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Unusual Fatty Acid Substitution in Lipids and Lipopolysaccharides of *Helicobacter pylori*

GABRIELE GEIS,* HERMANN LEYING, SEBASTIAN SUERBAUM, AND WOLFGANG OPFERKUCH

Department of Medical Microbiology and Immunology, Ruhr-Universität Bochum, D-4630 Bochum, Federal Republic of Germany

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Cellular fatty acids, phospholipid fatty acids, and lipopolysaccharide fatty acids of four strains of *Helicobacter pylori* were analyzed by gas-liquid chromatography. The presence of myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, 19-carbon cyclopropane fatty acid, β-hydroxypalmitic acid, and β-hydroxystearic acid was confirmed. In phospholipids, myristic acid and 19-carbon cyclopropane fatty acid were the major fatty acids. Hydroxy fatty acids and unsaturated fatty acids were not detected or occurred only in small amounts. The major fatty acids of lipopolysaccharides were stearic acid, β-hydroxypalmitic acid, and β-hydroxystearic acid. Unsaturated fatty acids and 19-carbon cyclopropane fatty acid were not found. The unusual compositions of *H. pylori* phospholipid and lipopolysaccharide fatty acids may have important implications for the taxonomy, physicochemical membrane properties, and biological activity of lipopolysaccharides.

Culture of *Helicobacter pylori* (formerly *Campylobacter pylori* [9]) was first reported by Warren and Marshall (25) in 1983. The bacterium is associated with gastritis, peptic ulcer disease (1, 18, 19), and possibly gastric cancer (F. Sitas, D. Forman, J. Chen, D. Newell, and A. Stacey, Abstr. 2nd Meet. Eur. *Campylobacter pylori* Study Group, Klin. Wochenschr. **67**[Suppl. XVIII]:S64, 1989) and has been the subject of a large number of both clinical and microbiological studies. However, relatively few components of the *H. pylori* cell have been purified and subjected to detailed analysis; among those structures that are characterized in some detail are urease (5, 20), the filament of the flagellum (7), the neuraminyllactose-binding hemagglutinin (4), and cellular fatty acids (10, 12, 16). Cellular fatty acid analysis has been performed by several groups who found unusual but characteristic profiles with high amounts of myristic acid and 19-carbon cyclopropane fatty acid (10, 12, 16) and with β-hydroxypalmitic and β-hydroxystearic acids as further constituents (10, 16). This unusual cellular fatty acid profile was a major criterion for the recent exclusion of *H. pylori* from the genus *Campylobacter* (9).

No data on the fatty acid substitution of isolated cell envelope components of *H. pylori* have been published to date. We have analyzed the fatty acid substitution of isolated lipids and lipopolysaccharides (LPSs) of *H. pylori* by gas-liquid chromatography. Implications of the findings for the taxonomy, physicochemical membrane properties, and biological activities of LPSs of *H. pylori* are discussed.

MATERIALS AND METHODS

Bacteria. Four strains of *H. pylori* were used. Strains 8981 and 9015 were isolated from gastric biopsies. Identification was based on typical colony morphology; Gram stain; positive tests for oxidase, catalase, and urease; negative nitrate reduction and hippurate hydrolysis; and susceptibility to cephalothin. National Collection of Type Cultures strains 11637 and 11639 were obtained from the Public Health Laboratory Service, London, United Kingdom. Bacteria were grown for 3 days in media and under conditions as previously described (7). To prepare coccoid cells, cultures were harvested, washed, and stored in phosphate-buffered saline (pH 7.4) at 4°C for 6 weeks.

Isolation and purification of LPSs and phospholipids. LPSs were isolated from about 0.5 g (wet weight) of bacteria by the phenol-water extraction method (26) and further purified by fractional cetavlon precipitation by procedure III of Westphal and Jann (26). Phospholipids were isolated from a similar weight of bacteria by the method of Folch et al. (6) and were evaporated with a gentle flow of nitrogen.

