STRUCTURAL ANALYSIS OF THE VARIABLE MAJOR
PROTEINS OF *BORRELIA HERMSII*

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Borrelia hermsii, a spirochete and an agent of relapsing fever, undergoes spontaneous antigenic variation both in vivo (1, 2) and in vitro (2). The rate of serotypic change is estimated to be 10^{-3}–10^{-4} per cell per generation (2). The antigenic repertoire of *B. hermsii* (2) may be as extensive as that of another vector-transmitted and blood-borne pathogen, *Trypanosoma brucei*, a eucaryote (3, 4).

Examination of four variants or serotypes found among the progeny of a single *B. hermsii* organism revealed that each serotype had an abundant protein of unique apparent molecular weight (5). Immunofluorescence and radioiodine-labeling studies indicated that these variable proteins, the pI proteins, were located on the surface. Serotype-specific monoclonal antibodies bound to homologous but not heterologous pI proteins (5). The pII protein was the other major protein found in whole cell lysate of each serotype (5). In contrast to pI proteins, the pII proteins had the same apparent molecular weight in each serotype, were less accessible than the pI proteins for iodine labeling, and were recognized in western blots by heterologous as well as homologous polyclonal antiserotype sera (5).

As it appears likely that antigenic variation is based on the spontaneous appearance in a borrellia population of an organism possessing an antigenically "novel" pI protein, we are studying the structure of the pI proteins. Of particular importance for models of the genetic mechanism of antigenic variation is the amount of structural relatedness among the different pI proteins.

We analyzed the pI and pII proteins of serotypes C, 7, and 21 with one- and two-dimensional (1-D, 2-D)[1] peptide-mapping procedures. The results indicated that whereas all pII proteins are identical, the pI proteins have little, if any, apparent sequence homology among them.

Materials and Methods

Organisms. The origin of *B. hermsii* HS1 and the derivation of the different serotypes have been previously described (2). Serotypes 7 and 21 were passed from frozen stocks

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[1] *Abbreviations used in this paper:* MWS, molecular weight standards; 1-D, 2-D, one- and two-dimensional; PAGE, polyacrylamide gel electrophoresis; PBS/Mg, phosphate-buffered saline with 5 mm MgCl₂; SDS, sodium dodecyl sulfate.

(5) into adult BALB/c mice that had been x-irradiated (900 rad) 1–3 h before intraperitoneal inoculation. 3–4 d later, heavily spirochetemic mice were exsanguinated by aseptic intracardiac puncture, and 0.2 ml of citrated blood was added to tightly capped plastic culture tubes (3033; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) containing 9 ml of BSK medium (6). After 2–3-d incubation at 35°C, 1-μl loopfuls of medium from individual cultures were removed, placed under an 18 × 18-mm coverglass, and examined by phase-contrast microscopy. The concentration of spirochetes was estimated by counting organisms in ten 640× fields. The medium in tubes containing moderate to heavy growth (10^7–10^8 borreliae/ml) was aspirated, taking care to avoid the blood elements and fibrin clots at the bottom of the tubes. The aspirated contents of 5–20 tubes were pooled and transferred to capped, 650-ml capacity glass bottles containing 532 ml BSK medium. These batch cultures were incubated at 35°C for 3–5 d before harvest. Populations of the culture-adapted serotype C had been passed continuously in vitro (2, 5). Serotype C organisms were transferred directly without animal passage from tube cultues to batch cultures.

At harvest, the broth was centrifuged at 9,500 g for 20 min at 20°C, and the pellet was resuspended in 1/50 vol of M/15 phosphate-buffered saline, pH 7.4, with 5 mm $MgCl_2$ (PBS/Mg). Glycerol was added to a final concentration of 16% (vol/vol) to borrelial suspensions to be stored at −76°C. Fresh, i.e., never frozen, spirochetes or thawed spirochetes were centrifuged and washed twice with PBS/Mg before use. In this procedure and in others described below, unless otherwise indicated, "centrifuged" means a 3-min centrifugation in a Microfuge (Beckman Instruments, Inc., Fullerton, CA) and "washed" means resuspension in 1 ml of PBS/Mg.

Serotype homogeneity of the harvests was assessed by direct immunofluorescence with polyclonal antisera (2) and by indirect immunofluorescence with monoclonal antibodies (5; A. G. Barbour and S. L. Tessier, manuscript in preparation). Only harvests estimated to have <2% "contamination" with heterologous serotypes (usually serotype C) were used in experiments described below. The protein content of washed spirochetes in suspension was determined by the Bradford method (7).

