Thermoregulation of Protein Synthesis in Borrelia burgdorferi

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Borrelia burgdorferi, the etiological agent of Lyme disease, infects humans via the bite of a tick. The microbe survives in at least two vastly different environments: an arthropod vector and a warm-blooded host. We examined protein synthesis in B. burgdorferi B31 in response to sudden heat stress, which is similar to that which occurs during the transmission from vector to host. Proteins synthesized after shifts from 28°C to higher temperatures and in pulse-chase experiments were labeled with 3H-labeled amino acids for 4 h and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. The synthesis of four proteins we designated as heat stress proteins (HSPs) was increased by shifts to higher temperatures (HSP-1, 75 kilodaltons [kDa]; HSP-2, 42 kDa; HSP-3, 39 kDa; and HSP-4, 27 kDa); and the amount of one protein we designated as heat-labile protein 1 (29.5 kDa) was decreased at higher temperatures. At 37 to 40°C, the major heat stress protein, HSP-1, represented 14 to 18% of the total cell protein compared with 1 to 2% of the total cell protein at 28°C. HSP-1 was stable during a 4-h chase at either 40 or 28°C. Demonstration of similar HSPs in low-passage, pathogenic strains of B. burgdorferi suggests that the heat stress response may be common among B. burgdorferi strains and may play a role in Lyme disease.

Borrelia burgdorferi, the causative agent of Lyme disease, induces a complex multisystem disorder in humans. The geographic distribution of B. burgdorferi and the incidence of Lyme disease is growing. In its natural life cycle, the spirochete must adapt to at least two major environments that are dramatically different: the various developmental stages of Ixodes ticks, which serve as the primary arthropod vector, and the mammalian host. The ability of B. burgdorferi to undergo structural changes, metabolic changes, or both in response to its varied environments may explain why many infected individuals fail to resolve their infections, leading to serious clinical manifestations. Among the myriad of differences between these two environments, and one which is known to modulate a number of characteristics in a wide variety of cells, is temperature. The dramatic increase in synthesis of a small number of highly conserved proteins by whole organisms or cultured cells in response to an elevation in temperature has been defined as the heat shock response (23, 29). It is well established that heat shock genes and their regulatory sequences are highly conserved, and much is known about the structure of these genes and their products. The heat shock proteins which are best described include hsp70, hsp83 and a number of small heat shock proteins in the range of 16 to 30 kilodaltons (kDa) (for reviews, see references 6 and 17).

Temperature-modulated protein synthesis and the influence of growth temperature on the expression of virulence factors have been studied in human pathogens. Production of the K1 capsular antigen (3) and adherency factors that mediate colonization by pathogenic Escherichia coli (7-9, 15, 21) and Salmonella typhimurium (14), as well as expression of invasive virulence of pathogenic Shigella (20), Listeria (25), and Yersinia (13) species, depend on environmental temperature. B. burgdorferi may undergo significant changes in vivo as a result of the sudden temperature shift during the mammalian infection. In this study we establish that significant alterations in in vitro protein synthesis occur during temperature shifts from 28°C to higher temperatures.

MATERIALS AND METHODS

B. burgdorferi strains. B. burgdorferi B31 (type strain, ATCC 35210) was obtained from Robert Lane (Department of Entomological Sciences, University of California, Berkeley, Calif.). Low-passage, pathogenic strains of B. burgdorferi B31, HB19, and N40 were kindly provided by Steven Barthold (Section of Comparative Medicine, Yale University, New Haven, Conn.). Strains B31 and HB19 were human isolates; strain N40 was recovered from the midgut of an Ixodes dammini tick (2). Spirochetes were cultivated in BSK II medium as described by Barbour (1), except that bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.) was reduced to 1% (wt/vol) in medium used to cultivate the type strain.

Culture conditions for analysis of heat stress responses and preparation of samples for SDS-PAGE. Cultures of B. burgdorferi were grown at 28°C to a concentration of 1 x 10⁸ to 2 x 10⁹ cells per ml, harvested by centrifugation (17,000 × g), and immediately suspended to a concentration of 3 x 10⁸ to 1 x 10⁹ cells per ml in BSK II medium containing a 3H-labeled amino acid mixture (ICN Pharmaceuticals Inc., Irvine, Calif.) at 20 µCi/ml or [35S]methionine (ICN) at 50 µCi/ml. The BSK II labeling medium was formulated as described above except that it contained 0.1% CMRL 1066 (GIBCO Laboratories, Grand Island, N.Y.). Different portions of the suspended spirochetes were then incubated at temperatures of between 28 and 42°C for 4 h in a specially constructed polythermic aluminum block. These radiolabeled cells were then harvested as described above, washed three times in phosphate-buffered saline, and suspended in 50 to 100 µl of Tris hydrochloride (0.5 M, pH 6.8). The protein concentration of each sample was determined by the method of Lowry et al. (18), and the samples were stored at −80°C. For sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), samples were thawed, suspended in sample buffer with 2-mercaptoethanol (5% [wt/vol]), and heated for 5 min at 95°C.

