PRELIMINARY CHEMICAL CHARACTERIZATION OF THE SPORE COATS OF BACILLUS LICHENIFORMIS

Robert W. Bernlohr[1] and Carl Sievert

Department of Agricultural Biochemistry, Ohio State University
Columbus, Ohio and Chemistry Department, Capital University,
Columbus, Ohio

Received July 23, 1962

Bernlohr and Novelli (1960, 1960a) have established that the polypeptide antibiotic, bacitracin, is produced during presporulation in post log phase cells of Bacillus licheniformis. During this same stage, these cells also incorporate radioactive bacitracin; the label appearing in the subsequently formed spores. Using C-14, H-3 - doubly labeled bacitracin, it was shown that the antibiotic is incorporated into the spore coats with a strict retention of the original C-14 to H-3 ratio (Bernlohr and Novelli, 1960b). Preliminary evidence reported here indicates that bacitracin may be a primary structural unit of the spore coat of this bacterium.

The quantitative significance of bacitracin incorporation into spore coats could not be determined using isotope techniques, as the cells continue to elaborate the antibiotic during spore formation. Therefore, coats were isolated by mechanical means and a preliminary chemical characterization has been performed. Glucose, 20 mM, and ammonium lactate, 50 mM, were added to the salts medium described previously (Bernlohr and Novelli,

---

[1] Present address: Department of Microbiology, University of Minnesota, Minneapolis, Minnesota.

REFERENCES

Hurlbert, R.B., Schmitz, H., Brumm, A.F., and Potter, V.R., J. Biol. Chem., 209, 23 (1954).

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).

Nirenberg, M.W., and Matthei, J.H., Proc. Nat. Acad. Sci. (U.S.), 47, 1588 (1961)

Nomura, M., Hall, B.D., and Spiegelman, S., J. Mol. Biol., 2, 306 (1960).

Tsugita, A., Fraenkel-Conrat, H., Nirenberg, M.W., and Matthei, J.H., Proc. Nat. Acad. Sci. (U.S.), 48, 846 (1962).

only 0.3% (Dubois, et al, 1956), while 3.1% hexosamine was detected (Cessi and Piliego, 1960). By extracting whole spore coats with ether and ethanol or mixtures of ethanol and chloroform, a maximum of 2.0% of the dry weight was lost. Because of the inherent difficulties in such a proceedure, it is doubtful that any lipid-like material is present. Extracting whole spore coats with perchloric acid and using the method of Armstrong, et al, (1960) for the determination of teichoic acids results in no detection of this polymer. The spore coat hydrolysates contain no dipicolinic acid as determined by a modification of the methods of Perry and Foster (1955) and Murty and Halvorson (1957). Combustion of whole spore coats has led to variable results in the weight of the residue. The significance of this may be important because differing quantities of inorganic ions can be found in spores, depending on the medium in which they were grown (Walker, et al, 1961). It should be mentioned that inorganic phosphate has been detected in significant quantities in the hydrolysates. Finally, upon acid hydrolysis, an insoluble residue forms that is also resistant to hydrolysis in 2.0 N KOH at 100° C. This residue account for about 9.1% of the weight of the spore coat.

The unusually large quantity of amino acids found in the hydrolysates of spore coats has prompted an investigation of the separation and determination of them. Three separate two-dimensional ascending paper chromatographic systems were utilized */, and the components of the hydrolysates were compared with both a bacitracin hydrolysate and a mixture of standard amino acids. In addition, high-voltage electrophoresis

*/ t-Butanol: water: methylethylketone (2:2:1) vs. n-Butanol: acetic acid: water (12:3:5); t-Butanol: water: methylethylketone (2:2:1) vs. Methanol: pyridine: water (40:10:1); and n-Butanol: acetic acid: water (12:3:5) vs. Methanol: pyridine: water (40:10:1).

of the hydrolysates was performed (Atfield and Morris, 1961). Three buffer systems **/ were used and the separations were performed in a Gilson Medical Electronics High Voltage Electrophorator. The use of these six systems allowed the complete resolution of all of the amino acids present in the spore coat hydrolysates. Table II shows the results of this semi-quantitative study.