Polyacrylamide Gel Electrophoresis (PAGE). Cell suspensions in water were solubilized in sodium dodecyl sulfate (SDS) and subjected to the PAGE system of Laemmli and Favre (8). The conditions of solubilization and electrophoresis have been previously described (5); the pH of the separating gel buffer was 8.6. After electrophoresis, gels were fixed in 50% methanol-10% acetic acid for 1 h, stained in 0.125% Coomassie brilliant blue-50% methanol-10% acetic acid for 2 h, and destained in 50% methanol-10% acetic acid followed by 5% methanol-10% acetic acid. Molecular weight standards (MWS) labeled with ^{14}C were phosphorylase B (93,000 mol wt [93 K]), bovine albumin (69 K), ovalbumin (46 K), carbonic anhydrase (30 K), and β-lactoglobulin (18 K) (New England Nuclear, Boston, MA).

Labeling of Borreliae with [^{14}C]Leucine. Fresh, washed borreliae were resuspended at a concentration of 1–2 × 10^9/ml in a RPMI 1640-based labeling medium that lacked leucine (9). [^{14}C]leucine (10 μCi; 3 μg; New England Nuclear) was added to 1.0 ml of the suspension. The cells and label were incubated for 4 h at 35°C. The spirochetes were harvested by centrifugation, washed twice with PBS/Mg, and resuspended in SDS-PAGE sample buffer. To examine the labeled proteins, the whole cell lysates were subjected to SDS-PAGE. Gels were fixed as described above and analyzed by fluorography as described by Bonner and Laskey (10). Dried fluorograms were exposed to Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY) at −76°C.

1-D Peptide Mapping. The procedure used for peptide mapping by limited proteolysis and PAGE was essentially that described by Cleveland et al. (11), with some modification. 15 μl samples of borrelial lysates in PAGE buffer (2 × 10^4 cpm of ^{14}C-labeled borreliae or 10 μg protein of unlabeled borreliae) were subjected to PAGE; the acrylamide concentrations were 7.5% for serotypes 7 and 21 and 10% for serotype C. The gels were fixed, stained, and destained as described above. The bands of interest were excised from gels, which were in 5% methanol-10% acetic acid, and placed in 1 ml of 0.125 M Tris/HCl, pH 6.8, 0.1% SDS, and 1 mM EDTA. After 60 min and one change of buffer, the slices

were put at the bottom of sample wells of a gel consisting of a 2-cm stacking gel of 6% acrylamide (30:0.8, wt/wt, acrylamide/bisacrylamide) and a 9-cm separating gel of 20% acrylamide (30:0.1, wt/wt, acrylamide/bisacrylamide). Each gel fragment was overlaid with 10 μl of 0.125 M Tris/HCl, pH 6.8, 0.1% SDS, 1 mM EDTA, 10% glycerol, 0.001% bromphenol blue, and 0, 8, or 40 μg/ml of *Staphylococcus aureus* V8 protease (Miles Laboratories Inc., Elkhart, IN). Electrophoresis was carried out at 10 mA constant current until the dye marker had migrated 1.5 cm into the stacking gel. The electrophoresis was stopped for 30 min and then continued at 10 mA until the dye markers entered the separating gel. The current was increased to 25 mA and the electrophoresis continued for another 2.5–3 h. After fixation, gels containing unlabeled proteins were processed using the silver stain of Poehling and Neuhoff (12). Gels with ^{14}C-labeled proteins were analyzed by fluorography. In addition to the MWS described above, we also used, in 20% acrylamide gels, prestained bovine trypsin inhibitor (6 K) and insulin (3 K) (Bethesda Research Laboratories, Inc., Rockville, MD) as markers.

Surface-labeling of Borreliae with Radioactive Iodine. Fresh, washed borreliae were surface labeled with ^{125}I in the presence of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce Chemical Co., Rockford, IL) (13). A pellet of borreliae ($\sim$0.5 mg protein) was resuspended in 50 μl of PBS/Mg and transferred to a glass tube containing 10 μg Iodogen. ^{125}I (0.5 mCi as NaI, 50 μCi/μl; ICN Pharmaceuticals, Inc., Irvine, CA) was added. After 5 min at room temperature and occasional shaking, the suspensions were removed to a microfuge tube containing 900 μl PBS/Mg and 50 μl KI (10 μg/ml). The cells were centrifuged and washed with PBS/Mg twice before final suspension of the pellet in PAGE sample buffer. Autoradiography was performed with X-Omat AR film on wet or dried gels.

Labeling of Proteins in Gel Fragments with Radioactive Iodine. Coomassie brilliant blue-stained bands representing proteins pI and pII were excised from 7.5% (serotypes 7 and 21) or 10% (serotype C) acrylamide gels and radiolabeled by the chloramine-T procedure (14, 15). Electrophoresis had been performed the previous day, and after fixation, staining, and destaining, the gels had been placed in 7% acetic acid for 1–3 h before slicing of gel. The gel fragments were further soaked for 1 h in 7% acetic acid and then in distilled water for 1 h. The slices were then dried in a Savant Speed-Vac (Savant Instruments, Inc., Hicksville, NY). A 10-μl vol of chloramine-T solution (1 mg/ml) was added to the gel slice. Then 100 μCi of ^{125}I (as NaI at 50 μCi/μl) was added and the reaction allowed to proceed for 1 h at room temperature. The reaction was terminated by adding 1 ml of sodium metabisulfite (1 mg/ml). After 15 min, unbound ^{125}I was removed by soaking the gel slices in a slurry of Dowex 1X-8 (20–50 mesh) anion-exchange resin (Bio-Rad Laboratories, Richmond, CA) in 2.5 ml 15% methanol. The gel slices were removed after 24 h and dried in the Speed-Vac. Surface-iodinated protein bands, which had been identified by Coomassie brilliant blue staining and by autoradiography of wet gels, were excised and treated as above except that unlabeled NaI was added.