Preparation of FAMEs. Fatty acid methyl esters (FAMEs) were prepared by method A as described by Gmeiner and Martin (8). Briefly, the dried samples (lyophilized LPSs, dried lipids, 0.15 g of bacteria [wet weight]) were suspended in 1 ml of 4 N NaOH and held at 100°C for 5 h. Two milliliters of 4 N HCl was added, and fatty acids were extracted with three chloroform washes. Chloroform phases were pooled and dried. One milliliter of BF_3-methanol reagent (Merck, Darmstadt, Federal Republic of Germany) was added, and the solution was heated to 100°C for 5 min. After cooling of the samples, 0.6 ml of double-distilled water was added and FAMEs were extracted three times with petroleum ether (boiling range, 30 to 50°C; Riedel-de Haën, Seelze, Federal Republic of Germany). Combined petroleum ether phases were evaporated with nitrogen and suspended in 50 μl of a mixture containing one part methanol and two parts chloroform.

Gas-liquid chromatography. FAMEs were detected in a gas-liquid chromatograph equipped with a flame ionization detector (model 3700; Varian, Palo Alto, Calif.). Samples of 5 μl were analyzed on a fused-silica capillary column (FS-FFAP-CB-0.25; Macherey-Nagel, Düren, Federal Republic of Germany). The column temperature was programmed from 140 to 220°C at 2°C/min and was maintained at 220°C for 10 min before recycling. The injector temperature was 220°C, and the detector temperature was 250°C. Nitrogen was used as the carrier gas. FAMEs were identified by comparing retention times to known standards. Quantitation of peak areas was done with a model 4270 integrator (Varian). Determinations were performed in triplicate; the coefficient of variation was always less than 0.1.

* Corresponding author.

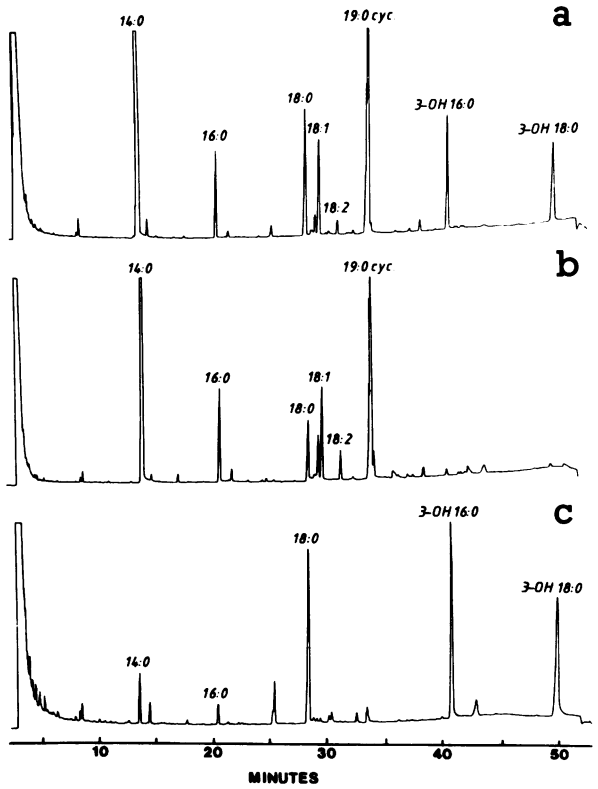

FIG. 1. Gas chromatograms of *H. pylori* fatty acid methyl esters of whole cells (a), phospholipids (b), and LPSs (c) (strain 8981). See text for explanations of compound abbreviations. Note the predominant presence of myristic acid and 19-carbon cyclopropane fatty acid in isolated phospholipids and of stearic acid and β-hydroxy fatty acids in isolated LPSs.

RESULTS AND DISCUSSION

Qualitative analysis of *H. pylori* fatty acids. Figure 1 shows the gas chromatograms of cellular fatty acids, phospholipid fatty acids, and LPS fatty acids of *H. pylori*. The fatty acids were identified as myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), 19-carbon cyclopropane fatty acid ($C_{19:0}$cyc), β-hydroxypalmitic acid (3-OH-$C_{16:0}$), and β-hydroxystearic acid (3-OH-$C_{18:0}$). The same fatty acids were identified in the studies by others (10, 12, 16), with the exceptions that linoleic acid ($C_{18:2}$) was not detected by Lambert et al. (16) and that the hydroxy fatty acids were not observed by Itoh et al. (12).

One LPS fatty acid with a retention time of about 25 min (Fig. 1c) could not be identified; its amount was smaller than 4% of total LPS fatty acids.

