Fatty Acids in Phospholipids of Cells, Cysts, and Germinating Cysts of *Azotobacter vinelandii*†

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Cyclopropane fatty acids constitute 25% of the phospholipid acyl groups in cysts of *Azotobacter vinelandii*. These are lost by dilution during germination when the synthesis of those fatty acids characteristic of vegetative cell phospholipids commences.

*Azotobacter vinelandii* can be induced to encyst by shifting an exponentially growing culture from glucose as a carbon source to 0.2% β-hydroxybutyrate (9). In tracing the metabolic rate of β-hydroxybutyrate during this cell-to-cyst transition, we have noted (i) that a significant amount of β-hydroxybutyrate is incorporated into membrane as acyl groups of phospholipids and (ii) that the cyclopropane fatty acids cis-9,10-methylenehexadecanoic acid (C₁₇:₈) and lactobacillic acid (cis-11,12-methyleneoctanoic acid; C₁₇:₇) are formed in the phospholipids during encystment (15). The significance of these acids with respect to encystment is unknown, and our initial attempts at its assessment were to examine the fatty acid composition of phospholipids, and thus of cell membrane, throughout the organism's cell cycle.

*A. vinelandii* 12837 was grown with aeration at 30°C in Burk N-free buffer (18) with 1% glucose as the carbon source. Samples (40 ml) were taken at appropriate growth stages. Encystment was induced in 3 liters of a late exponentially growing culture by harvesting it, washing the cells in Burk buffer, and suspending them in 3 liters of Burk buffer containing 0.2% β-hydroxybutyrate. These cells were then incubated at 30°C with aeration for 72 h. Mature cysts which formed were washed once with Burk buffer and stored at −20°C. For germination, cysts were suspended to a concentration of 6.3 × 10⁶ cells per ml in 400 ml of Burk buffer containing 1% glucose and incubated with aeration at 30°C. Samples (40 ml) were taken immediately after suspension and at intervals over a 12-h period.

Cell or cyst samples were harvested, suspended in 3.2 ml of water, cooled in an ice bath, and disrupted by sonic treatment in eight 15-s pulses (MSE sonic oscillator). The lipids were extracted in CHCl₃-CH₃OH (2:1, vol/vol) by the single-phase procedure of Bligh and Dyer (1) and dried with a benzene–ethanol mixture (4:1, vol/vol). The extracted lipids were separated into neutral lipids, glycolipids, and phospholipids by chromatography on silicic acid columns eluted sequentially with chloroform, acetone, and methanol (8). The phospholipids were subjected to mild alkaline methanolysis (17), and the fatty acid methyl esters were extracted twice with CHCl₃, dried in an N₂ stream, and dissolved in ethylacetate. These were initially analyzed by gas-liquid chromatography in a Varian Aerograph 1400 chromatograph equipped with a 180-cm column of 1.5% SE-30 and an H₂ flame ionization detector which had been calibrated with known methyl esters. Positive identification of fatty acids was made by subjecting the methyl esters to gas chromatography-mass spectrometry.

The profiles of fatty acids in vegetative cells and cysts are shown in Fig. 1. The principal fatty acids in phospholipids of vegetative cells were palmitoleic (C₁₆:₁), palmitic (C₁₆:₀), and cis-vaccenic (C₁₈:₁) acids. Total extractable lipid accounted for 10.8% of the dry weight of whole cells, a result corresponding to that published by Kaneshiro and Marr (7). Total extractable lipids accounted for 17.5% of the dry weight of cysts, which is close to the result of Lin and Sadoff (10). The principal fatty acids in cysts were palmitic, palmitoleic, cis-9,10-methylene hexadecanoic, cis-vaccenic, and lactobacillic acids; the minor fatty acids (less than 5% each) were myristic (C₁₄:₀) and octadecanoic (C₁₈:₀) acids.

Cyclopropane fatty acids have been found in a variety of bacteria and higher plants (2). They are synthesized from the corresponding unsaturated acids by addition of a methylene group, donated by 5-adenosylmethylene, across the double bond (13, 19). This conversion is illustrated qualitatively in Fig. 1.
In Table 1 are presented typical fatty acid compositions of phospholipids from *A. vinelandii* vegetative cells during exponential growth phase and at various times in the cell cycle. The content of C16:1, C16:0, and C18:1 fatty acids increased steadily during germination, with a corresponding decrease in the percentages of C17:3, C18:0, and C20:0 fatty acids. The total percentage of C16:1 plus C17:3 and C18:1 plus C19:5 in all samples was relatively constant. C17:3 is not a constituent of vegetative cells, but it does occur in very young vegetative cells derived from cysts. The cyclopropane fatty acids are metabolically stable (14), and the amount of C17:3 present in the phospholipids was used as an internal standard in calculating the rate of synthesis of other fatty acids during germination (Fig. 2). The variability of the amount of C19:5 present during the period of observation (relative to C17:3) lends credence to this approach. The increase of C14:0, C16:0, C16:1, and C18:1 fatty acids found in vegetative cells correlated with the cell size increase during the germination of cysts. Synthesis oc-

![Graph](http://jb.asm.org/)

**Fig. 1.** Gas chromatography elution profile of the methyl esters of fatty acids in phospholipids of cells (-----) and cysts (-----) of *A. vinelandii*. The instrument was programmed to heat 10°C/min over the range of 120 to 220°C and, after reaching the higher temperature, to operate isothermally for 5 min. The injection temperature was 250°C, the detection temperature was 275°C, and helium was used as the carrier gas with a flow rate of 25 ml/min.

**Fig. 2.** Amount of fatty acid relative to C17:3 at various times during the germination and vegetative growth of *A. vinelandii* cysts.

<table>
<thead>
<tr>
<th>TABLE 1. Percentage of fatty acids in phospholipids at various times during the cell cycle of <em>A. vinelandii</em>&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td><strong>Time of determination</strong></td>
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<td>--------------------------</td>
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<td>% Of total as:</td>
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<td>C14:0</td>
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<td>C16:0</td>
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<td>C19:5</td>
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<td>C16:1 + C17:3</td>
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<td>C18:1 + C19:5</td>
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<sup>a</sup>The percent composition for each fatty acid was calculated from recorded gas chromatographic data (area under curve). Data are averages of two determinations.

<sup>b</sup>The times listed are those after the initiation of germination by the addition of 1% glucose to a cyst suspension.

<sup>c</sup>This culture had a generation time of 3 h and a turbidity (optical density at 600 nm) of 0.40.

<sup>d</sup>The times listed are those after the initiation of encystment by the addition of 0.2% β-hydroxybutyrate to a washed, exponential culture.
curred at a low exponential rate and lacked the critical transition in rate between 4 and 5 h characteristic of both protein and RNA synthesis during germination (11). All four fatty acids found in membrane phospholipids of vegetative cells of *A. vinelandii* were synthesized upon initiation of germination, then maintained distinct synthetic rates throughout germination and outgrowth, and were then all synthesized at the same relative rate upon initiation of exponential growth at 8 h.

The precise physiological role of cyclopropane fatty acids is unknown, but, since they are resistant to peroxidation (4, 16) and function much like their unsaturated precursors (3, 6), they would seem logical components of metabolically dormant resting cells. Marr and Ingraham (12) suggest that nitrogen limitation promotes cyclopropane fatty acid formation. *A. vinelandii* cells cease N fixation immediately upon induction of encystment (5), resulting in just such nitrogen starvation.

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LITERATURE CITED