Analysis of B. burgdorferi protein synthesis under various conditions.
temperature conditions by SDS-PAGE, fluorography, and scanning laser densitometry. The labeled *B. burgdorferi* cells (approximately 150 μg) were applied to SDS-polyacrylamide gels by the method of Laemml and Favre (16) by using a 5% acrylamide stacking gel and a 10% acrylamide running gel and were electrophoresed at 12 mA overnight under reducing conditions. After electrophoresis the gels were stained with Coomassie brilliant blue, enhanced for fluorography (En3Hance; Dupont, NEN Research Products, Boston, Mass.), and exposed to photographic film (XAR-5; Eastman Kodak Co., Rochester, N.Y.) at −80°C. Autoradiographed film was developed and quantified with a scanning laser densitometer (UltraScan XL; LKB Instruments, Bromma, Sweden). All labeling experiments were performed two to five times, and the incorporation of labeled amino acid into individual heat stress proteins (HSPs) was consistent with the results presented here. The relative amount of a given HSP synthesized at the same temperature varied by 5 to 20% from experiment to experiment.

Stability of HSPs 1 through 4 and HLP-1 at 28 and 40°C. A pulse-chase experiment was performed to determine the relative stabilities of the HSPs and heat-labile protein (HLP), HLP-1 compared with those of the other proteins that were resolvable by SDS-PAGE. Spirochetes were harvested, suspended as described above, and then divided into two portions and placed in two tubes containing labeling medium and 3H-labeled amino acids. The organisms were pulsed at 28 or 40°C for 4 h, and the volumes of cells that were pulsed at each temperature were then divided into three parts. One portion was harvested immediately (pulsed only), and the remaining two portions were pelleted and suspended (chased) in unlabeled medium for 4 h at 28 or 40°C, respectively. The cells were then prepared for SDS-PAGE and fluorography as described above.

RESULTS

Heat stress response in high-passage *B. burgdorferi* B31. The SDS-PAGE-derived fluorogram of newly synthesized proteins from high-passage *B. burgdorferi* B31 indicated that the synthesis of at least five proteins was affected by temperature (Fig. 1). Increased synthesis of the four proteins that we designated HSPs (HSPs 1 through 4) occurred with increasing temperature from 28 to 40°C. At 42°C, protein synthesis was negligible (Fig. 1). At 40°C, HSP-1 made up 11.4% of the labeled protein detected by scanning laser densitometry compared with 0.8% at 28°C, a 14-fold increase (Table 1). In similar labeling experiments with either 3H-labeled amino acids or [35S]methionine, HSP-1 accounted for 12 to 15% of the labeled protein from spirochetes grown at 38 to 40°C. At the five temperatures studied, total protein synthesis was greatest at 38°C. Incubation at 35 and 40°C yielded 90 to 95% of the total labeled protein seen at 38°C, while the lowest temperatures, 28 and 30°C, yielded only 42 and 52%, respectively, of the maximum amount (data not shown). The intensity of a 29.5-kDa band that we designated HLP-1 decreased as the incubation temperature was increased (Fig. 1). At 40°C, HLP-1 represented only 1% of the total labeled protein compared with 6% at 28°C (Table 1).

Pulse-chase of HSPs and HLP in high-passage *B. burgdorferi* B31. HSPs 1 through 4 in high-passage *B. burgdorferi* B31 pulsed at 40°C were cell associated and stable during a 4-h chase period at either 28 or 40°C (Fig. 2 and Table 2). After a 40°C pulse-chase, the HSPs remained the same or increased as a percentage of total labeled protein compared with cells which were pulsed but not chased (Fig. 2). The HSPs were present at much lower levels in cells that were pulsed at 28°C, but remained stable after chases at both high and low temperatures. In sharp contrast, HLP-1 was present at relatively low levels when *B. burgdorferi* was labeled at temperatures of 35°C and above. This protein disappeared from cells that were pulsed at 28°C during a 40°C chase period of 4 h, but the band did not diminish during an equivalent chase at 28°C (Fig. 2).