Quantitative analysis of cellular fatty acids. Table 1 shows the distribution of cellular fatty acids for four strains of *H. pylori*. Values are given only for those exceeding 2% of total fatty acids. The major cellular fatty acids were myristic acid (31 to 45%) and 19-carbon cyclopropane fatty acid (20 to 24%). All other fatty acids were found in amounts of 12% or less of total cellular fatty acids.

Although similar profiles were found for all strains tested, there was some strain-to-strain variation in profiles. Data for cellular fatty acids were consistent with those of other investigators (10, 12, 16).

Quantitative analysis of phospholipid fatty acids. Table 1 shows the distribution of fatty acids in *H. pylori* phospholipids. Myristic acid (41 to 55%) and 19-carbon cyclopropane fatty acid (22 to 30%) were the major components, with β-hydroxy fatty acids and unsaturated fatty acids either not detected or present in small amounts.

This composition of fatty acids in the phospholipids of *H. pylori* is very unusual compared with other bacterial phospholipids (8, 24). The fatty acid substitution of bacterial phospholipids is a major determinant of physicochemical properties of membranes, properties such as fluidity or phase transition temperature (2). It seems very likely that the unique fatty acid substitution of *H. pylori* phospholipids leads to unusual membrane properties that warrant further investigation.

It is known that culture conditions such as growth phase or temperature affect the fatty acid profiles of bacterial phospholipids (3, 14, 17). When *H. pylori* cultures were

TABLE 1. Fatty acid composition of *H. pylori*

Strain	Fatty acids (% of total fatty acids)[a]							
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{19:0}$cyc	3-OH-$C_{16:0}$	3-OH-$C_{18:0}$
Cellular fatty acids								
8981	43	3	7	6	tr	22	6	7
9015	44	4	9	2	tr	21	4	6
NCTC 11637	45	2	7	5	tr	24	4	7
NCTC 11639	31	5	12	5	3	20	10	7
Fatty acids in isolated phospholipids								
8981	41	7	5	7	2	28	—	—
9015	55	4	5	2	tr	22	—	—
NCTC 11637	49	3	4	5	tr	30	—	—
NCTC 11639	45	5	6	6	2	28	—	—
Fatty acids in isolated LPSs								
8981	4	2	21	—	—	—	25	24
9015	4	2	29	—	—	—	20	27
NCTC 11637	3	10	23	—	—	—	27	22
NCTC 11639	3	2	18	—	—	—	37	23

[a] Abbreviations: tr, 0.5 to 1.9%; —, less than 0.5%. The number before the colon refers to the number of carbon atoms, and the number after the colon refers to the number of double bonds; cyc refers to a cyclopropane fatty acid; OH refers to a hydroxyl group.

grown for longer periods of time, most of the spiral cells transformed into coccoid cells. We have analyzed logarithmic-phase cultures which contained predominantly spiral cells and compared these with cultures which contained more than 90% coccoid cells. We found no significant differences in the phospholipid fatty acid profiles (data not shown).

Quantitative analysis of LPS fatty acids. Table 1 shows the distribution of fatty acids in LPSs of *H. pylori*. Stearic acid (18 to 29%), β-hydroxypalmitic acid (20 to 37%), and β-hydroxystearic acid (22 to 27%) were the major fatty acids. Unsaturated fatty acids and 19-carbon cyclopropane fatty acid were not detected. As with the phospholipid fatty acids, there was some strain-to-strain variability of the LPS fatty acid profiles.

β-Hydroxystearic acid is rarely detected in bacterial membranes and has been found only in very few species, such as *Francisella tularensis* (13, 15). Its presence in *H. pylori* LPS as one of the major fatty acids is a very characteristic and unusual feature of this species.

Also unusual was the presence of stearic acid, a common constituent of bacterial phospholipids (8, 24) but rarely found in LPSs (e.g., *Brucella* sp. [21]). The structure of lipid A is a highly conserved feature of gram-negative bacteria (11), and LPS properties are regarded as important taxonomic markers (23). The fatty acid composition of LPSs will be useful to determine the phylogenetic relationship of *H. pylori* with current and future members of the new genus *Helicobacter*.

It was demonstrated that even small changes in the lipid A structure can result in major differences in endotoxin activity (for a review, see reference 22). *H. pylori* LPS, because of its unusual structure, may also be a tool to get further information on the relationship between LPS structure and biological activity.

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