2-D ^{125}I-Labeled Peptide Mapping. The dried gel slices were rehydrated in 250 μl of 50 mM NH$_4$HCO$_3$ buffer, pH 8.5. A 25-μl vol of α-chymotrypsin solution (1 mg/ml of 0.01 N HCl; Calbiochem-Behring Corp., La Jolla, CA) was added. After 4 h at 37°C, the supernatant containing the ^{125}I-labeled peptides was aspirated and frozen. This process was repeated four times. The pooled supernatants were dried and washed eight times with distilled water. High voltage thin-layer electrophoresis in the first dimension and ascending thin-layer chromatography in the second dimension were performed as previously described (14, 15). Autoradiography was performed with X-Omat AR film. We compared the peptide patterns of different proteins, which were examined on the same day, by adjusting the location of radioemitting peptides in reference to a standard included in each map, the ninhydrin-stained spot of isoleucine. Composite, idealized peptide maps of two or more proteins were produced by expressing the vectors, i.e., angles and distances from origin, of a given peptide in terms relative to the vector of isoleucine.

Western Blot Analysis with Rabbit Antisera to Excised Gel Fragments. After PAGE (10% acrylamide) of a serotype C lysate, the gels were briefly stained (14) to identify pI$_C$ and pII proteins. Slices containing these bands were emulsified first in 0.1% SDS and then in

complete Freund's adjuvant. New Zealand white rabbits were injected intramuscularly with the emulsion. 35 d later the rabbits were injected subcutaneously with a similar preparation containing incomplete Freund's adjuvant instead of complete. Rabbits were bled 8 d later. Harlan Caldwell (Rocky Mountain Laboratories) provided sera from rabbits that had been immunized in like manner to ovalbumin-containing slices from PAGE gels. Reactivity of the antibodies against whole cell proteins was determined by a previously described modification (5) of the western blot procedure of Towbin et al. (16).

Results

We used two "finger-printing" techniques, 1-D and 2-D peptide mapping, to analyze the structure of the pI proteins. The pII proteins from each of the three serotypes were also examined, because the molecular weight invariability and antigenic cross-reactivity of these proteins suggested that they had similar structure (5) and could, therefore, probably serve as controls in the various procedures.

Fig. 1 (first panel) shows the relative electrophoretic mobilities of the pI proteins in PAGE. The apparent subunit molecular weights of pI_C, pI_7, and pI_{21} were 21,000, 40,000, and 38,000, respectively, in this gel. Each preparation exhibited a pII protein; all had apparent molecular weights of 41,000. Use of a 7.5% acrylamide gel permitted discriminating excision of pI_7 and the pII (Fig. 1, second panel).

Fig. 1 (third panel) is a fluorogram of [^{14}C]leucine-labeled proteins that have been separated by PAGE. The pI proteins were the most abundantly labeled
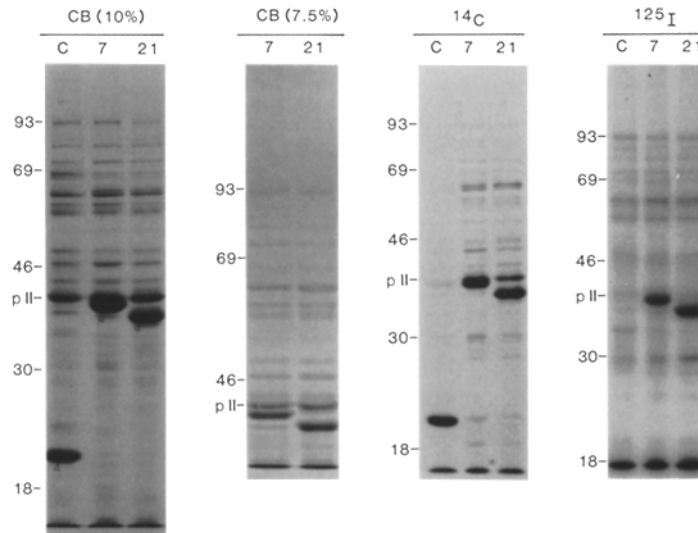

