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Localization and Quantitation of the Ornithine Lipid of *Thiobacillus thiooxidans*

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The ornithine lipid of *Thiobacillus thiooxidans* was found to be 1.9% of the total polar lipids. Approximately 80% of this lipid was found to be localized in the outer membrane.

Thiobacillus thiooxidans is a chemoautotropic bacterium that oxidizes elemental sulfur and reduced sulfur compounds, which results in a highly acid growth environment (12). The optimal pH for growth of *T. thiooxidans* is 3.5, but the organism can survive an environmental pH of 0 (12). The mechanism(s) by which the cell envelope can resist high concentrations of hydrogen ions is unknown (4). Noguchi et al. (8) have however, investigated the acidostability of spheroplasted cells of *T. thiooxidans* and theorized that acid resistance is due to proteins in the cell membranes and is energy dependent. Shively et al. (1, 6, 11) characterized the polar lipids of *T. thiooxidans* and discovered an unusual ornithine lipid. It was postulated that this lipid might form a positively charged "protein shield" in the membrane, thus protecting the cell from the highly acid environment. This study was designated to determine the total amount of this lipid present in the cell and to determine its precise cellular location.

T. thiooxidans cells were grown in 15-liter carboys as described previously (1, 11). Cells were allowed to grow until the pH of the growth medium reached levels below 0.5 and no sulfur was macroscopically visible in the medium. Spheroplasts of the cells were formed by a modification of the method of Noguchi et al. (8). Removal of the peptidoglycan was accomplished by purified beta-glucuronidase (Sigma Chemical Co., St. Louis, Mo.) instead of snail intestinal juice (8). Membrane samples were obtained as described by Osborn et al. (9). The cytoplasmic and outer membranes were separated by sucrose density step gradient centrifugation The outer and cytoplasmic membrane fractions were removed from the gradient by a micropipette and diluted with distilled water. Membranes were pelleted by centrifugation, using an L5-65 preparative ultracentrifuge with a Ti-75 rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 6.5 $\times$ 10^4 rpm for 3 h. Membrane pellets were washed three times with distilled water and collected by centrifugation. Succinate dehydrogenase (9) and 2-keto-3-deoxyoctulosonic acid (10) assays demonstrated less than 2% cross-contamination of the inner and outer membrane. Membrane lipids were extracted by the method of Bligh and Dyer (3) and dried with nitrogen. Lipid extracts were returned to a known volume with chloroform-methanol, and samples were analyzed for total lipid phosphorus by the Fiske-Subbarow method as modified by Bartlett (2). The remainder of the lipid extracts were dried with nitrogen and subjected to a 6 N HCl hydrolysis at 100°C for 14 h. Hydrolysates were washed with three volumes of chloroform, and the water-soluble moieties were dried in a vacuum over sodium hydroxide. Vacuum-dried hydrolysates were returned to a known volume with 0.1 N HCl and used for ornithine quantitation. Levels of ornithine were determined by gas-liquid chromatography by the method of Gehrke and Liemer (5) and by analysis with a Phoenix amino acid autoanalyzer (Mark Instruments, Villanova, Pa.).

The ornithine lipid was 1.9% of the total polar lipids of the whole cell (Table 1). Furthermore, 97% of all ornithine lipid was membrane associated, and approximately 80% was in the outer membrane. Sment et al. (K. A. Sment, T. L. Brockman, and B. J. Wilkinson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K178, p. 167) recently found that the majority of the ornithine lipid of *Paracoccus denitrificans* was localized in the outer membrane. Makula and Finnerty (7), however, reported an ornithine lipid to be equally distributed between the outer and cytoplasmic membrane in *Desulfovibrio gigas*.

These data show that the ornithine lipid discovered by Shively et al. (1, 6, 11) is almost exclusively contained in the membranes, with the majority residing in the outer membrane. The function of this amino lipid is currently unknown, but the presence of a positively

TABLE 1. Ornithine lipid composition of whole cells and membranes of *T. thiooxidans*

Location	Amt (μmol) of:		% Total polar lipid as ornithine lipid	% Localization
	Phospholipid	Ornithine lipid		
Whole cell	1,128 ± 13	21.3 ± 0.01	1.9	
Cytoplasmic	796 ± 25	4.1 ± 0.04	0.51	20
Outer	319 ± 3	16.6 ± 0.03	4.9	80

charged group in this lipid and its selective localization suggests a possible role in acid resistance.

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