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Acylated Proteins in *Borrelia hermsii*, *Borrelia parkeri*, *Borrelia anserina*, and *Borrelia coriaceae*

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***Borrelia hermsii*, *Borrelia parkeri*, *Borrelia anserina*, and *Borrelia coriaceae* produced several lipoproteins identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of bacteria grown in [^{3}H]palmitate. Five major acylated proteins were demonstrated by sequential alkaline and acid hydrolysis. High-pressure liquid chromatography of isolated proteins confirmed that covalently bound radioactivity was represented by fatty acids.**

Bacteria of the genus *Borrelia* belonging to the order *Spirochaetales* are transmitted to vertebrate hosts by the bite of an arthropod vector and, like other spirochetes, are helical (10). *Borrelia hermsii* and *Borrelia parkeri* are responsible for cases of American tick-borne relapsing fever (12), *Borrelia anserina* is a bird pathogen spread worldwide (2), and *Borrelia coriaceae* is a putative agent of epizootic bovine abortions (14).

Previous studies have shown that major outer surface proteins A and B (i.e., OSP-A and OSP-B) of *Borrelia burgdorferi*, the causative agent of Lyme disease (3), are lipoproteins (1) and that the differences in cysteine incorporation observed between *B. burgdorferi* and *B. hermsii* are correlated with differences in lipoproteins (19). These observations prompted us to search for acylated proteins in other *Borrelia* species.

Bacterial strains. The following *Borrelia* strains were used: *B. hermsii* HS-1 (ATCC 35209) (21); *B. parkeri* M3001, isolated from *Ornithodoros parkeri* in the western United States (17); *B. anserina* Es, isolated from White Leghorn chickens in California (7); and *B. coriaceae* Co 53 (ATCC 43381), isolated from *Ornithodoros coriaceus* in California (14). (All the strains were kindly provided by Russell C. Johnson, Minneapolis, Minn., and by Michael A. Lovett, Los Angeles, Calif.) Borreliae were cultured in BSK II medium as previously reported (20).

SDS-PAGE and autoradiography. To determine the incorporation of ^{14}C-labeled amino acids, [^{35}S]cysteine, and [^{3}H]palmitate into proteins, borreliae were grown in the presence of 1 μCi of ^{14}C-labeled amino acid mixture (>50 mCi/ml; Amersham Co., Amersham, United Kingdom) per ml, 5 μCi of L-[^{35}S]cysteine (>600 Ci/mmol; NEN-Du Pont, Bad Homburg, Germany) per ml, or 250 μCi of [9,10-(n)-^{3}H]palmitic acid (53 Ci/mmol; Amersham) per ml. Spirochetes were then harvested and processed for sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (13) as previously described (5). Fluorograms were obtained by following standard procedures as previously described (19).

Lipid analysis of proteins. The lipid analysis was performed on proteins excised from SDS-PAGE gels after Coomassie brilliant blue staining and destaining of the gels. Sequential alkaline and acid hydrolyses were performed directly on each gel slice previously extracted (4, 6) with diethyl ether-ethanol (2:1) twice and were followed by three extractions with chloroform-methanol-concentrated HCl (6.6:3.3:0.1) controlling the radioactivity in the organic extracts. After drying with N_2, gel slices were incubated in 3 ml of 1.5 N NaOH under N_2 at 30°C for 3 h, acidified to pH 1 to 2 with 6 N HCl, and extracted twice with diethyl ether-ethanol (2:1). The organic phases were then pooled. The aqueous phase containing the gel slice was dried, suspended in 3 ml of 6 N HCl, and incubated at 100°C for 4 h. The samples were then extracted twice again, and the organic phases were pooled. The organic extracts were then dried under a flux of nitrogen and redissolved in 1.0 ml of chloroform for radioactivity counting; 0.5 ml was dissolved in 5 ml of toluene containing 0.1% PPO (2,5-diphenyloxazole) (Merck, Darmstadt, Germany), and tritium radioactivity was counted by liquid scintillation in a Tri Carb 4450 instrument (Packard, Meridien, Conn.). The remaining fraction of hydrolysate was used for fatty acid determination.