FIGURE 1.   Coomassie blue-stained (CB) PAGE gels and autoradiographs (^{14}C and ^{125}I) of dried PAGE gels of *B. hermsii* HS1 serotypes C, 7, and 21. Gels contained either 10% acrylamide (first, third, and fourth panels) or 7.5% acrylamide (second panel). Borrelia proteins were labeled intrinsically with [^{14}C]leucine (^{14}C) or extrinsically with ^{125}I in the presence of Iodogen (see text). The locations of the pI proteins in each serotype are indicated by open triangles in the CB (10%) panel. The location of the pII proteins in each gel is indicated to the left of each panel. The MWS were phosphorylase B (93 K), bovine ablumin (69 K), ovalbumin (46 K), carbonic anhydrase (30 K), and β-lactoglobulin (18 K), and their relative mobilities are shown to the left of each gel.

proteins after the 4-h incubation of cells with [^{14}C]leucine. 4 h is one-third of the approximate generation time of *B. hermsii* HS1 (A. G. Barbour, unpublished observation). Compared with the amount of pII proteins revealed by Coomassie brilliant blue stain in gels, there was relatively less radioemission by the pII proteins. The differences between the amounts of [^{14}C]leucine in the pI protein and in the rest of the proteins was greatest in serotype C cells.

Fig. 1 (fourth panel) demonstrates the radioiodination of pI$_7$ and pI$_{21}$ under conditions in which surface-exposed proteins are more likely to take up available ^{125}I than are cytoplasmic proteins (20). As expected, there was relatively little incorporation of ^{125}I by pII proteins, but we found poor or no labeling of pI$_C$ as well. Similar results were obtained when vectorial radioiodination of pI$_C$ with solid-phase chloramine-T (17) was attempted (data not shown).

1-D Peptide Mapping. Limited proteolysis of labeled or unlabeled pI and pII proteins contained in excised gel fragments yielded peptides that were separated one-dimensionally by PAGE and identified by fluorography or silver stain (Fig. 2*A* and *B*). We found that for a given protein the two methods revealed peptides that were virtually identical in their patterns. There were more minor bands in the silver-stained gels than in the fluorograms. Repeat experiments gave peptide patterns that were very similar to those shown in Fig. 2. The location of the V8 protease in the silver-stained gels was determined through incubation of the protease with a gel slice that did not contain any protein.

The patterns of the peptides produced by exposure of the pI proteins to either 0.08 or 0.4 μg of protease were unique for each pI protein (Fig. 2*A*). There were only four pairs of peptides among the 30 or so major peptides of pI$_C$, pI$_7$, and pI$_{21}$ that had similar migrations in the maps of two pI proteins. The apparent molecular weights of these peptide pairs (and the respective pI proteins) were 15,000 (pI$_C$ and pI$_{21}$), 8,000 (pI$_C$ and pI$_7$), 6,000 (pI$_7$ and pI$_{21}$), and 5,000 (pI$_C$ and pI$_7$). Moreover, no discrete peptide was shared by all three pI proteins. The apparent greater protease susceptibility of pI$_{21}$ in comparison with pI$_C$ and pI$_7$ was seen in repeat experiments. The minor band below pI$_{21}$ in the protease-free sample of the silver-stained gel was present in the analogous lane of fluorograms exposed to film for longer periods of time.

The 1-D maps of pII proteins from serotypes C, 7, and 21 were identical to one another in the silver-stained gels (Fig. 2*B*). We did not attempt fluorography because of the relatively poor labeling of the pII proteins with [^{14}C]leucine. Like pI$_{21}$, the pII proteins were very susceptible to V8 protease and appeared to have been cleaved to some extent in the absence of added protease during the interval between the first and second PAGE gels. The cleavage of pII without protease was even more extensive than that of pI$_{21}$, and the patterns of resultant peptides were the same in serotypes 7 and 21 as in serotype C.

