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A Glyceraldehyde-3-Phosphate Dehydrogenase Homolog in *Borrelia burgdorferi* and *Borrelia hermsii*

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A polyreactive monoclonal antibody recognized a 38.5-kDa polypeptide with amino-terminal sequence identity to conserved regions of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in *Borrelia burgdorferi*, the Lyme disease agent, and *Borrelia hermsii*, an agent of American relapsing fever. This monoclonal antibody also recognized GAPDH from other pathogenic spirochetes and other prokaryotes and eukaryotes as well. GAPDH activity was detected in sonicates of both *B. burgdorferi* and *B. hermsii* but not in live, intact organisms, indicating the possibility of a subsurface localization for the *Borrelia* GAPDH activity. Degenerate primers constructed from highly conserved regions of *gapdh* of other prokaryotes successfully amplified this gene homolog in both *B. burgdorferi* and *B. hermsii*. Nucleic acid and deduced amino acid sequence analysis of the 838-bp probes for each borrelia indicated 93.9% identity between *B. burgdorferi* and *B. hermsii* at the amino acid level. Amino acid identities of *B. burgdorferi* and *B. hermsii* with *Bacillus stearothermophilus* were 59.2% and 58.8%, respectively. Southern hybridization studies indicated that the gene encoding GAPDH is located on the chromosome of each borrelia. In other bacterial species, GAPDH has other functions in addition to its traditional enzymatic role in the glycolytic pathway. GAPDH may play a similar role in borrelias.

A polyreactive murine immunoglobulin M (IgM) monoclonal antibody recognizes an acidic epitope in *Borrelia burgdorferi* (2), the agent of Lyme disease (4, 10, 24, 46), which is shared by several conserved antigens, including p93 (29, 40), DnaK (3, 49), and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) homolog with an approximate molecular mass of 38.5 kDa. The mapped linear epitope for p93 and DnaK had an absolute requirement for a pair of glutamic and aspartic acid residues without which reactivity would not be present. The fact that this polyreactive monoclonal antibody is of the IgM class, recognizes conserved antigens, and has reactivity that appears to be dependent on the charge of the epitope suggests characteristics which can be found in association with autoreactivity (31, 48).

GAPDH is an enzyme of the glycolytic pathway whose main function is to catalyze the conversion of glyceraldehyde-3-phosphate (G-3-P) into 1-3-diphosphoglycerate. This is a fundamental step for the production of energy in all living systems, and so it stands to reason that enzymes involved in this pathway are conserved, at least in the functionally constrained domains of the molecule (21). GAPDHs from organisms ranging from bacteria to plants and animals have shown a high degree of interspecies identity (34), particularly in regions such as the ATP binding site and the S-loop which forms the core of the tetrameric structure of the active enzyme.

In recent years, a number of other physiologic functions have been associated with GAPDH enzymes. For eukaryotes, some of these functions include assembly of microtubules (23) and DNA binding (37, 41). For prokaryotes, newly identified functions of GAPDH include ADP-ribosylation (39); adhesion to fibronectin, myosin, and actin (38); and the ability to serve as a receptor for plasmin (9, 32, 33).

The fact that GAPDH or a similar molecule(s) can act as a cell membrane receptor or adhesin requires that it be membrane bound and exposed on the surface. Evidence for a membrane-bound GAPDH in erythrocytes has been presented (27, 50). Likewise, a surface location of GAPDH or a related molecule in streptococci enabling it to function as a receptor for plasmin (9, 32, 33) and as an adhesin for matrix and cytoskeletal proteins (38) has been demonstrated.

The presence of a GAPDH homolog in *B. burgdorferi* was inferred by NH_2-terminal sequencing of an antigen which was recognized by the IgM polyreactive monoclonal antibody. This antigen had a molecular mass within the range of those of other GAPDH monomers (~36 to 40 kDa). The possibility that such a conserved enzyme in borrelias could be a target for antibodies in Lyme disease with a potential for cross-reactivity with human GAPDH was of interest in terms of immunopathogenesis. Of further functional interest is that fact that *B. burgdorferi* can bind plasmin(ogen), whereupon activation by exogenous plasminogen activators can lead to the formation of proteolytically active plasmin bound to the surface of the organism (14, 19, 26).

For these reasons, we initiated a study to determine the extent of reactivity for GAPDH of the polyreactive IgM monoclonal antibody with other pathogenic spirochetes, to determine whether two species of the genus *Borrelia* had GAPDH enzymatic activity, and lastly to document the presence of *gapdh* homologs in *B. burgdorferi* and *Borrelia hermsii*.

MATERIALS AND METHODS

Bacteria and reagents. Strains B31 (high passage [10]) and BEP-4 (low passage [13, 20]) of *B. burgdorferi* were both tick derived and were used for most experiments. All *B. burgdorferi* organisms were grown in serum-free BSK medium (12). *B. hermsii* and *Borrelia anserina* were gifts from Russell C. Johnson

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(University of Minnesota) and were grown in complete BSK medium. *Treponema pallidum* used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was a gift of Sheila Lukehart (University of Washington). CAB, a murine IgM polyreactive monoclonal antibody made by immunization with a whole-cell lysate of strain BEP-4 of *B. burgdorferi*, was purified from ascites as described previously (2).

