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Isolation of an Ornithine-containing Lipid from *Thiobacillus thiooxidans*

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The ornithine-containing lipid was separated from the other lipids of *Thiobacillus thiooxidans* by thin-layer chromatography. The aminolipid possesses both amide and ester linkages.

Chloroform-methanol extracts of *Thiobacillus thiooxidans* have been reported to contain a lipid which reacts with ninhydrin but is devoid of phosphorus (5). The isolation and preliminary characterization of this lipid are described.

Cultures of *T. thiooxidans* ATCC 8085 were grown in carboys containing 15 liters of a medium composed of 5.0 g of $Na_2S_2O_3 \cdot 5H_2O$, 3.0 g of KH_2PO_4, 0.4 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.25 g of $CaCl_2 \cdot 2H_2O$, and 0.01 g of $FeSO_4 \cdot 7H_2O$ per liter of distilled water. A concentrated solution of $Na_2S_2O_3$ was sterilized by filtration and added aseptically to the remainder of the medium, which had been autoclaved at 121 C for 2 hr. Finally, the *p*H was adjusted to 4.5 with H_2SO_4. Each carboy was inoculated with 300 ml of a 48-hr culture which had been grown in 250-ml Erlenmeyer flasks (50 ml of the above medium per flask). The flasks were incubated at 30 C on a rotary shaker. The inoculated carboys were incubated at 30 C with vigorous aeration.

After the cultures had reached the maximal stationary growth phase, the cells were harvested by centrifugation and washed with 0.01 N H_2SO_4. The lipids were extracted from the cells with chloroform-methanol (7). The extract was washed with a sodium chloride solution (2) and evaporated to dryness. The lipids were redissolved in chloroform-methanol (2:1, v/v).

The aminolipid was separated from phospholipids by two-dimensional thin-layer chromatography (Eastman Chromagram, type 6061, Distillation Products Industries, Rochester, N.Y.). Chloroform-methanol-14.8 M ammonium hydroxide (75:25:2, v/v/v) and chloroform-methanol-water (66:30:4, v/v/v) were used as developing solvents. The aminolipid exhibited R_F values of 0.11 and 0.5 in these solvents, respectively. Phospholipids were detected by molybdenum blue reagent (1), and the aminolipid by ninhydrin. Larger quantities of the aminolipid were prepared by chromatographing the lipid extract in the chloroform-methanol-ammonium hydroxide solvent system, locating the aminolipid by spraying a small strip of the chromatogram with ninhydrin solution, and eluting the aminolipid with chloroform-methanol (2:1, v/v). After rechromatography by means of the chloroform-methanol-water solvent system, the aminolipid was located and eluted as before. The eluate was evaporated to dryness, and the purified aminolipid was redissolved in chloroform-methanol (2:1, v/v). Rechromatography of the aminolipid with either solvent yielded only one spot when the chromatograms were sprayed with 2',7'-dichlorofluorescein or ninhydrin.

Acid hydrolysis (6 N HCl at 100 C for 12 hr) of the purified product and two-dimensional chromatography of the resulting water-soluble material(s) on Whatman no. 1 paper with phenol-water (100:38, v/v) and *n*-butanol-propionic acid-water (142:71:100, v/v/v) revealed one ninhydrin-positive spot. This matreial exhibited the same mobility as ornithine (Nutritional Biochemicals Corp., Cleveland, Ohio). Glycerol could not be detected with alkaline silver nitrate reagent (6). The presence of ornithine as the only amino acid in the acid hydrolysate was confirmed by means of an amino acid analyzer (Beckman/Spinco model 120C).

The purified aminolipid was subjected to transesterification with 0.02 N sodium methoxide for 1 hr, and the resulting products were analyzed by thin-layer chromatography (chloroform-methanol-water system). Two 2',7'-dichlorofluorescein-staining spots were observed (R_F 0.9 and 0.37), with only the more polar material (R_F 0.37) being ninhydrin-positive. This ninhydrin-positive substance was eluted with chloroform-methanol (2:1, v/v), evaporated to dryness,

and hydrolyzed in 6 N HCl at 100 C for 12 hr. Thin-layer chromatography of the acid hydrolysate in the chloroform-methanol-water system yielded two spots. One, found at the origin, was identified as ornithine. The other (R_F 0.9) was ninhydrin-negative and 2',7'-dichlorofluorescein-positive.

Thus, it appears that *T. thiooxidans* produces an ornithine-containing lipid which possesses both amide and ester linkages. This aminolipid seems to be similar to the ones reported in *Rhodopseudomonas* (3) and *Streptomyces* (4), which contain ornithine and lysine, respectively. A complete characterization of the ornithine-containing lipid of *T. thiooxidans* is currently in progress.

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