HPLC analysis of ^{3}H-labeled fatty acids. Tritiated fatty acids released by alkaline and acid hydrolyses were analyzed by high-pressure liquid chromatography (HPLC) with a liquid chromatograph from Waters Chromatography Division (Milford, Mass.). Twenty-microliter samples were injected onto an Ultrasphere octyldecyl silane reversed-phase column (25 by 0.46 cm; Beckman, Fullerton, Calif.) and eluted with methanol–0.1 M triethylammonium formate (85:1) at a flow rate of 1.5 ml/min. The radioactivity in the eluate was monitored with a Radiomatic Flo-One detector (Packard), and the identity of peaks was based on cochromatography with standards of tritiated fatty acids.

Acylated proteins in *Borrelia* species. The analysis of bacteria radiolabeled with ^{14}C-amino acids, [^{35}S]cysteine, and [^{3}H]palmitate by SDS-PAGE and autoradiography showed that a relatively small number of borrelial proteins labeled with ^{14}C-amino acids were also biosynthetically labeled with [^{35}S]cysteine (Fig. 1). Few major cysteine-containing proteins could be detected in borrelial strains, and some of these proteins were also those preferentially radiolabeled by [^{3}H]palmitate (Fig. 1, lanes C). *B. hermsii* showed two cysteine-containing proteins of 22 and 24 kDa (Fig. 1, frame 1, lane B), and *B. parkeri* showed a doublet of 40 to 42 kDa that incorporated [^{3}H]palmitate (Fig. 1, frame 4, lanes B and C). Of several weak [^{35}S]cysteine-labeled proteins of the

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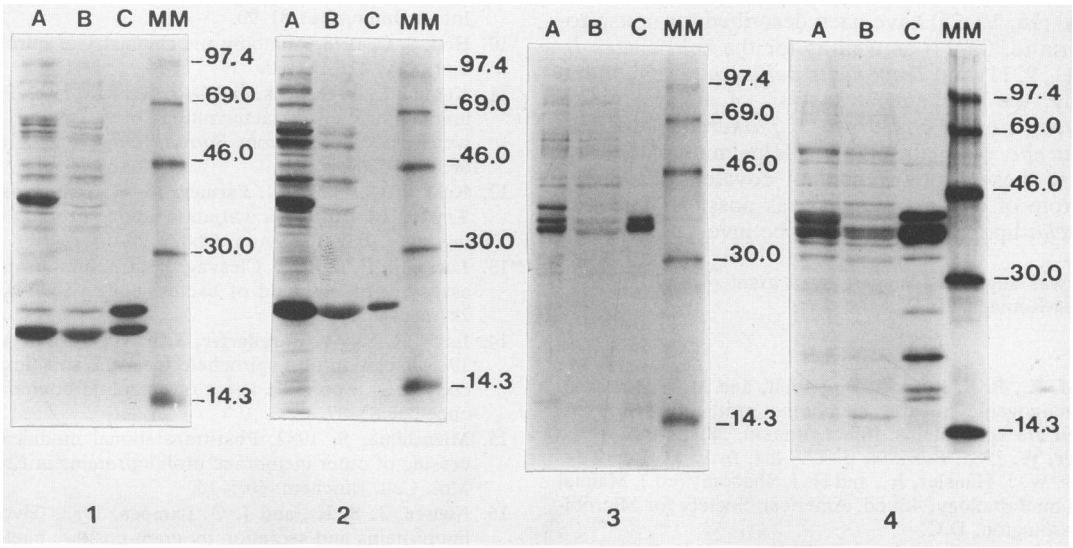

FIG. 1. Fluorographs of SDS-polyacrylamide gels showing incorporation of ^{14}C-amino acids (lanes A), [^{35}S]cysteine (lanes B), and [^{3}H]palmitic acid (lanes C) into proteins of *B. hermsii* (1), *B. anserina* (2), *B. coriaceae* (3), and *B. parkeri* (4). Molecular weight markers (MM) are shown. Molecular masses of proteins are expressed in kilodaltons.

animal-pathogenic *B. coriaceae*, a 34- to 36-kDa doublet was the only one to incorporate [^{3}H]palmitate (Fig. 1, frame 3, lanes B and C). Finally, *B. anserina* demonstrated a major 20-kDa cysteine-incorporating protein that was also identified by [^{3}H]palmitate (Fig. 1, frame 2, lanes B and C). The radioactive palmitate was not degraded before incorporation into borrelial proteins, since only a discrete subset of polypeptides labeled with ^{14}C-amino acids was also labeled with [^{3}H]palmitate in all borrelial strains.