SDS-PAGE and immunoblotting. The Laemmli buffer system as adapted for *B. burgdorferi* (12) was used for all the experiments, and electrophoresis was done with gels containing 7.5 or 12.5% polyacrylamide under reducing conditions (28). A whole-cell lysate of the spirochetes was prepared by washing the organisms in phosphate-buffered saline (PBS) supplemented with 5 mM $MgCl_2$, sonicating the pellet on ice until intact organisms were no longer visible, determining the protein content, and suspending the desired amount in SDS-PAGE sample buffer. Purified GAPDH from human erythrocytes, rabbit smooth muscle, and *Bacillus stearothermophilus*, as well as β-galactosidase and cytochrome β-oxidase (Sigma, St. Louis, Mo.) as controls, was also subjected to electrophoresis in the manner described above. Two-dimensional gel electrophoresis was carried out for some experiments by procedures already described for whole-cell lysates of these organisms (2, 12). Transfer of *B. burgdorferi* whole-cell lysates as well as enzymes from one- and two-dimensional SDS-PAGE gels to nitrocellulose or polyvinylidene difluoride membranes (0.45-μm pore size; Bio-Rad Laboratories, Hercules, Calif.) was done according to standard procedures for borrelias (12).

Enzyme-linked immunosorbent assay (ELISA). Flat-bottom 96-well polystyrene plates (Becton Dickinson, Oxnard, Calif.) were coated with *B. burgdorferi* whole-cell lysate and each of the following enzymes: GAPDH from human erythrocytes, rabbit smooth muscle, and *B. stearothermophilus*; β-galactosidase; and cytochrome β-oxidase (Sigma). The plates were coated with the enzymes at a concentration (7) of 5 μg/ml in 0.1 M carbonate buffer (pH 9.6) and incubated for 16 h at 4°C. Plates were washed in PBS-Tween (PBS supplemented with 0.05% Tween 20 [pH 7.2]) and blocked with PBS-bovine serum albumin (BSA) (2%). Monoclonal antibody, diluted in PBS-Tween, was added at the appropriate concentrations, in triplicate, incubated for 3 h at 37°C and then subjected to three washes and an incubation with affinity-purified alkaline-conjugated goat anti-mouse IgM (Cooper Biomedical, Malvern, Pa.), and the reaction was developed with *p*-nitrophenyl phosphate (Sigma). Optical density was determined with an MR580 micro ELISA reader (Dynatech, Alexandria, Va.) at 406 nm.

Enzymatic activity for borrelial GAPDH. The assay to measure the oxidative phosphorylation of D-G-3-P to form 1,3-diphosphoglycerate in the presence of both P_i and NAD^+ was conducted as described initially (17) with subsequent modifications (38). Briefly, *B. burgdorferi* and *B. hermsii* were adjusted to a final concentration of 2×10^8 spirochetes per 100 μl in GAPDH assay buffer (50 mM Na_2HPO_4, 5 mM EDTA, 40 mM triethanolamine [pH 8.6] [Sigma]). In two separate experiments, 100 μl of whole organisms (2×10^8) and the same concentration of freshly sonicated spirochetes (with a protein concentration of 180 to 200 μg/ml) were used as possible sources of enzyme. The spirochete samples were added to 1.9 ml of GAPDH assay buffer with a 0.8 mM final concentration of G-3-P (Sigma). The samples were first measured for baseline readings at a wavelength of 340 nm in a spectrophotometer (LKB Biochrom, Piscataway, N.J.). After 3 min of reading to detect any NADH release due to the presence in the system of NAD^+ from other sources, NAD^+ (Sigma) was added to the cuvette (Bio-Rad) to a final concentration of 1 mM and the NADH release was measured under the same conditions every 5 s for a maximum of 3 min. Positive controls included GAPDHs from human erythrocytes and from *B. stearothermophilus* (both from Sigma) at various concentrations. Negative controls included the reaction mixtures lacking a possible enzyme source (spirochetes) and the cosubstrates (NAD^+, as described above, and/or G-3-P).

The kinetics of GAPDH enzymatic activity of sonicated (from 2×10^8 organisms) *B. burgdorferi* and *B. hermsii* was determined with a range of concentrations of G-3-P substrate and a fixed concentration of NAD and alternatively with a range of concentrations of NAD and a fixed concentration of G-3-P. The V_{max} and K_m (the substrate concentration at which the velocity of the reaction is one-half of the V_{max}) were determined for both G-3-P and NAD by using Lineweaver-Burk double reciprocal plots (30). In these plots, the *y*-axis intercept represents $1/V_{max}$, while the slope represents K_m/V_{max}. Taking into account the fact that the measurements had to be taken in a region of the curve where the increments of NADH release were still exponential, absorbance values were selected at 1 min. To convert A_{340} to moles of NADH per min, we used the molar extinction coefficient of NADH 6.22×10^3 (17).

