PHOSPHOTIDYLINOSITOL AS A WETTING AGENT IN SULFUR OXIDATION BY *THIOBACILLUS THIOOXIDANS*

W. I. SCHAEFFER AND W. W. UMBREIT

Department of Bacteriology, Rutgers, The State University, New Brunswick, New Jersey

Received for publication 30 August 1962

Jones and Starkey (J. Bacteriol. **82**:788, 1961) recently demonstrated that cultures of *Thiobacillus thiooxidans* produce a wetting agent which permits wetting of the sulfur and thus its more ready attack by the microorganisms. We were able to identify a major component of this wetting agent as phosphotidylinositol. This substance is also known as inositol phosphotide or as phosphoinositide but will be referred to in this paper as phosphotidylinositol, as recommended by Hawthorne (J. Lipid Res. **1**:255, 1960).

T. thiooxidans was grown on a mineral salts medium (Starkey, J. Bacteriol. **10**:135, 1925) at pH 3 with sterile sulfur on a rotary shaking machine at room temperature. For CO₂ fixation, 100-ml portions of the medium plus 2 g of sulfur were placed in 250-ml flasks, and the flasks were closed with a rubber stopper, radioactive Na₂CO₃ was added, and incubation continued. At intervals, 10-ml samples were removed, centrifuged, and the radioactivity of the organic carbon found in the medium and in the cells was determined by a Tracerlab gas-flow counter standardized against C¹⁴ samples of known activity.

Figure 1 shows that a portion (of the order of 10 to 15%) of the CO₂ fixed appears as organic compounds in the medium. Over a 4-hr period the average amount was 17%. But what is more important is that the radioactive carbon makes its appearance in the "excreted" matter even in the first 15 min after exposure to CO₂ and is, therefore, a product of newly fixed CO₂ and is not a result of cell autolysis or breakdown.

It was found that between 40 and 60% of the excreted material was soluble in ether under acid (but not under neutral or alkaline) conditions, and, at the suggestion of A. J. Merola, we examined this ether extract for phospholipids. For this purpose we used silica-impregnated paper at 4 C with *n*-butyl ether, acetic acid, chloroform, and water (40:35:6:5; v/v) in an ascending system according to the method of Marinetti and Stotz (Biochim. Biophys. Acta **21**:168, 1956) and Marinetti et al. (Federation Proc. **16**:837, 1957).

FIG. 1. Time course of excretion of C¹⁴-labeled material by *Thiobacillus thiooxidans*. At 15-min intervals, 10-ml samples were removed from the incubation flask containing 100 ml of *T. thiooxidans* culture plus C¹⁴O₂, centrifuged, and the radioactivity in the medium and in the cells was determined.

All of the radioactivity was found at an *R_F* value of 0.08 (as determined by a Vanguard Automatic Scanner) and this same spot fluoresced blue when sprayed with rhodamine G. Organic phosphorus was present in the same spot. This information indicates that the substance was a phosphotidylinositol. We therefore compared the lipid isolated from *T. thiooxidans* with various known phospholipids, e.g., phosphotidylethanolamine, lecithin, phosphotidylcholine, as well as others, in a variety of thin-layer chromatographic systems (especially on Kieselguhr and silica plates) using methanol, benzene, *n*-butyl alcohol, and water (2:1:1:1; v/v). In addition to spotting of individual known and unknown phospholipids on chromatograms, we mixed quantities of the labeled unknown with a solution of known phosphotidylinositol. In each of the above cases we found that the radioactivity, chemical properties (reaction with sprays such as molybdate-per-

chloric acid), and physical properties (ultraviolet fluorescence) corresponded exactly to those of phosphatidylinositol.

Phosphatidylinositol isolated from brain proved to be an excellent wetting agent for

sulfur, and sulfur treated with phosphatidylinositol was oxidized more readily than untreated sulfur.

This research was supported in part by National Science Foundation grant G-9085.

ATTEMPTED STABILIZATION OF AUTOCLAVED BACITRACIN BY METALLIC IONS

MERRILL L. CRISLER¹ AND EUGENE D. WEINBERG

Department of Bacteriology, Indiana University, Bloomington, Indiana

Received for publication 10 September 1962

An important and useful role of metallic ions in living systems is that of enabling biological structures and macromolecules to withstand destruction by deleterious agents. Examples include prevention by calcium ions of (i) fragmen-

5:417, 1961), and (iii) proteolysis of animal and bacterial α -amylases (Vallee et al., *J. Biol. Chem.* **234**:2901, 1959); and suppression by zinc ions of heat and storage inactivation of bacitracin powder (Gross, *Drug Cosmetic Ind.* **75**:612, 1954).

Fresh solutions of bacitracin are activated by ions of cobalt (Trace and Edds, *Am. J. Vet. Res.* **15**:639, 1955), zinc and cadmium (Weinberg,

FIG. 1. Effect of presence of added metallic chloride or EDTA on antibacterial activity of bacitracin. The numbers on the abscissa indicate the molar ratio of the additive to bacitracin: 0.1:1.0, 1.0:1.0, 10:1.0, and 25:1.0. The molecular weight of bacitracin is 1,470. In the tests on autoclaved bacitracin, the additive was included prior to autoclaving. The base line represents an activity of 15 and 105 $\mu\text{g/ml}$ for raw and autoclaved samples, respectively. *Staphylococcus aureus* S-14 was the assay organism.

tation of meiotic plant chromosomes (Steffensen, Brookhaven Symp. Biol. **12**:103, 1959), (ii) heat inactivation of bacterial spores (Levinson, Hyatt, and Moore, *Biochem. Biophys. Res. Commun.*

¹ Present address: Science Department, Bloomington High School, Bloomington, Ind.

TABLE 1. Effect of zinc chloride on antibacterial activity against *Staphylococcus aureus*, strain S-14, of raw and autoclaved bacitracin

Addition of zinc chloride*	Treatment of bacitracin	Minimal inhibitory concentration $\mu\text{g/ml}$
Not added	Raw	15
	Autoclaved	105
Added	Raw	1.5
Added prior to autoclaving	Autoclaved	9.5
Added after autoclaving	Autoclaved	9.5

* Molar ratio of zinc chloride to bacitracin was 25:1.

Antibiotics Ann. 1958-59, p. 924), and manganese (Adler and Snoke, *J. Bacteriol.* **83**:1315, 1962), and are inactivated by ethylenediaminetetraacetate (EDTA; Weinberg, *Antibiotics Ann.* 1958-59, p. 924) and by autoclaving (Smith and Weinberg, *J. Gen. Microbiol.* **28**:559, 1962). In the present study, experiments were performed to learn whether metallic ions can prevent heat-induced loss of antibacterial activity of solutions of the antibiotic.