THE ENZYMIC SYNTHESIS BY A PNEUMOCOCCAL EXTRACT OF A SEROLOGICALLY REACTIVE POLYMER FROM URIDINE DIPHOSPHATE GALACTURONIC ACID

Evelyn E. B. Smith, Brian Galloway and George T. Mills
Department of Medicine, State University of New York College of Medicine at New York City, Brooklyn 3, New York

Received March 24, 1961

The synthesis of Type III pneumococcal capsular polysaccharide (SIII) from UDP-glucose and UDP-glucuronic acid by a cell free extract of Diplococcus pneumoniae Type III has been described previously (1). In addition, genetic and biochemical investigations have shown the importance of UDP-galacturonic acid for the synthesis of Type I capsular polysaccharide (SI) by intact Type I pneumococci (2, 3).

The structure of SI has not yet been elucidated but it is known to contain a high percentage of galacturonic acid (4). Galactose, acetyl glucosamine and a methyl pentose have also been identified as constituent sugars of a preparation of SI (5) and the liberation of these latter sugars during acid hydrolysis follows a course which suggests that the polysaccharide may contain a backbone of polygalacturonic acid. The present paper reports the synthesis of a serologically reactive polymer from UDP-galacturonic acid, a finding which lends support to this hypothesis.

The substrate, UDP-galacturonic acid, was isolated from the cells of a fully capsulated Type I pneumococcus (strain I41S) by the methods of ion exchange and of paper chromatography described previously (6). The major

proportion of the UDP-galacturonic acid was eluted from a Dowex 1 Cl^{1} column with the 0.01 N HCl-0.03 M NaCl solvent and, on chromatography in the neutral ethanol-ammonium acetate solvent of Paladini and Leloir (7), had an R_{UMP} value of 0.61 (UDP = 0.63; UDP-glucuronic acid = 0.73). 30 µmoles of UDP-galacturonic acid can be obtained from a 4 litre culture of Type I pneumococcus when the organisms are grown and harvested in the manner described by Smith et al. (8).

The enzyme extract was prepared from the non-capsulated mutant $S\text{-}I_3$ (9) in the manner described (1). Genetic and biochemical data show that strain $S\text{-}I_3$ is deficient in the enzyme UDP-glucuronic acid-4-epimerase (2, 3). The use of this strain avoids the high background of polysaccharide which is present in enzyme extracts from a fully capsulated Type I organism. The final volume of the enzyme preparation obtained from a 500 ml neutralized culture was 3 ml.

The reaction mixture consisted of 2 µmoles of UDP-galacturonic acid, 15 µmoles of $MgCl_2$, 0.5 ml of enzyme extract and 0.1 M TRIS buffer, pH 8.35, to a final volume of 3 ml. Incubation was carried out at 32° for 1 hour and was followed by heating of the reaction mixture in a boiling water bath for 2 min. The mixture was rapidly cooled, centrifuged and the supernatant assayed for serologically reactive material by the standard precipitin reaction in the presence of excess Type I pneumococcal anticapsular rabbit serum. The complex formed after incubation of an aliquot of the supernatant at 37° for 1 hour in the presence of antiserum was centrifuged and washed three times with ice-cold isotonic saline. After the final washing and centrifugation, the complex was dissolved in 0.1 ml of N/100 NaOH and protein estimated by the Biuret-Folin reaction (10).

Table I shows the results of a typical experiment with the requisite

TABLE I

The enzymic synthesis of serologically reactive material from UDP-galacturonic acid

	Substrate	Reaction time min.	Protein precipitated from Type I anticapsular serum mg.
1.	UDP-galacturonic acid (2 μmoles)	0	0.77
2.	None	60	0.87
3.	UDP-galacturonic acid (2 μmoles)	60	2.07
4.	UDP-glucuronic acid (2 μmoles)	60	0.84

control reactions. Also shown in Table I is an experiment in which UDP-glucuronic acid was substituted for UDP-galacturonic acid as substrate. This latter experiment shows the specificity of UDP-galacturonic acid in the production of serologically reactive material.

Table II shows the cross-reactivity of Type III anticapsular serum with the material synthesized. Some cross-reactivity is evident and this cross-reactivity is not shown by pure Type I capsular polysaccharide.

When the product of the enzyme reaction was dialyzed against running water for 24 hours, there was no reduction in the amount of antibody protein precipitated from Type I anticapsular serum. The antigen-antibody complex gave a positive carbazole reaction (11) for uronic acid.

It is suggested that the serologically active material formed in the enzyme reaction described is polygalacturonic acid and that this acid may constitute

TABLE II

Cross-reactivity of Type I and Type III anticapsular sera with the material synthesized enzymically from UDP-galacturonic acid

Material tested	Protein precipitated from Type I anticapsular serum mg.	Protein precipitated from Type III anticapsular serum mg.
Enzyme reaction with no substrate	0.55	0.11
Enzyme reaction with UDP-galacturonic acid (2 μmoles)	1.56	0.34
4 μg. Type I capsular polysaccharide	0.32	< 0.01
8 μg. Type I capsular polysaccharide	0.64	< 0.01

the backbone of Type I pneumococcal capsular polysaccharide. The cross-reactivity with Type III anticapsular serum is most probably due to the presence of a large number of free carboxyl groups in this simple structure.

ACKNOWLEDGMENTS

This work was supported by Grant E-1018(C5) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service, and was carried out in close collaboration with Dr. Harriet P. Bernheimer, and with the excellent technical assistance of Miss Ingbritt Blomstrand.

The preparation of Type I capsular polysaccharide used here was kindly presented by Dr. Michael Heidelberger.

The Type I anticapsular serum was made available through the courtesy of Dr. E. F. Roberts, Wyeth Laboratories, American Home Products Corporation.

REFERENCES

1. Smith, E. E. B., Mills, G. T., Bernheimer, H. P., and Austrian, R., J. Biol. Chem. 235, 1876 (1960)

2. Austrian, R., Bernheimer, H. P., Smith, E. E. B., and Mills, G. T., J. Exptl. Med., 110, 585 (1959)

3. Smith, E. E. B., Mills, G. T., Austrian, R., and Bernheimer, H. P., J. Gen. Microbiol., 22, 265 (1960)

4. Heidelberger, M., Kendall, F. E., and Scherp, H. W., J. Exptl. Med. 64, 559 (1936)

5. Smith, E. E. B., Galloway, B., and Mills, G. T., Biochem. J., 76, 35P (1960)

6. Smith, E. E. B., Mills, G. T., and Harper, E. M., Biochim. Biophys. Acta, 23, 662 (1957)

7. Paladini, A. C., and Leloir, L. F., Biochem. J., 51, 426 (1952)

8. Smith, E. E. B., Mills, G. T., Bernheimer, H. P., and Austrian, R., J. Gen. Microbiol., 20, 654 (1959)

9. Austrian, R., and Bernheimer, H. P., J. Exptl. Med., 110, 571 (1959)

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

11. Dische, Z., Methods of Biochemical Analysis, 2, 313 (1955)