PCR and nucleotide sequencing. The GeneAmp PCR reagent kit (Perkin-Elmer Cetus, Norwalk, Conn.) was used according to the directions, with 1 μl of template and 1 μl of each primer (30 pM) per 100 μl of reaction mixture. The degenerate primers used for the initial PCR are shown in Fig. 1. Thermal cycling conditions for the PCR with degenerate primers consisted of 15 s of denaturation at 94°C, 75 s of annealing at 50°C, and 15 s of extension at 65°C for 30 cycles in a Perkin-Elmer GeneAmp 9600 thermal cycler. Thermal cycling conditions for PCR using specific primers were as follows: denaturation for 24 s at 93°C, annealing for 10 s at 54°C, and extension for 2 min at 70°C for 25 cycles. The amplification products were separated by 0.8% agarose gel electrophoresis and stained with 0.5 μg of ethidium bromide per ml. DNA size standards (1-kb DNA ladder) were from GIBCO/BRL, Gaithersburg, Md. PCR amplification products were excised from the stained agarose gels and purified (Magic PCR Preps; Promega Corp., Madison, Wis.). The DNA was reamplified in triplicate by PCR and purified as indicated above. The resulting DNA was tested for purity by spectrophotometry, sequenced with the *Taq* DyeDeoxy Terminator Cycle sequencing kit (43) (Applied Biosystems, Inc., Foster City, Calif.), purified (CentriSep columns; Princeton Separations, Adelphia, N.J.), and analyzed with an Applied Biosystems model 373A DNA sequencing system.

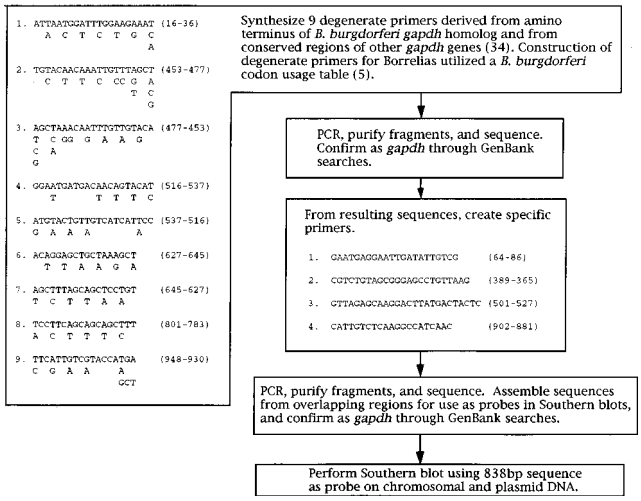

FIG. 1. Degenerate PCR primer sequences and schematic for the strategy used to develop a probe for Southern blot hybridization to determine the location of the *gapdh* gene homolog in the genomes of *B. burgdorferi* and *B. hermsii*.

DNA preparation and field inversion gel electrophoresis. Probes utilized were amplified by PCR using the above-described conditions, excised from low-melt agarose gels, purified as described above, and labeled with [^{32}P]dCTP by using a Random Primed Labeling Kit (Boehringer Mannheim, Indianapolis, Ind.). Prior to hybridization, the labeled probes were denatured at 100°C for 10 min and then quickly placed on ice for another 5 min.

Total genomic DNA was prepared in agarose plugs as described earlier (18) and separated at 200 V (6.9 V/cm) for 28 h in a reverse mode (pulse time, 1 to 50 s) on 1% agarose gels with an HE 100 Super Sub horizontal electrophoresis unit equipped with a switchback pulse controller (Hoefer Scientific Instruments, San Francisco, Calif.). Gels were stained with 0.5 μg of ethidium bromide per ml, visualized by UV illumination, and photographed.

DNA transfer and hybridization. Electrophoretically separated genomic DNA from *B. burgdorferi* and *B. hermsii* was transferred to nitrocellulose with a Vacugene XL vacuum blotting system (Pharmacia LKB Biotechnology, Uppsala, Sweden) according to the manufacturer's specifications. Transferred DNA was cross-linked with UV light (Stratalinker 1800; Stratagene, La Jolla, Calif.) and incubated with 5 ml of hybridization buffer (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.5% SDS, 50% formamide, 5× Denhardt's solution) for 2 h at 40°C. Hybridization with random primed ^{32}P-labeled probes for both *B. burgdorferi* and *B. hermsii* was carried out in 5 ml of fresh hybridization buffer. The specific primer sequences used to construct the probe are listed in Fig. 1. A final concentration of 10^6 cpm of the radiolabeled probe per ml was incubated at 42°C for 15 h. After hybridization, the nitrocellulose membranes were washed at 53°C (2× SSC plus 0.1% SDS) for 1 h with buffer changes every 20 min and the membranes were dried on 3MM Whatman filter paper. Hybridization of probes was visualized after exposure of membranes to XAR X-ray film (Eastman Kodak Company, Rochester, N.Y.).

NH_2-terminal sequence analysis. Polypeptides used for sequencing were derived from affinity-purified antigens subjected to SDS-PAGE or from whole-cell lysates and affinity-purified antigens subjected to two-dimensional gel electrophoresis. Transfer of antigens to polyvinylidene difluoride membranes and staining of the resulting bands were done as described previously (2, 12, 35). NH_2-terminal sequencing was done at the Center for the Analysis and Synthesis of Macromolecules at the State University of New York at Stony Brook and at the Department of Physiology, Tufts University School of Medicine, Boston, Mass. Homology searches were conducted by using the Protein Identification Resource Center, Georgetown University Medical Center, Washington, D.C. (22).

Computer analysis. MacVector version 4.1.1 for Apple Macintosh computers (Eastman Kodak Company, Laboratory and Research Products, New Haven, Conn.) was used for DNA sequence alignments and analyses. Comparisons with the GenBank sequence database and sequence retrieval were done by using the BLAST and RETRIEVE electronic mail servers, respectively (National Center for Biotechnology Information, Bethesda, Md.).