2-D Peptide Mapping. The results of the 1-D peptide mapping experiments led us to examine, using a 2-D mapping procedure, more exhaustively proteolyzed proteins. Our initial 2-D maps showed much apparent homology between the pI proteins. However, these experiments were performed using gels that had been stored in 7% acetic acid for weeks before the excision of bands. It was difficult to recover radioactivity from the gel fragments, and long film exposures were required. Subsequent studies and those shown here were performed on
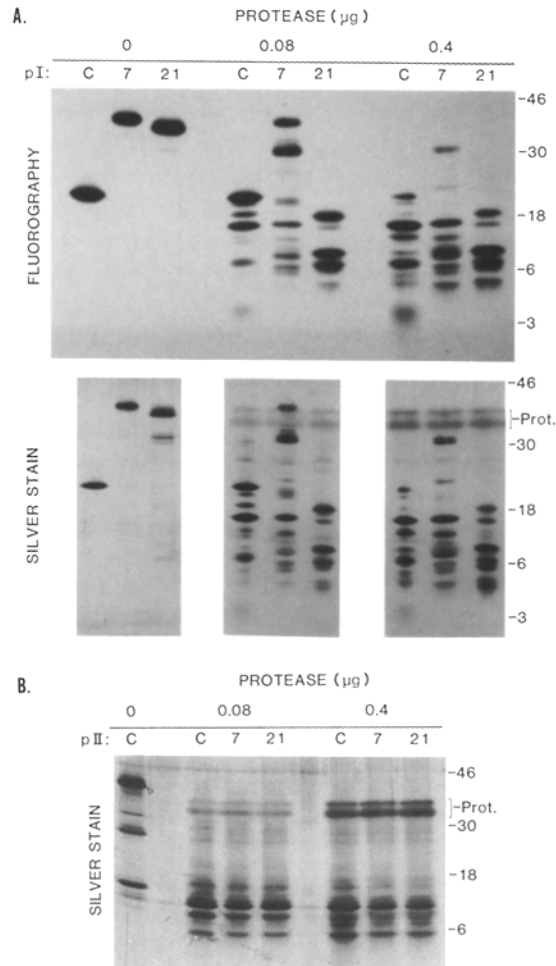
FIGURE 2.   1-D peptide maps of pI and pII proteins of *B. hermsii* HS1 serotypes C, 7, and 21. See text for methods. Proteins were incubated with 0, 0.08, or 0.4 μg of *S. aureus* V8 protease. The locations of the MSW are shown at the right. In addition to the standards of Fig. 1, we also used bovine trypsin inhibitor (6 K) and insulin (3 K; see text). (*A*) pI proteins and their peptides visualized by fluorography of intrinsically [^{14}C]leucine-labeled proteins or by silver stain of unlabeled proteins. (*B*) pII protein of serotype C and the peptides of pII proteins from serotypes C, 7, and 21 visualized by silver stain. There is cleavage of the pII protein in the absence of added protease (left-most lane). The open triangle indicates the location of uncleaved pII protein.

gels that had been in fixative no longer than 16 h. In the cases of pI$_C$ and pII$_C$, nearly identical 2-D maps to those shown in Figs. 3 and 4 were obtained with pI$_C$ and pII$_C$ proteins that had been purified by column chromatography instead of PAGE (A. Barbour, P. Barstad, and O. Barrera, unpublished observations).

Isoleucine, a neutral, nonpolar amino acid, was an internal marker and served as a reference point for standardization of the maps. As was the finding with the 1-D maps, the 2-D maps of pII proteins from serotypes C, 7, and 21 were essentially identical (Fig. 3). There were differences in the intensity of the
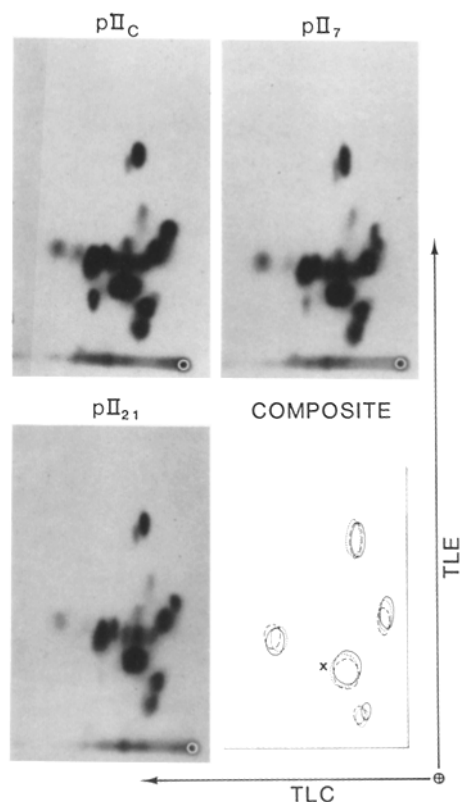
FIGURE 3. 2-D peptide maps of chloramine-T-radioiodinated pII proteins of *B. hermsii* HS1 serotypes C (pII$_C$), 7 (pII$_7$), and 21 (pII$_{21}$). pII bands were excised from PAGE gels, radioiodinated with chloramine-T, and digested with α-chymotrypsin. The digestion sample was applied at the location indicated by the open white circle in the lower right of each map. Thin-layer electrophoresis (TLE) was performed in the 1-D (the cathode is at the top of the figure). Thin-layer chromatography (TLC) was performed in the 2-D (toward the left in the figure). The locations of selected major peptides of pII proteins from serotypes C (———), 7 (———), and 21 (———) in an idealized, composite map is shown at the lower right. The migrations of the peptides were adjusted with respect to the marker isoleucine, indicated by an "X" in the map (see text).

emission of a given peptide seen in each of the three maps, but we did not find any major peptide that could not be accounted for in all maps. A selection of five peptides, which have been standardized as to migration with reference to isoleucine, is idealistically shown in the last panel of Fig. 3.