Heat stress response in low-passage, pathogenic strains of *B. burgdorferi*. Low-passage, pathogenic strains of *B. burgdorferi*.

![Fluorogram of newly synthesized 3H amino acid-labeled proteins from whole-cell lysates of high-passage *B. burgdorferi*. Approximately 150 μg of protein was loaded per lane and the film was exposed to the enhanced gel for 17 days. The following HSPs were tested: HSP-1, 75 kDa; HSP-2, 42 kDa; HSP-3, 39 kDa; HSP-4, 27 kDa; HLP-1, 29.5 kDa. Molecular mass standards were as follows: phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31 kDa), and soybean trypsin inhibitor (21.5 kDa). Other proteins are indicated as follows: flagellin (41 kDa) and outer surface proteins (OSPs) A and B, OspA (31 kDa) and OspB (34 kDa).](http://iai.asm.org/)

![FIG. 1. Fluorogram of newly synthesized 3H amino acid-labeled proteins from whole-cell lysates of high-passage *B. burgdorferi*. Approximately 150 μg of protein was loaded per lane and the film was exposed to the enhanced gel for 17 days. The following HSPs were tested: HSP-1, 75 kDa; HSP-2, 42 kDa; HSP-3, 39 kDa; HSP-4, 27 kDa; HLP-1, 29.5 kDa. Molecular mass standards were as follows: phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31 kDa), and soybean trypsin inhibitor (21.5 kDa). Other proteins are indicated as follows: flagellin (41 kDa) and outer surface proteins (OSPs) A and B, OspA (31 kDa) and OspB (34 kDa).](http://iai.asm.org/)

<p>| TABLE 1. Quantification of HSP synthesis in high-passage <em>B. burgdorferi</em> B31 at different temperatures* |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Temp (°C)</th>
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<th>HSP-3</th>
<th>HSP-4</th>
<th>HLP-1</th>
<th>OspB</th>
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* As determined by scanning laser densitometry of Fig. 1. Abbreviations: OSP, outer surface protein; CA, common antigen.
feri (B31, HB19, and N40) synthesized [35S]methionine-labeled HSPs similar to the 3H amino acid-labeled proteins found in the high-passage strain (Fig. 3). HSPs 1, 3, and 4 had electrophoretic mobilities identical to those obtained in the high-passage, nonpathogenic type strain; however, HSP-2 and HLP-1 were not evident (Fig. 3). One HSP of approximately 36 kDa, designated HSP-5, which was not seen in the high-passage, nonpathogenic strain, was apparent in the three pathogenic strains that were examined (Fig. 3). Synthesis of the HSPs in the low-passage, pathogenic strains was enhanced at the higher temperature, especially for HSP-1 in strains B31 and HB19 (Table 3). Generally, the differences in the labeling patterns of the HSPs at 28 and 39°C were similar to those seen at the same two temperatures with the type strain. The synthesis of HSPs in isolate N40 from ticks was not affected dramatically by temperature. However, HSPs 4 and 5 were synthesized at much higher levels at 28°C in the low-passage, pathogenic strains than they were in the laboratory-adapted type strain. In the three pathogenic strains studied, synthesis of OspA, OspB, and common antigen was not affected by temperature (Table 3).

**FIG. 2.** Fluorogram of newly synthesized proteins from whole-cell lysates of high-passage *B. burgdorferi* B31. Bacteria were grown at 28°C prior to the 4-h labeling period (pulsed) at 28 or 40°C and then (after removal of the soluble 3H-labeled amino acids) were grown in the same medium without labeled amino acids (chased) at 28 or 40°C, as indicated. Molecular mass standards are as described in the legend to Fig. 1.

**FIG. 3.** Fluorogram of newly synthesized [35S]methionine-labeled proteins from whole-cell lysates of low-passage, pathogenic strains (HB19, B31, and N40) of *B. burgdorferi*. Spirochetes were grown at 31°C prior to the 4-h labeling period at either 28 or 39°C. HSPs and molecular mass markers as described in the legend to Fig. 1. Each lane contained 80 μg of protein.

<table>
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<th>Labeled protein</th>
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* As determined by scanning laser densitometry of Fig. 2.