Nucleotide sequence accession numbers. The nucleotide sequences of the

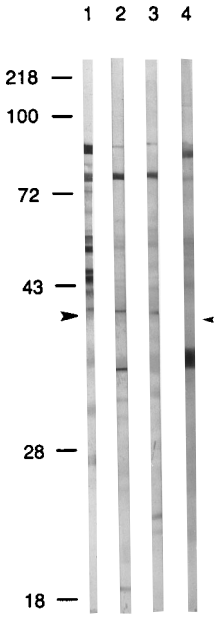

FIG. 2. Reactivity of CAB in immunoblots to antigens of *B. burgdorferi* BEP-4 (lane 1), *B. hermsii* (lane 2), *B. anserina* (lane 3), and *T. pallidum* (lane 4). Arrowheads denote the GAPDH homologs. Molecular mass markers in kilodaltons are shown on the left.

probes generated are under GenBank accession numbers U28760 for *B. burgdorferi* and U28761 for *B. hermsii*.

RESULTS

A polyreactive IgM murine monoclonal antibody (CAB) to *B. burgdorferi* recognizes antigens in other spirochete species. In addition to the polyreactivity demonstrated for CAB with antigens from several strains of *B. burgdorferi* (2), this monoclonal antibody also showed a polyreactive pattern with several antigens of *B. hermsii*, *B. anserina*, and *T. pallidum* (Fig. 2). Amino-terminal sequencing of the reactive polypeptides at ≈37 to 38 kDa showed a close identity with amino-terminal sequences of several prokaryotic and eukaryotic GAPDHs (Table 1), demonstrating the presence of GAPDH homologs in two other species of *Borrelia* and in *T. pallidum*. The electrophoretic mobility of the GAPDH homolog of *T. pallidum* was less than those of the borrelial homologs (Fig. 2).

Monoclonal antibody CAB recognizes GAPDHs from other species as determined by ELISA and Western blotting (immunoblotting). SDS-PAGE-separated human erythrocyte GAPDH, rabbit muscle GAPDH, and GAPDH from *B. stearothermophilus* (Fig. 3A) as well as two control enzymes, β-galactosidase and cytochrome β-oxidase, were transferred to nitrocellulose and probed with 2 μg of CAB per ml. At this concentration, CAB reacted weakly with the *B. stearothermophilus* GAPDH in this immunoblot (Fig. 3B) but did not react with other mammalian GAPDHs or with the control enzymes. At a concentration of 10 μg/ml, CAB provided a stronger signal with the GAPDH of *B. stearothermophilus* (Fig. 3C) but was still not reactive with the mammalian GAPDHs or the controls.

The polyreactive monoclonal antibody CAB, however, reacted with both the eukaryotic and the prokaryotic GAPDHs as determined by ELISA. The mean optical densities for CAB (2 μg/ml) with rabbit muscle GAPDH (0.314 ± 0.030), *B. stearothermophilus* GAPDH (0.398 ± 0.037), and human erythrocyte

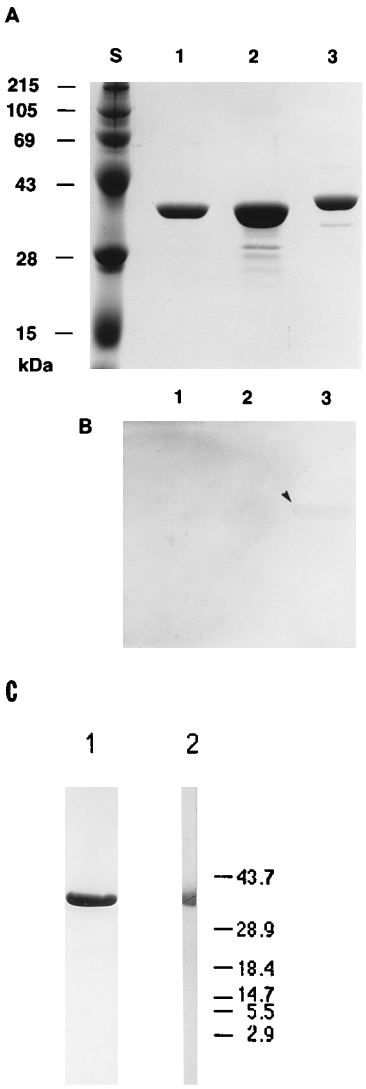

FIG. 3. (A) Coomassie blue-stained SDS-PAGE gel containing human erythrocyte GAPDH (lane 1), rabbit muscle GAPDH (lane 2), and *B. stearothermophilus* GAPDH (lane 3). (B) Reactivity of 2 μg of monoclonal antibody CAB per ml in immunoblot with the nonborrelial *B. stearothermophilus* GAPDH (arrowhead). (C) Coomassie blue-stained SDS-PAGE gel containing *B. stearothermophilus* GAPDH (lane 1) and reactivity of 10 μg of monoclonal antibody CAB per ml in immunoblot with the nonborrelial *B. stearothermophilus* GAPDH (lane 2). Molecular mass markers in kilodaltons are shown on the left of panel A and on the right of panel C.