The 2-D peptide maps of the pI proteins confirmed the 1-D map results (Fig. 4). There were few peptides that conceivably were shared by any two of pI proteins and no major peptide that was shared by all three pI proteins. The composite of several major peptides selected from the maps pI$_C$, pI$_7$, and pI$_{21}$ and standardized with reference to isoleucine shows the dissimilarities between the patterns and the possibly identical peptides (Fig. 4). In general, there was less overlap between peptides in the hydrophilic regions of the maps (18). Because pI$_{21}$ had more detectable major peptides than pI$_7$, we considered the possibility that a co-migrating protein had been proteolysed at the same time as pI$_{21}$ during
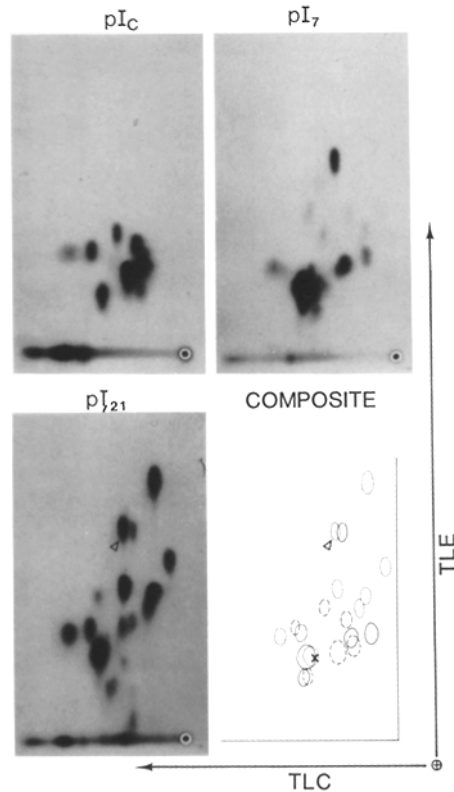
FIGURE 4.   2-D peptide maps of chloramine-T-Radioiodinated pI proteins of *B. hermsii* HS1 serotypes C (pI$_C$), 7 (pI$_7$), and 21 (pI$_{21}$). See legend to Fig. 3 for methods, conventions, and abbreviations. Open triangles indicate which of two similarly migrating pI$_{21}$ peptides were used for the composite map. The peptide to the right of the one indicated by the triangle in the pI$_{21}$ maps migrates similarly to a peptide in the pI$_7$ map.

the procedure. A minor protein does migrate with about the same mobility as pI$_{21}$; this protein was seen in lanes containing serotypes C or 7 (Fig. 1, first panel). However, when this minor band was excised, proteolysed, and mapped separately, we found that this protein contributed only a few faint spots to the pI$_{21}$ map (data not shown).

The surface-labeled pI$_7$ and pI$_{21}$ proteins also gave 2-D maps with different patterns (Fig. 5). A map of pI$_C$ was not attempted because of the poor Iodogen-mediated labeling of pI$_C$ (see above). The migrations of the major peptides were standardized with reagent to isoleucine, and the resultant idealized map was compared with the composite map shown in Fig. 4. Although a peptide from each of pI$_7$ and pI$_{21}$ did correspond in migration with respective peptides seen in maps of chloramine-T labeled, SDS-denatured proteins (identified by arrows in Fig. 5), we found, as have others (19, 20), that there may be little correlation in the patterns of a protein that had been labeled under these different conditions.

Western Blotting.   To see if detergent-mediated denaturation of pI$_C$ could expose a hypothetical conserved region that pI$_C$ shared with other pI proteins, we immunized rabbits against pI$_C$ and pII that had been separated by PAGE and
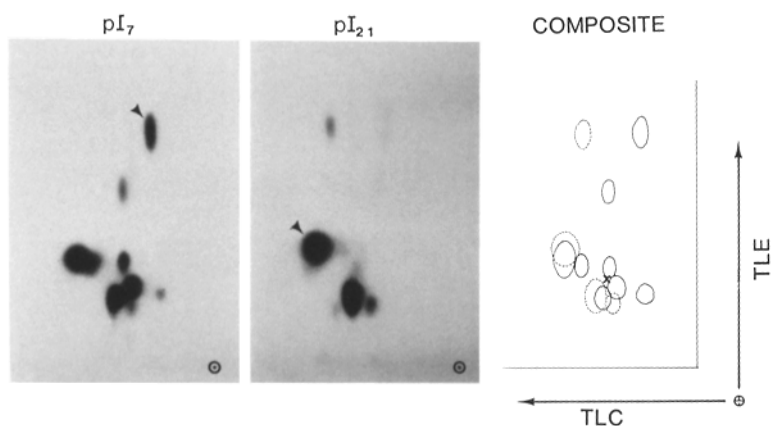
FIGURE 5. 2-D peptide maps of surface-radioiodinated pI proteins of *B. hermsii* HS1 serotypes 7 (pI$_7$) and 21 (pI$_{21}$). Except for the use of Iodogen (see text) and whole cells, instead of chloramine-T and gel fragments, to radioiodinate the pI proteins, the methods, conventions, and abbreviations are the same as in Fig. 3. Arrows indicate those peptides of surface-labeled pI$_7$ and pI$_{21}$ proteins that migrate similarly to peptides in the respective chloramine-T-radioiodinated peptide maps.

excised from the gels while still in the presence of SDS. Western blot anlaysis (Fig. 6) showed cross-reactivity as expected among the pII proteins of the three serotypes but only a homologous reaction by the anti-pI$_C$ serum shown in the figure and by another rabbit's serum (not shown). The antisera were diluted 1:2,000. Antiserum to denatured ovalbumin did not produce any detectable bands in the blots (data not shown).

