

CROSS-REACTIVITY OF THE MEMBRANE TEICHOIC ACID OF *Streptococcus faecalis* N.C.I.B. 8191 IN ANTIPNEUMOCOCCAL TYPE XII AND TYPE XVI SERA*†

MICHAEL HEIDELBERGER

New York University School of Medicine, New York, N.Y. 10016 (U. S. A.)

AND JAMES BADDILEY

Microbiological Chemistry Research Laboratory, University of Newcastle, Newcastle-upon-Tyne NE1 7RU (Great Britain)

(Received November 30th, 1973; accepted December 31st, 1973)

ABSTRACT

The membrane teichoic acid (MTA) precipitates heavily in the equine and rabbit antipneumococcal (anti-Pn) type XII sera tested. MTA contains multiple kojibiosyl residues and this reaction confirms the presence of kojibiosyl residues in the capsular polysaccharide, S XII, the antigenic determinant of pneumococcal type XII. MTA also precipitates one-third of the antibodies in an anti-Pn XVI serum: the poly(glycerol phosphate) residues of MTA may be involved in this reaction as well as D-glucose residues. Precipitation of MTA in anti-Pn XII is strongly inhibited by kojibiose, but the reaction in anti-Pn XVI is unaffected.

INTRODUCTION

The membrane teichoic acid (MTA) of *Streptococcus faecalis* N.C.I.B. 8191 is a poly(glycerol phosphate) in which some of the hydroxyl groups of glycerol are substituted by D-alanyl and kojibiosyl¹ [2-O- α -D-glucopyranosyl-(1 \rightarrow 2)-D-glucopyranosyl] residues. MTA precipitates antisera to the whole cells of this strain. The present study was undertaken because the occurrence of kojibiosyl residues in MTA suggested a possible cross-reaction in antipneumococcal (anti-Pn) type XII sera. There was also strong precipitation in anti-Pn XVI. Data on these reactions are given in Table I. After these experiments were completed, it was called to our attention that kojibiose had actually been isolated² from polysaccharide S XII.

MATERIALS AND METHODS

The equine antisera used were obtained from the Laboratories of the New York City and New York State Departments of Health. Quantitative microanalyses for

*Dedicated to the memory of Professor W. Z. Hassid.

†This investigation was supported by a grant from the National Science Foundation, U. S. A.

antibody nitrogen precipitated in the cross-reactions were carried out as described in previous papers^{3,4}.

RESULTS

Table I shows that MTA gives heavy precipitates in anti-Pn XII and anti-Pn XVI sera and (footnotes b and c) that the precipitates actually involve antibody and not non-specific protein. Inhibition tests with kojibiose, kindly provided by Dr. E. A. Kabat, showed a strong effect with MTA and anti-Pn XII, and no inhibition with MTA and anti-Pn XVI.

TABLE I

CROSS-REACTIONS OF THE MEMBRANE TEICHOIC ACID OF *Streptococcus faecalis* N.C.I.B. 8191

Substance	Weight (μ g)	Weight (μ g) of antibody N precipitated from antipneumococcal type ^a	
		XII296	XVI594
Homologous polysaccharide	250	1000	
	500		872
Membrane teichoic acid	100	222	55
	200	322	111
	300	410 ^b	
	400	465 ^b	174
	500	490 ^b	
	800		271 ^c
	1200		299 ^c
	1500		281

^aAt 0° per ml of equine antiserum. The sera were absorbed with group-specific pneumococcal C-substance. ^bSupernatants+180 μ g of Pn polysaccharide S XII gave 450 μ g N; total 905 μ g. ^cSupernatants+380 μ g of Pn polysaccharide S XVI gave 415 μ g N.

DISCUSSION

The strong reactivity of the membrane teichoic acid in antipneumococcal type XII sera is of interest for several reasons. It had been suspected⁵⁻⁷ that kojibiosyl residues occurred in the capsular polysaccharide, S XII, the type-specific determinant of pneumococcal type XII. Dextrans containing (1→2) linkages of D-glucose residues precipitated in anti-Pn XII serum more or less strongly and yielded kojibiose, among other products, on mild hydrolysis. This disaccharide was also the best inhibitor of the cross-reaction of such dextrans in anti-Pn XII serum. Kojibiosyl residues are present in MTA¹, so that the strong reaction of this substance in anti-Pn XII, added to previous evidence, confirms the presence of multiple⁸ kojibiosyl residues in polysaccharide S XII. Kojibiose has now been isolated² from S XII.

MTA was also found to give heavy precipitation in the anti-Pn XVI serum available (Table I). Polysaccharide S XVI, the type-specific antigenic determinant of

pneumococcal type XVI, is composed of galactose, glucose, rhamnose, galactosamine, glucosamine, and glycerophosphate residues⁹. Anti-Pn XVI serum had previously been found to precipitate weakly with streptococcal poly(glycerol phosphate)¹⁰. It is therefore possible that polysaccharide S XVI contains (1→2)-linked residues of D-glucose, the only sugar common to MTA and S XVI, and that these residues reinforce the reaction due to multiple residues of glycerol phosphate.

EXPERIMENTAL

The data obtained for cross-reactive precipitating antibody are given in Table I. Inhibition tests were performed in a total volume of 0.315 ml and the tubes were kept at 0° for 11 days. MTA, anti-Pn XII, saline: 45 µg N; MTA, anti-Pn XII, 3.8µM kojibiose: 4.5 µg N; inhibition, 90%. A similar experiment with anti-Pn XVI gave 32 and 31 µg N without and with 3.8µM kojibiose, respectively.

REFERENCES

- 1 P. TOON, P. E. BROWN, AND J. BADDILEY, *Biochem. J.*, 127 (1972) 399.
- 2 I. J. GOLDSTEIN, J. A. CIFONELLI, AND J. DUKE, *Biochemistry*, 13 (1974) 867.
- 3 M. HEIDELBERGER AND P. A. REBERS, *J. Bacteriol.*, 80 (1960) 145.
- 4 M. HEIDELBERGER AND J. M. TYLER, *J. Exp. Med.* 120 (1964) 711.
- 5 J. W. GOODMAN AND E. A. KABAT, *J. Immunol.*, 84 (1960) 347.
- 6 H. SUZUKI AND E. J. HEHRE, *Arch. Biochem. Biophys.*, 104 (1964) 305.
- 7 J. A. CIFONELLI, P. A. REBERS, M. B. PERRY, AND J. K. N. JONES, *Biochemistry*, 5 (1966) 3066.
- 8 M. HEIDELBERGER AND F. E. KENDALL, *J. Exp. Med.*, 61 (1935) 563.
- 9 Z. A. SHABAROVA, J. G. BUCHANAN, AND J. BADDILEY, *Biochim. Biophys. Acta*, 57 (1962) 146.
- 10 M. HEIDELBERGER AND S. ELLIOTT, *J. Bacteriol.*, 92 (1966) 281.