TABLE 1. Amino-terminal sequences of the 38.5-kDa polypeptides of *B. burgdorferi*, *B. hermsii*, and *T. pallidum* and GAPDHs from *B. stearothermophilus* (6) and *E. coli* (8) from the GenBank database

Species	Sequence[a]
B. burgdorferi	- - M K V L A I N G F G R I G
B. hermsii	- - * * * * * * * * * * * * *
T. pallidum	- - * X * - * * * * * * * X *
B. stearothermophilus	M A V * * - G * * * * * * * *
E. coli	M T I * * - G * * * * * * * *

[a] GenBank accession numbers are M30783 and X02662 for the *B. stearothermophilus* and *E. coli* sequences, respectively. X, unknown amino acid; ⋆, identity with *B. burgdorferi*; −, gap.

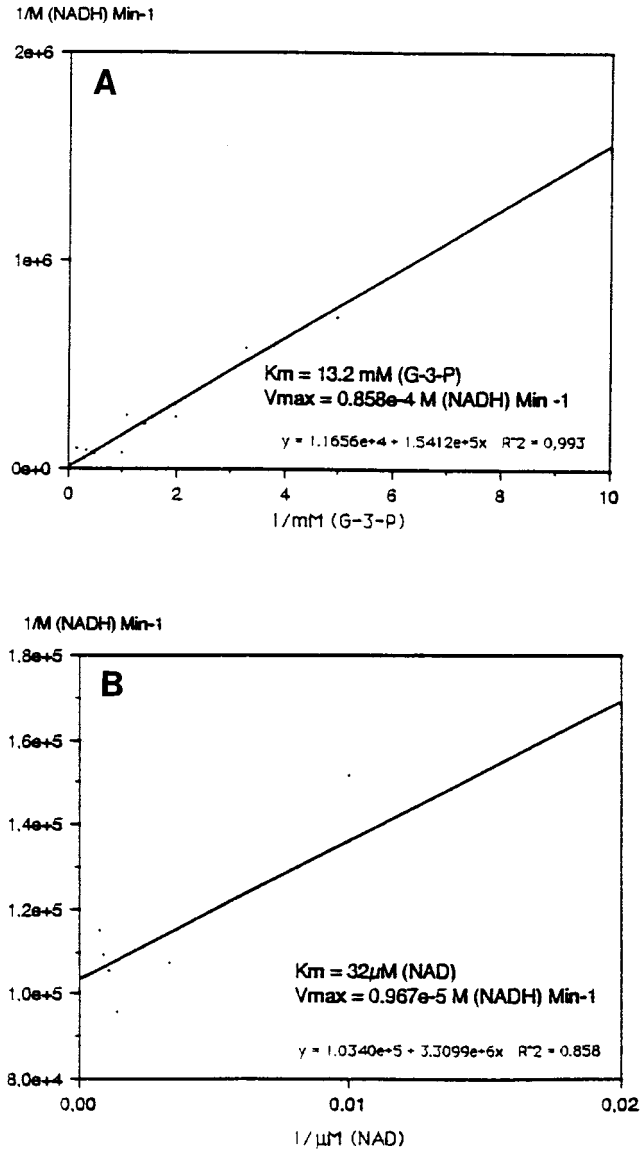

FIG. 4. Lineweaver-Burk double reciprocal plots for the kinetic analysis of the activity of GAPDH. (A) *B. burgdorferi* sonicate (enzyme source) was measured as a function of G-3-P in the presence of NAD. The intercept on the *y* axis ($1/V_{max}$) and the slope (K_m/V_{max}) are shown in the equation. (B) *B. burgdorferi* sonicate (enzyme source) was measured as a function of NAD in the presence of G-3-P. Intercept and slope values are shown in the equation.

GAPDH (0.280 ± 0.028) were significantly greater than the mean optical densities obtained for control enzymes and the BSA controls (0.101 ± 0.012 for β-galactosidase and 0.094 ± 0.010 for cytochrome β-oxidase; $P < 0.01$ by Student's *t* test).

***B. burgdorferi* and *B. hermsii* possess GAPDH activity.** Sonicate preparations from 2×10^8 *B. burgdorferi* (strain BEP-4) and *B. hermsii* spirochetes, but not live spirochetes, exhibited enzymatic activity for the conversion of NAD to NADH (Fig. 4 and 5, respectively). The kinetics of the GAPDH activity for both organisms was analyzed by the Lineweaver-Burk double reciprocal plot by varying the enzymatic reaction rate with different concentrations of G-3-P and NAD. The *B. burgdorferi* sonicate had K_ms for G-3-P and NAD of 13.2 mM and 32 μM, respectively. V_{max} values for *B. burgdorferi* are shown in Fig. 4. The K_m of the *B. hermsii* sonicate for G-3-P was 13.0 mM, and that for NAD was 77.7 μM. V_{max} values for *B. hermsii* are shown in Fig. 5.

***gapdh* homolog probes hybridize with borrelial chromosome.** The strategy followed to isolate the gene for the *gapdh* homolog of *B. burgdorferi* is outlined in Fig. 1. Degenerate primers were derived from the conserved sequences of other *gapdh* genes (1, 6, 8, 11, 15, 16, 33, 34, 36, 44, 47) according to the *B. burgdorferi* codon usage table (5).