Delipidated *Borrelia* proteins excised from gels were subjected to sequential alkaline and acid hydrolysis in order to release [^{3}H]palmitate in either ester or amide linkage. The results are reported in Table 1. As predicted from conventional bacterial lipoproteins, the ratio of alkali-labile (ester-linked) to acid-labile (amide-linked) radioactivity was about 2:1 for lipoproteins from *B. parkeri*, *B. anserina*, and *B. coriaceae*, whereas the ratio was higher for proteins extracted from *B. hermsii* (3.4 and 4.5 for the 22- and 24-kDa proteins, respectively).

The HPLC analysis of the organic extracts obtained after alkali or acid hydrolysis of all five proteins reported in Table 1 was performed to confirm that covalently bound radioactivity was represented by fatty acids. Radioactivity from all samples comigrated with [^{3}H]palmitate, generating similar chromatograms. A typical HPLC analysis of the 22-kDa protein of *B. hermsii* is shown in Fig. 2. No evidence of chain elongation, beta oxidation, or desaturation of radioactive fatty acids was found in various *Borrelia* species, as indicated by the recovery of unaltered [^{3}H]palmitate from proteins after alkaline and acid hydrolysis.

In recent years, proteins with covalently attached lipids

TABLE 1. Radioactivity released after sequential acid and alkaline hydrolysis of *Borrelia* lipoproteins

Species, protein size (kDa)	Radioactivity recovered after hydrolysis (%)		Ester-to-amide linkage ratio
	Alkaline	Acid	
B. hermsii, 22	77	23	3.4
B. hermsii, 24	81	19	4.5
B. parkeri, 40 to 42	69	31	2.2
B. anserina, 20	64	36	1.6
B. coriaceae, 34 to 36	61	39	1.8

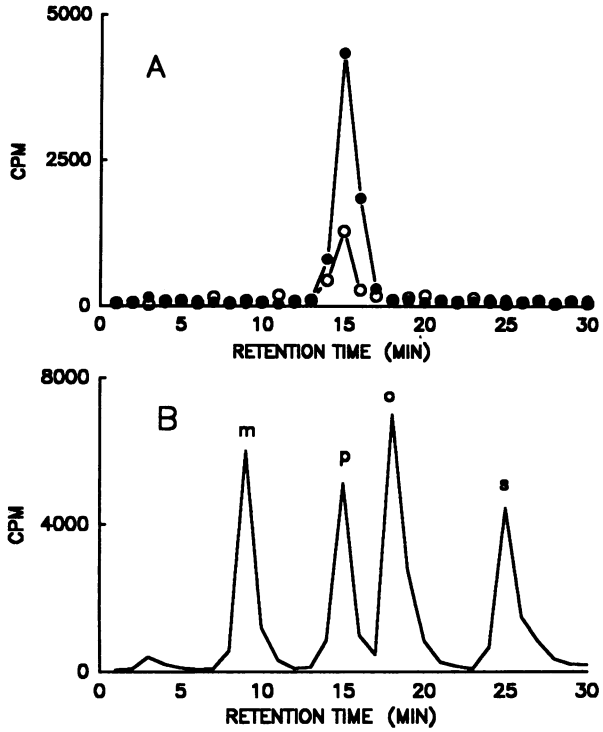

FIG. 2. HPLC analysis of fatty acids from the 22-kDa protein of *B. hermsii* labeled with [^{3}H]palmitic acid. Radioactivity recovered after sequential alkaline (●) and acid (○) hydrolysis of protein in polyacrylamide gel slices. (B) Fatty acid standards: myristate (m), 14:0; palmitate (p), 16:0; oleate (o), 18:1; stearate (s), 18:0.

(proteolipids) (15, 16, 25) have been described for nonspirochetal bacteria (8, 22–24) as well as for the spirochetes *B. burgdorferi* (1, 9, 11) and *Treponema pallidum* (6, 18). In the present study, the biochemical analysis of major proteins from prototype strains of *B. hermsii*, *B. parkeri*, *B. anserina*, and *B. coriaceae* radiolabeled by [^{3}H]palmitate provided evidence that these proteins contain covalently attached lipids. The role of cysteine residues as possible lipidation sites in *Borrelia* lipoproteins needs to be investigated.

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