Discussion

B. hermsii, a bacterium, can survive extracellularly in its mammalian host in spite of a vigorous production by the host of neutralizing antibodies (1, 2). Borrelial antigenic variation, which undoubtedly is the basis of the paradoxical persistence, has the following characteristics. (*a*) The appearance of a new serotype is spontaneous (2). (*b*) There is little, if any, cross-reactivity among the surface antigens of the 24 serotypes of *B. hermsii* HS1 (2, 5). (*c*) Serotype specificity is associated with the abundant, molecular weight-variable pI proteins at the surface of HS1 borreliae (5). (*d*) Populations of mouse-passaged borreliae once cultivated in vitro for more than a few passages appear to be overgrown by a single serotype, C (2, 5). Serotype C has a pI protein with a considerably lower apparent molecular weight than those of the mouse-passaged, numbered serotypes, such as 7 or 21.

The first two characteristics of borrelial antigenic variation could also describe the antigenic variation of the salivarian trypanosomes (3, 4). However, compared with trypanosomal variant-specific glycoproteins, borrelial variable proteins appear to have a greater range of electrophoretic mobilities (21, 22). In addition, trypanosomes do not seem to have the equivalent of serotype C. The "cultured" forms of *T. brucei* do not have any detectable variant glycoprotein (4, 23).

These attributes of borrelial antigenic variation suggest several possible molec-
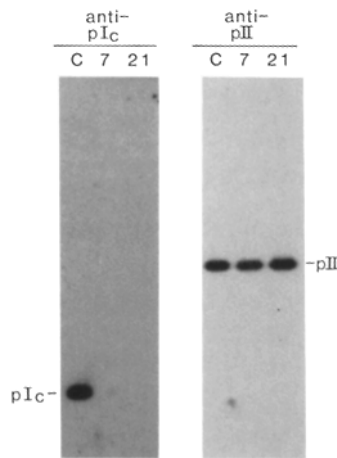
FIGURE 6.   Western blot analysis of rabbit antisera to pI$_C$ and pII$_C$ proteins of *B. hermsii* HS1 serotype C. See text for methods. Acrylamide concentration in the PAGE gel was 10%. The locations of pI$_C$ and pII$_C$ are indicated in the figure and were found in the experiment by amido black staining of the nitrocellulose after transfer (16). Antisera diluted 1:2,000 were reacted against PAGE-separated, whole cell proteins of serotypes C, 7, and 21.

ular mechanisms for the phenomenon. The majority of the models can be categorized into one of two groups. The first group's premise is that there is only one pI gene in the genome. A subgroup of the "one-gene" models hypothesize primary sequence alterations of the gene through mutation, deletion, transposition, or internal rearrangements. Another subgroup suggests post-translational modification of the proteins through, for instance, proteolysis or glycosylation. The pI proteins arising from any of the above mechanisms could vary in the major surface epitopes, but the pI proteins, except when they result from very extensive DNA rearrangement, would predictably share considerable amino acid sequence. A variation of the one-gene theory would have pI$_C$ be an unembellished core protein or constant region and the numbered pI proteins be variable regions in tandem with core or constant regions. In that case, there should be evidence of pI$_C$ sequence in the other pI proteins.

The second group of models suppose that there are many different pI genes, perhaps one for every pI protein, in the genome. Only one gene would be expressed at one time. This basic mechanism is analogous to *Salmonella* flagella-phase variation (24) in its simplest case and to trypanosome antigenic variation (25–27) in a more complex manifestation. Antigenic shift in the influenza A hemagglutinin proteins also involves a replacement of one hemagglutinin gene for another, but only one hemagglutinin gene is present in a given virus particle (28, 29). In the trypanosome and influenza examples, peptide map or amino acid sequencing studies have shown, in most instances, <50% homology between the expressed gene products (25, 26, 28, 30).

The primary tool in our first studies of pI and pII structure was peptide mapping in three forms: 1-D, 2-D of extensively labeled proteins, and 2-D of surface-labeled proteins. Although all three techniques generated peptides, we did not attempt to match peptides of one system with those of another. Even in

the case of the two 2-D mapping techniques in which the same protease was used, the dissimilar labeling conditions could produce significant differences in degree of iodination of the same part of the molecule. Use of three different techniques did allow independent confirmation of the results of any one of the techniques, however.