The amplified products were sequenced and confirmed to be *gapdh* homologs by GenBank comparisons, with identity values exceeding $P < 10^{-11}$. Once these products were confirmed to be homologs, specific primers were constructed and used to amplify 838-bp sequences, which were used as probes for Southern blots (Fig. 1). These probes were also identified as

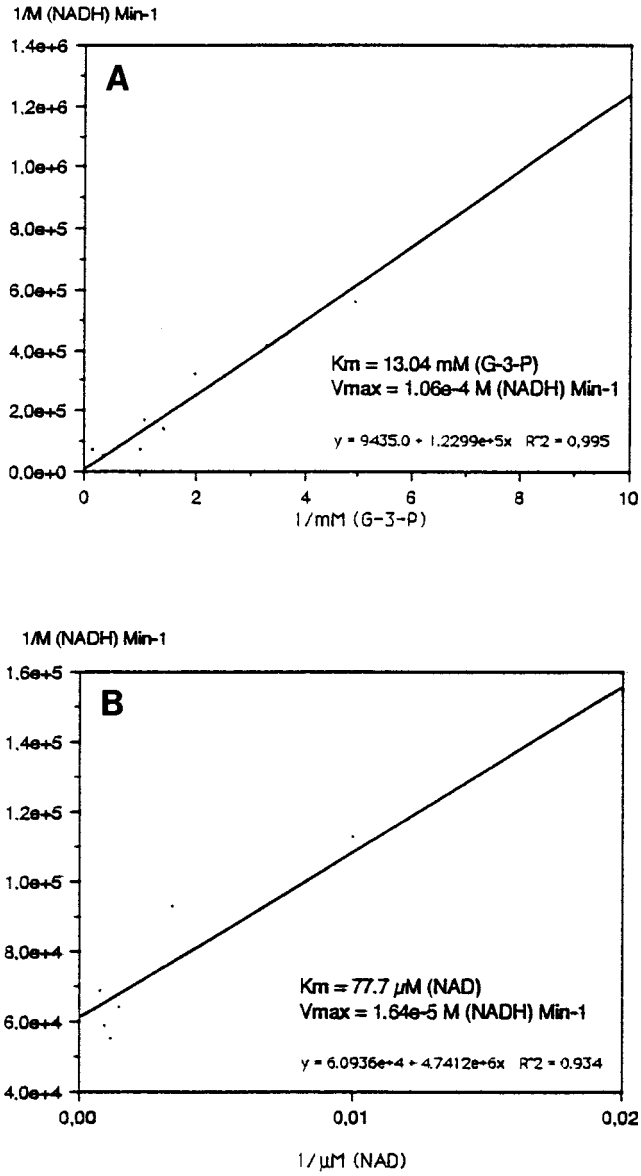

FIG. 5. Lineweaver-Burk double reciprocal plots for the kinetic analysis of the activity of GAPDH. (A) *B. hermsii* sonicate (enzyme source) was measured as a function of G-3-P in the presence of NAD. The intercept on the *y* axis ($1/V_{max}$) and the slope (K_m/V_{max}) are shown in the equation. (B) *B. hermsii* sonicate (enzyme source) was measured as a function of NAD in the presence of G-3-P. Intercept and slope values are shown as described above.

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                    1                                                  25
B.stearothermophilus  A V K V G I N G F G R I G R N V F R A A L K N P D
                                                                       50
B.stearothermophilus  I E V V A V N D L T N A D G L A H L L K Y D S V H
B.burgdorferi                 . . . D P K T . . . . . . . . . T F
B.hermsii                     . . . D P K T . . . . . . . . . T F
                                                                       75
B.stearothermophilus  G R L D A E V V V N D G D V S V N G K E I I V K A
B.burgdorferi         . V Y N K K . E S R . . A I V . D . R . . K I I .
B.hermsii             . V Y N K K . E S R . . A I V . D . R . . K I I .
                                                                       99
B.stearothermophilus  E R N P E N L A W G E I G V D I V V E S T G R F
B.burgdorferi         . . D . K . . P . A K L . I . V . I . . . . V . S
B.hermsii             . . D . K . . P . . K L . I . V . I . . . . V . S
                                                                      120
B.stearothermophilus            T K R E D A A K H L   E A G A K K V I I S A
B.burgdorferi         S A T S D K G G Y L D . V N H . . . . . . . L T V
B.hermsii             S A T S D K G G Y L D . V N G . . . . . . . L T .
                                                                      145
B.stearothermophilus  P A K V E N I T V V M G V N Q D K Y D P K A H H V
B.burgdorferi         . . . D . I K . I . L . . . D H D I N S   D L K A
B.hermsii             . . . D . I K . I E L . . . D H D I N T   D L K A
                                                                      170
B.stearothermophilus  I S N A S C T T N C L A P F A K V L H Q E F G I V
B.burgdorferi         V . . . . . . . . . . . . . L . . . . . E S . . . E
B.hermsii             V . . . . . . . . . . . . . L . . . . . E S . . . E
                                                                      195
B.stearothermophilus  R G M M T T V H S Y T N N Q R I L D L P H K D L R
B.burgdorferi         Q . L . . . . . A . . . D . . . . . . . S . . .
B.hermsii             Q . L . . . . . A . . . D . . . . . . . S . . .
                                                                      220
B.stearothermophilus  G A R A A A E S I I P T T T G A A K A V A L V L P
B.burgdorferi         R . . . . . L . . . . . S . . . . . . . G . . . .
B.hermsii             R . . . . . L . . . . . S . . . . . . . G . . . .
                                                                      245
B.stearothermophilus  E L K G K L N G M A M R V P T P N V S V V D L V A
B.burgdorferi         . . . . . T S . . . . V . T G . I . . . T G
B.hermsii             . . . . . T S . . . . V . T G . I . . . T V
                                                                      268
B.stearothermophilus  E L   E K E V T V E E V N A A L K A A A E G   E L
B.burgdorferi         Q . K K . D . . K . . I . P V . R K . S . T P . .
B.hermsii             Q . K K . D . . K . . I . S V . . K . S . T P . .
                                                                      293
B.stearothermophilus  K G I L A Y S E E P L V S R N Y N G S T V S S T I
B.burgdorferi         . . . . E . T . D . F . . S D I R . N S H . . I
B.hermsii             N . . . G . T . D . I . . S D I K . N S H . . I
                                                                      318
B.stearothermophilus  D A L S T M V I D G K M V K V V S W Y D N E T G Y
                                          335
B.stearothermophilus  S H R V V D L A A Y I N A K G L *
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FIG. 6. Deduced amino acid sequences of the *gapdh* homolog probes for *B. burgdorferi* and *B. hermsii*. A comparison with the sequence of the GAPDH from *B. stearothermophilus* (6) is shown. A dot indicates identity with *B. stearothermophilus*. The amino terminus corresponds to the NAD binding domain, and the carboxy terminus corresponds to the catalytic domain of the enzyme homolog. The overlined tract corresponds to the S-loop.