The constituent amino acids of bacitracin are listed, facilitating a comparison between the polypeptide antibiotic and the spore coat, both with regard to the amino acids present in each and to the relative quantity. Both the paper chromatographic and the electrophoresis experiments were performed by placing equal molar quantities of either a bacitracin hydrolysate or the spore coat hydrolysate on separate sheets of paper. After the amino acids had been separated by the appropriate method, 0.25% ninhydrin in acetone was sprayed on them and the papers were developed. Examination of the relative intensities of the spots on about 40 chromatograms and 25 electrophoresis patterns has led to the approximate values given in Table II.

It is immediately obvious that there is a very distinct similarity between the amino acid compositions of the bacitracin and spore coat hydrolysates. This similarity includes both the variety and the quantity of the amino acids. Since bacitracin is incorporated, *in toto*, into the spore coats and because the constituent amino acids of bacitracin comprise the great majority of the amino acids found in the coat hydrolysates, a hypothetical model of the coat can be constructed. Using bacitracin as the primary structural entity, cross linking can be envisioned with hexosamine and phosphate ions serving as

---

**/ 0.1 M Tris (hydroxymethylaminomethane) pH 7.8; pyridine, 20 ml/lit. + acetic acid, 9.5 ml/lit., pH 5.2; and formic acid, 11.5 ml/lit. + acetic acid, 39 ml/lit., pH 2.2.

bridges. This structure would be functionally analagous to the peptide substructure of Bacillus cereus cell walls (Church and Epstein, 1962).

TABLE II

COMPARISON OF THE AMINO ACID COMPOSITION OF BACITRACIN AND B. LICHENIFORMIS SPORE COATS

AMINO ACID	BACITRACIN CONSTITUENTS *	SPORE COAT CONSTITUENTS**
Alanine	-	1
Aspartic Acid	2	1
Cysteine	1	1/2
Glutamic Acid	1	1
Histidine	1	1
Isoleucine	3	2
Leucine	1	1
Lysine	1	1
Ornithine	1	1
Phenylalanine	1	1
Proline	-	Trace
Tyrosine	-	Trace
Valine	-	Trace
All others	-	-

* from: Lockhart and Abraham (1956).

** taking alanine as unity; other amino acids are related in color intensity to alanine.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. Helen Engel for her fine technical assistance. This investigation was supported in part by National Science Foundation Grant G9889, and by the Ohio State University Development Fund.

REFERENCES

Armstrong, J. J., Baddiley, J., and Buchanan, J. G., Biochem. J. 76: 610 (1960).
Atfield, G. N., and Morris, C. J. O. R., Biochem. J. 81:606 (1961).
Bernlohr, R. W., and Novelli, G. D., Arch. Biochem. Biophys. 87:232 (1960).
Bernlohr, R. W., and Novelli, G. D., Biochim. Biophys. Acta 41:541 (1960a).
Bernlohr, R. W., and Novelli, G. D., Bact. Proc. p. 149 (1960b).
Cessi, C., and Piliego, F., Biochem. J. 77:508 (1960).
Church, B. D., and Epstein, R. L., Bact. Proc. p. 61 (1962).
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., Anal. Chem. 28:350 (1956).
Lockhart, I. M., and Abraham, E. P., Biochem. J. 62:645 (1956).
Murty, G. G. K., and Halvorson, H. O., J. Bacteriol. 73:236 (1957).
Perry, J. J., and Foster, J. W., J. Bacteriol. 69:337 (1955).
Troll, W., and Cannan, R. K., J. Biol. Chem. 200:803 (1953).
Walker, H. W., Matches, J. R., and Ayres, J. C., J. Bacteriol. 82:960 (1961).