An additional way to examine structure was the production of antibodies to pI_C and pII_C through immunization of rabbits with SDS-denatured proteins. We knew that both polyclonal antisera and monoclonal antibodies raised against in situ pI proteins did not cross-react with heterologous pI proteins (5), but perhaps the more conserved regions of the molecule would be exposed by SDS treatment. Such exposure has been demonstrated with serotype-specific outer membrane proteins of *Chlamydia trachomatis* (31) and outer membrane proteins of *Neisseria gonorrhoeae* (32).

Our studies showed that the pII proteins of the three serotypes were identical in their 1-D and 2-D peptide maps. The function of the pII protein is not known, but it probably is not surface exposed and does not contribute to antigenic variation. The cleavage of pII in the absence of protease during the 1-D mapping procedure may indicate an acid-labile bond or bonds in this molecule. With the exception of pI_{21}, which was cleaved to limited extent, we did not detect cleavage products of pI proteins under these conditions.

The pI proteins clearly have considerable differences in primary structure as assessed by peptide mapping. Only a minority of the peptides produced by each of mapping procedures appear to be shared between any pair of pI proteins. Between pI_7 and pI_{21}, there were about as many overlapping (2-D) or similarly migrating (1-D) peptides as there were between pI_C and either of the numbered pI proteins. We find little evidence, therefore, for the representation of pI_C as the core or constant region of the other pI proteins. The lack of detectable reactivity of anti-pI_C antibodies against pI_7 and pI_{21} supports this conclusion.

As stated above, the presence of a pI protein in the culture-adapted form, serotype C, distinguishes *B. hermsii* from trypanosomes. We have also seen proteins similar in electrophoretic mobility to pI_C in in vitro-passaged *Borrelia parkeri* and *Borrelia turicatae* (A. G. Barbour, unpublished observations). But is pI_C truly a pI protein? For the present time, we include pI_C in the pI family for these reasons. (*a*) pI_C is the most abundant and apparently most rapidly produced protein in serotype C cells and, in this sense, replaces a numbered pI protein. (*b*) Although pI_C is poorly iodinated under surface-specific labeling conditions, pI_C-specific monoclonal antibodies bind to the outer membranes of serotype C borreliae (A. G. Barbour and S. L. Tessier, manuscript in preparation). Thus, pI_C like pI_7 and pI_{21} is an outer membrane-associated protein.

These studies indicate that some of the aforementioned models of borrelial antigenic variation are less likely to be true than others are. If pI proteins differed only in hypothetical constituent carbohydrates or in the length of their polypeptide chains, we should have seen more shared peptides among the pI proteins than we did. Therefore, posttranslation modification through glycosylation or proteolysis is probably not the mechanism.

Although mutations in protein-encoding genes can confer changes in antigenicity (33, 34), the present data suggest that the "antigenic shift" of influenza

hemagglutinins (28, 29) is a closer analogy to borrelial antigenic variation than is "antigenic drift" of the hemagglutinins (29, 35). In the latter phenomenon, mutations probably accumulate during the interpandemic period but the peptide maps of representative hemagglutinins are very similar to each other (36).

Our findings are rather more consistent with either (*a*) models specifying extensive rearrangements of the nucleotide sequence of a single pI gene through deletions, transpositions, internal inversions ("flip flops"), or combinations of these events, or (*b*) models specifying a different pI gene for each pI protein, as is the case with the salivarian trypanosomes (25–27). In a mixture of these two primary mechanisms, considerable sequence variety might also be achieved through recombinations of genes from different loci. Finally, we cannot exclude additions to and alterations of the borrelial genome through lysogenic conversion. An isolate of the closely related Lyme disease spirochete was shown to have bacteriophage (37).

Summary

Borrelia hermsii undergoes spontaneous antigenic variation in vivo and in vitro. Serotype specificity is associated with expression of one of a family of molecular weight-variable proteins, the pI proteins. We studied the structure of the pI proteins as well as the molecular weight-invariable pII proteins of three serotypes of *B. hermsii* HS1: C, 7, and 21. The techniques used were one-dimensional (1-D) mapping of *Staphylococcus aureus* V8 protease-generated peptides and two-dimensional (2-D) mapping of α-chymotrypsin-generated peptides. The pI and pII proteins were isolated by excision of polypeptides from stained polyacrylamide gel electropherograms. The 1-D peptide patterns were visualized by fluorography of intrinsically [^{14}C]leucine-labeled proteins or by silver stain. Before 2-D mapping, polypeptides in excised gel fragments were labeled with ^{125}I in the presence of chloramine-T. We also compared the 2-D peptide maps of pI proteins, pI$_7$ and pI$_{21}$, after their surface-exposed portions were radioiodinated using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen). The 1-D and 2-D peptide maps demonstrated the following: (*a*) pI proteins of the three serotypes have few V8 protease- or chymotrypsin-generated peptides in common, and (*b*) pI proteins of each serotype appear to be identical. The findings suggest that pI protein variability derives from extensive differences in the amino acid sequences of these proteins.

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