gapdh homologs by GenBank comparisons with similar or greater identity values. The deduced amino acid sequence of each probe (for *B. burgdorferi* and for *B. hermsii*) is shown in Fig. 6, and the sequences are aligned with the published GAPDH sequence of *B. stearothermophilus* (6). The deduced amino acid sequence of the *B. burgdorferi* probe was 59.2% identical and 78.8% similar to the corresponding segments of *B. stearothermophilus*; the deduced amino acid sequence of the *B. hermsii* probe was 58.8% identical and 77.3% similar. The *B. burgdorferi* probe was 53% identical to the GAPDH of group A streptococci (GenBank accession number M95569 [33]), while the *B. hermsii* probe was 52.3% identical. The identity of the *B. burgdorferi* and *B. hermsii* probes was 93.9% and the similarity was 97%, indicating a high degree of conservation in these two species of pathogenic borrelias. In addition to the conserved amino terminus obtained by Edman degradation (Table 1), the deduced amino acid sequences of the probes contained extensive tracts of complete identity, such as the region of amino acids 147 to 158, and the functionally important S-loop (amino acids 180 to 201). The amino-terminal halves of the deduced amino acid sequences of the probes, which would be within the NAD binding domain of the enzyme, were less conserved (52.2 and 51.5% for *B. burgdorferi* and *B. hermsii*, respectively) than the carboxy-terminal halves (66.9% identical for both *Borrelia* spp.), which would correspond to the catalytic domain of GAPDH. The probes hybridized with the chromosomes of *B. burgdorferi* and *B. hermsii* electrophoresed on agarose gels (18) (Fig. 7).

DISCUSSION

We have shown that *B. burgdorferi* and *B. hermsii* have a chromosomal gene with strong identity to prokaryotic (1, 6, 8, 32, 38, 44) and eukaryotic (15, 16, 34, 36, 47) GAPDH. We have also demonstrated that sonicated borrelias of both species possess the enzymatic activity to catalyze the oxidative phosphorylation of G-3-P into 1-3-diphosphoglycerate in the presence of P_i and NAD^+ (Fig. 4 and 5).

Our interest in demonstrating the presence of a *gapdh* gene as well as the borrelial enzymatic activity stemmed from our previous finding of a 38.5-kDa antigen in *B. burgdorferi* with a marked identity with the amino termini of other GAPDH enzymes. The amino termini of GAPDHs have a conserved sequence, GFGRIG (residues 8 to 13), which is thought to be the ATP binding site of the enzyme (Table 1) (25). This antigen was recognized by a polyreactive murine IgM monoclonal antibody which recognized several other conserved antigens in *B. burgdorferi* (2) as well as in other pathogenic spirochetes (Fig. 2). This IgM antibody had the capacity to recognize multiple unrelated antigens which had acidic epitopes on highly conserved molecules. Such antigens included DnaK and a GAPDH homolog in borrelias. In addition, this antibody can recognize GAPDHs from eukaryotic sources as determined by ELISA and from *B. stearothermophilus* as determined by both ELISA and immunoblotting (Fig. 3). IgM-class antibodies with the capability of binding multiple unrelated antigens through ionic interactions have been described previously (31, 48). The multiple reactivities of these polyreactive natural antibodies are often directed to negatively charged epitopes in highly conserved molecules, such as nucleic acids, cytoskeletal proteins, the Fc portion of IgG, and common surface antigens (31, 48). These natural autoantibodies could serve as precursors for antibodies with autoreactive potential. Thus, polyreactive autoantibodies could enable the immune system to respond rapidly and nonspecifically to a wide variety of foreign invaders. This hypothesis has been supported by the finding of higher levels of these natural autoantibodies in sera of healthy per-