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</table>

* As determined by scanning laser densitometry of Fig. 3. Abbreviations: Osp, outer surface protein; CA, common antigen.

* Sum of the 27-kDa doublet.
**DISCUSSION**

Lyme disease results from infection with *B. burgdorferi* via the bite of an *Ixodes* tick. Remissions, exacerbations, and different clinical manifestations may occur at various stages following infection. Infected individuals may experience an extensive array of symptoms, despite the presence of only a small number of spirochetes (5, 22, 26–28). The chronicity of the disease and the extended duration of the infection indicate that *B. burgdorferi* can avoid clearance mechanisms of the host immune system for considerable time periods. In fact, elements of the immune response, such as immune complexes, interleukin-1, and autoimmune components, may actually induce or accentuate pathogenicity (10, 12, 19). Furthermore, thermoregulated proteins are important in the virulence of microbial pathogens, and similar heat shock proteins are involved in the immune response and may be crucial in combating such infections (4).

In this study we examined the changes in *B. burgdorferi* protein synthesis that occur in response to a shift in temperature, from 28°C (similar to the temperature experienced by the spirochete in poikilotherm arthropod vectors) to 37 to 40°C (the temperature of homeothermic hosts). During shifts to higher temperatures, the synthesis of at least four major proteins increased, and the amount of at least one protein decreased. The lack of significant synthesis of these proteins above 40°C distinguishes them from true heat shock proteins, and they were designated HSPs. The major HSP-1, increased from approximately 1% of the total cell protein at 28°C to 12 to 18% during a 4-h labeling period at 38°C to 40°C. In one-dimensional SDS-PAGE, HSP-1 appeared to be produced constitutively at low temperatures and was strongly induced at higher temperatures. Two-dimensional electrophoresis of labeled, heat-stressed *B. burgdorferi* will reveal if HSP-1 is truly constitutive or if the band designated HSP-1 comigrates with other proteins which are not heat inducible. Furthermore, isoforms of proteins in the heat shock protein hsp70 family have been described. If HSP-1 is composed of a family of isoforms, then it will be of interest to determine which isoforms are preferentially synthesized during heat stress.

Hansen et al. (11) established that the immunodominant 60-kDa antigen of *B. burgdorferi* is the common antigen, which cross-reacts with 59- to 65-kDa antigens from numerous bacteria. While the common antigen of *Coxiella burnetii* (30) and the *E. coli* GroEL common antigen are heat shock proteins (21, 24), synthesis of the 60-kDa protein of *B. burgdorferi* increased only slightly at higher temperatures in the type strain, from 5.1% of the total protein detected at 28°C to 7.8% at 40°C (Fig. 1), and was unchanged in the pathogenic strains (Fig. 3). Interestingly, while the common antigen from *B. burgdorferi* is antigenically similar to heat shock proteins with similar subunit molecular masses, results of this study indicate that this protein is not strongly induced during temperature stress. Rather, in *B. burgdorferi*, synthesis of HSP-1 is the major response to heat stress and thus may satisfy the role that common antigen serves in other organisms.

The synthesis and maintenance of HLP-1 are in contrast to those of the HSPs. A band which decreases in intensity during the chase, as HLP-1 does at high temperatures, suggests two possibilities: (i) the protein is actively degraded or (ii) the protein is selectively exported. If HLP-1 is degraded or is inherently heat labile, then the breakdown products must be less than 14 kDa since new low-molecular-mass bands did not appear during the 28 and 40°C pulse-chase. Considering the antagonistic levels of the HSPs and HLP-1, it is tempting to speculate that HLP-1 is important in maintaining *B. burgdorferi* at low temperatures.

The synthesis of HSPs may represent a significant modulation of antigens that influence the immune response. If these proteins are highly conserved, the immune system would be faced with responding to the self-proteins that are induced during infection (leading to autoimmunity) or with failing to respond to proteins presented by the infecting spirochete with which it shares a significant number of determinants (leading to chronic infection). The presence of similar proteins, particularly HSP-1, in several strains of low-passage, pathogenic *B. burgdorferi* may help to explain some of the arthritis, central nervous system, and cardiac pathologies which are common among chronic Lyme disease sufferers. Demonstration of HSPs 1 through 4 in pathogenic strains of *B. burgdorferi* suggests that this common response is important in the survival of the spirochete within homeothermic hosts. The rapid synthesis of these proteins immediately after infection may affect pathogenesis and complicated serodiagnosis.

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**LITERATURE CITED**


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