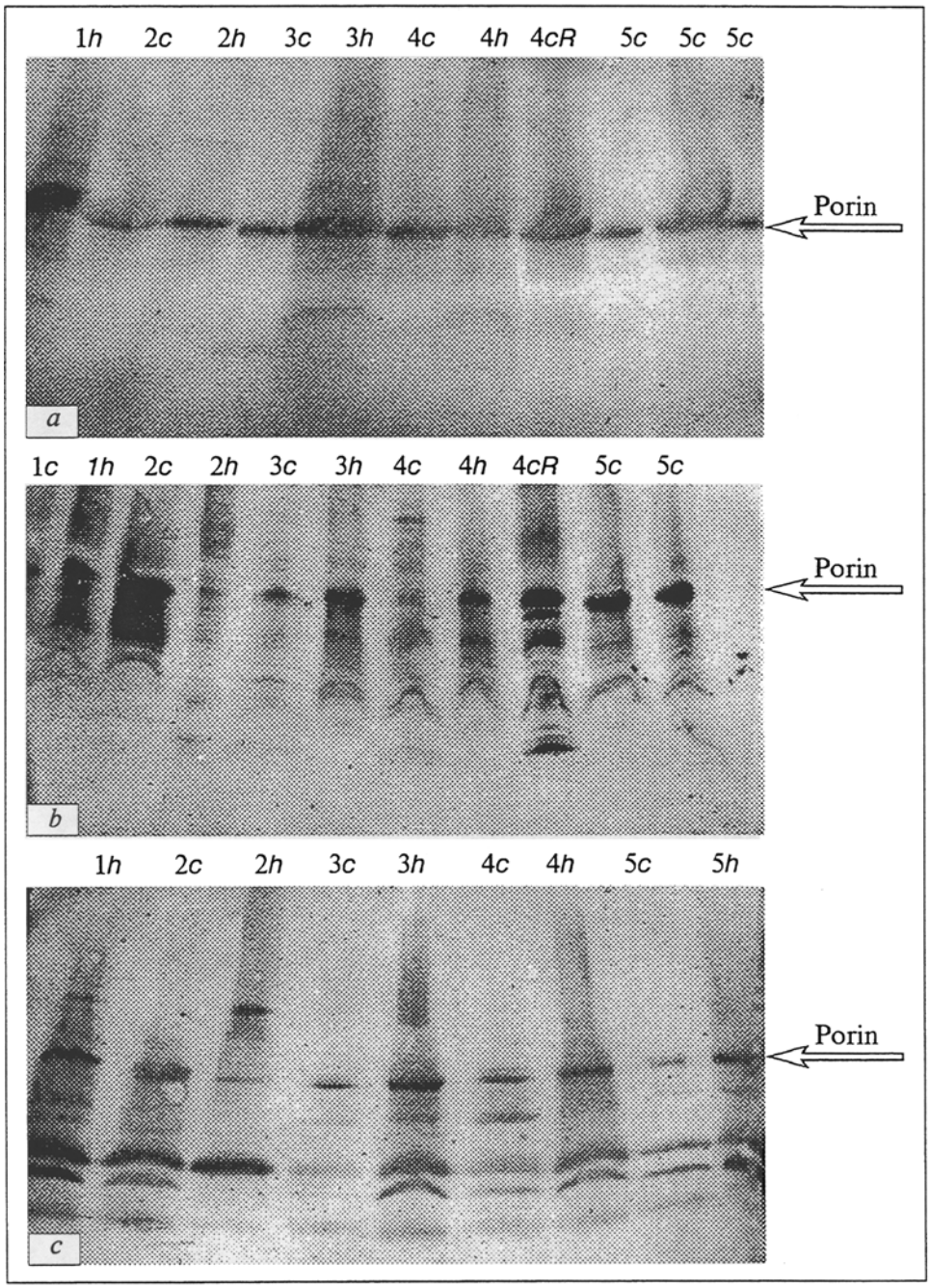

Fig. 2. Immunoblotting assays of sera from rabbits immunized with the monomeric form of *Y. pseudotuberculosis* porin (*a*), total *Y. pseudotuberculosis* outer membrane proteins (*b*), or whole *Y. kristensenii* cells (*c*). Shown are electrophoregrams of porin monomers from *Y. intermedia* (*1*), *Y. enterocolitica* (*2*), *Y. kristensenii* (*3*), *Y. frederiksenii* (*4*), and *Y. pseudotuberculosis* (*5*). Micoroorganisms were grown at 4–6°C (*c* = "cold" variant) or at 37°C (*h* = "hot" variant); *R* = repeat.

that the *Y. enterocolitica* porin detected antibodies in the blood of animals immunized with *Y. pseudotuberculosis* serovars 1-6 (Fig. 1, *a*), and that the *Y. pseudotuberculosis* porin reacted with sera to *Y. enterocolitica* bacteria of various serovars, as well as with sera against *Y. kristensenii*, *Y. intermedia*, and *Y. frederiksenii* (Fig. 1, *b*). However, the cross-reactions of these proteins with antibodies to some members of the families Enterobacteriaceae and Brucellaceae occurred at much lower levels (Fig. 1, *c*).

In order to confirm that the *Yersinia* porins are genus-specific, thermostable proteins isolated from bacteria of five *Yersinia* species were used in immunoblotting assays of rabbit antisera to the *Y. pseudotuberculosis* porin, total *Y. pseudotuberculosis* outer membrane proteins, and whole *Y. kristensenii* cells.

Analysis of the immunoblots (Fig. 2) showed that the antisera detected all of the *Yersinia* porins.

Yersinia porins are thus genus-specific antigens giving only low levels of cross-reactions with other members of the family Enterobacteriaceae. The findings from this study open a way to developing *Yersinia* porin-based test systems sufficiently specific and sensitive for use to identify *Yersinia* infections among other intestinal diseases and to devise species- or genus-specific vaccine preparations.

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