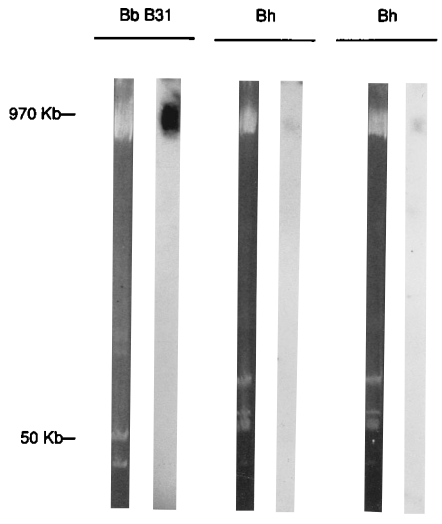

FIG. 7. Southern blot hybridization of *gapdh* homolog probes of *B. burgdorferi* B31 and *B. hermsii*. The left lane of each pair is an ethidium bromide-stained field inversion electrophoresis gel containing total genomic DNA; the right lane is the corresponding autoradiograph of Southern blot hybridization with chromosomal DNA using a *B. burgdorferi gapdh* homolog probe for the first twopairs and a *B. hemsii gapdh* homolog probe for the third and last pair. DNA size standards (size range, 48 to 970 kbp) were from Bio-Rad Laboratories.

sons during exposure to exogenous antigens (31, 48). The implications that polyreactive antibodies may have for the pathogenesis of human Lyme disease are unknown but merit further study.

The demonstration that a GAPDH or GAPDH-related molecule can be located on the surface of group A streptococci and function as a plasmin receptor (9, 32, 33) and as an adhesin for fibronectin and cytoskeletal proteins (38) was also of interest, since it has recently been found that *B. burgdorferi* can bind plasmin(ogen) and urokinase-type plasminogen activator (14, 19, 26). At present, it is not known whether the borrelial GAPDH homolog functions in any of these capacities. Furthermore, the location of the borrelial GAPDH homolog is not known with certainty. Previous studies showed that some of the antigens recognized by the polyreactive monoclonal antibody were trypsin resistant and that most of the antigens were partitioned in the insoluble phase after Triton X-114 phase partitioning (2). On the basis of these observations, we suspect a cytosolic location for the borrelial GAPDH homolog. Nonetheless, it has been suggested that there is a family of *gapdh*-related genes (32, 39, 42). In this regard, *Escherichia coli* has at least two *gapdh* genes (1, 8), with the possibility of the gene products being located in the cytoplasm as well as associated with the membrane.

That GAPDH activity could not be demonstrated for live, intact *B. burgdorferi* or *B. hermsii* spirochetes also supports an internal location for this enzyme homolog or at least a subsurface location of the catalytic domain of the enzyme. The enzymatic activity of group A streptococci can be detected with intact organisms as well as with purified enzyme (38). Borrelial sonicates, however, did have GAPDH activity (Fig. 4 and 5), with K_m values for G-3-P of 13.2 and 13.0 mM for *B. burgdorferi* and *B. hermsii*, respectively, and K_m values for NAD of 32 μM for *B. burgdorferi* and 77.7 μM for *B. hermsii*. These K_m values for G-3-P are higher than those obtained for group A streptococci (1.3 mM) (38). However, a purified enzyme (streptococcal surface dehydrogenase) from streptococci was used for the enzymatic assay rather than a sonicate, as we used with both *Borrelia* spp., and so direct comparisons may not be useful. Likewise, the K_ms for NAD obtained for *B. burgdorferi* and *B. hermsii* were one-fifth and one-half of the K_m obtained for streptococcal surface dehydrogenase, respectively (38). Better comparisons of enzymatic activities with those of other bacteria will be made when the borrelial *gapdh* homologs are cloned and expressed.

The *gapdh* homologs were mapped to the chromosomes of both *B. burgdorferi* and *B. hermsii* by Southern blot hybridization (Fig. 7) utilizing probes derived from PCR products obtained with degenerate primers specific to the amino terminus (based on our sequence [Table 1]) and to other conserved regions of GAPDH according to a *B. burgdorferi* codon usage table (Fig. 1). Subsequent nucleotide sequence analysis of the 838-bp probes showed that the GAPDH homologs of *B. burgdorferi* and *B. hermsii* have many features in common with previously published sequences of GAPDH enzymes. GAPDH of the moderately thermophilic bacterium *B. stearothermophilus* was the first of the prokaryotic enzymes to be sequenced, and its three-dimensional structure is known (6). Therefore, it is this sequence with which alignments of the borrelial GAPDH homolog probes were compared. Numerous motifs of the enzyme were found in both probes in addition to the ATP binding site at the amino terminus. The probes also showed a conserved S-loop, which forms the core of the tetramer (amino acids 180 to 202) but contains a serine at position 192. This is characteristic of eukaryotic GAPDHs (34) as well as one *E. coli* GAPDH (8). Our probes also showed extensive tracts of complete identity as well as the presence of histidine 176 (45), which is required for catalysis.

In addition to GAPDH's critical role in glycolysis, the numerous new functions associated with both eukaryotic and prokaryotic GAPDHs provide important reasons to study this enzyme's biological relevance in borrelias.

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