A Serological Comparison of the Membrane Teichoic Acids from Lactobacilli of Different Serological Groups

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Membrane glycerol teichoic acids have been isolated by phenol extraction from lactobacilli belonging to the different serological groups defined by Sharpe (1955). The preparations, referred to as lipoteichoic acids, differ from those obtained by extraction with trichloracetic acid in that they are antigenic when injected with adjuvant, and also contain glycolipid and protein (Knox, Hewett & Wicken, 1970; Knox & Wicken, 1970; Wicken & Knox, 1970).

The lactobacilli studied in detail were representatives of group A (Lactobacillus helveticus NCIB 8025), group D (L. plantarum NCIB 7220) and group F (L. fermenti NCTC 6991); preparations of lipoteichoic acid have also been obtained from strains of L. casei NIRD H831 (group B), NIRD R094 and NCTC 6375 (group C) (A. J. Wicken & K. W. Knox, unpublished observations). The teichoic acids differed in structure but cross-reacted to varying extents as shown by the quantitative precipitin method (Knox et al. 1970) and also haemagglutination (Hewett, Knox & Wicken, 1970). This report extends the study of these serological cross-reactions, and also compares the ability of glycerol-phosphoryl-glycerol-phosphoryl-glycerol (G₃P₂) to inhibit the various cross-reactions.

The lipoteichoic acids used differ in their sugar substitution. The preparation from Lactobacillus plantarum contains a low proportion of α-D-glucosyl substituents (Glu: P = 0.11:1.00), whereas that from L. helveticus contains a higher proportion of the same substituent (Glu: P = 0.45:1.00); the product from L. fermenti contains both glucose and galactose as minor though serologically important substituents (Glu: Gal: P = 0.06:0.12:1.00); preparations from L. casei strains also contain glucose and galactose, the one from strain NIRD R094 used in this study having a ratio of Glu: Gal: P of 0.12:0.07:1.00.

Table I compares the cross-reactions between these lipoteichoic acid preparations and antisera obtained by injecting them with Freund’s adjuvant into rabbits. Preparations were compared by the quantitative precipitin method using an appropriate amount of antigen (5 to 30 μg.) and antiserum (generally 0.05 to 0.2 ml. containing 120 to 160 μg. antibody); the results are expressed as a percentage of the antibody precipitated in the homologous reaction. Results for cross-reactions of Lactobacillus casei are presented only for strain NIRD R094, preparations from the other strains giving similar results (cf. Knox et al. 1970). This lipoteichoic acid had been prepared by an aqueous extraction procedure that gives a product with enhanced antigenicity (A. J. Wicken, J. W. Gibbens & K. W. Knox, unpublished observations). The common structural feature for all these preparations is the
glycerol phosphate ‘backbone’, and inhibition of the cross-reactions by units of the ‘backbone’ was also studied (Table I). The inhibitor used was glycerol-phosphoryl-glycerol-phosphoryl-glycerol (G3P2) prepared from cardiolipin (General Biochemicals, Chagrin Falls, Ohio, U.S.A.) by mild deacylation (Wilkinson, 1968).

Table I. *Cross-reactions of membrane lipoteichoic acids from lactobacilli, and inhibition of the reactions by glycerol-phosphoryl-glycerol-phosphoryl-glycerol (G3P2)*

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>% Cross-reaction with lipoteichoic acid from</th>
<th>(^a)L. helveticus</th>
<th>(^b)L. plantarum</th>
<th>(^c)L. fermenti</th>
<th>(^d)L. casei</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus helveticus</em></td>
<td></td>
<td>100</td>
<td>60</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>L. plantarum</td>
<td></td>
<td>110</td>
<td>100</td>
<td>82</td>
<td>42</td>
</tr>
<tr>
<td>L. fermenti</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit 146</td>
<td></td>
<td></td>
<td></td>
<td>(^*)100</td>
<td>35</td>
</tr>
<tr>
<td>Rabbit 148</td>
<td></td>
<td></td>
<td></td>
<td>(^*)100</td>
<td>30</td>
</tr>
<tr>
<td>L. casei</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain NIRD RO94</td>
<td></td>
<td>64</td>
<td>67</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>Strain NCTC 6375</td>
<td></td>
<td>100</td>
<td>100</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^b\) W. Knox & A. J. Wicken (unpublished observations).  
\(^*\) Not examined.  
\(^d\) A. J. Wicken, J. W. Gibbens & K. W. Knox (unpublished observations).

The specificity of the antiserum to *Lactobacillus helveticus* lipoteichoic acid depends primarily on \(\alpha\)-d-glucosyl substituents (Knox & Wicken, 1970), and the cross-reaction with *L. plantarum* lipoteichoic acid is therefore not unexpected. The weaker reaction with *L. fermenti* lipoteichoic acid is at least partly due to the common glycerol-phosphate ‘backbone’ as G3P2 is an effective inhibitor. It is unlikely that there are two groups of antibodies, one \(\alpha\)-d-glucosyl specific and the other glycerol phosphate specific, as absorption with *L. fermenti* lipoteichoic acid removes 90% of the reaction with *L. helveticus* lipoteichoic acid (Knox & Wicken, 1970).

The specificity of antiserum to *Lactobacillus plantarum* lipoteichoic acid also involves the \(\alpha\)-d-glucosyl substituents (K. W. Knox & A. J. Wicken, to be published). However, in this case G3P2 is an effective inhibitor of the homologous reaction indicating that the glycerol phosphate ‘backbone’ is also an important component of the antigenic determinant. This would account for the stronger cross-reaction with *L. fermenti* and *L. casei* lipoteichoic acids when compared with the results for *L. helveticus* antiserum.

Antiseras to *Lactobacillus fermenti* lipoteichoic acid differ in their specificity, some sera (e.g. 148) being inhibited by D-galactose and D-glucose, whereas in other cases (e.g. serum 146) neither sugar is an effective inhibitor (Knox et al. 1970). These differences are also reflected in the cross-reactions of lipoteichoic acids from *L. helveticus* and *L. casei* with
antisera to *L. fermenti* (Knox *et al.* 1970; Knox & Wicken, 1970). The cross-reactions of *L. plantarum* lipoteichoic acid with antisera 146 and 148 are consistent with the earlier observations, while the inhibitions with G₃P₂ indicate the role of the glycerol phosphate ‘backbone’ in the cross-reactions.

Antisera to lipoteichoic acid from *Lactobacillus casei* NIRD R094 reacted strongly with all the preparations of lipoteichoic acid tested, and G₃P₂ was a very effective inhibitor of each of the cross-reactions. Neither D-glucose nor D-galactose (100 µmoles) gave a detectable inhibition of the homologous precipitin reaction, supporting the conclusion that the antibodies are primarily specific for the glycerol phosphate ‘backbone’ of the teichoic acid. The specificity apparently extends to the requirement that the glycerol units be joined by phosphodiester bonds involving positions 1 and 3 of glycerol, as the wall teichoic acid from *Bacillus stearothermophilus* 865 did not react with the antisera; this teichoic acid is a glucosyl substituted glycerol phosphate polymer but the phosphodiester bonds involve positions 2 and 3 of glycerol (Wicken, 1966).

To confirm the results obtained with *Lactobacillus casei* NIRD R094, antiserum was also prepared to the lipoteichoic acid from *L. casei* NCTC 6375. Tests with this antiserum (Table I) showed even stronger cross-reactions with lipoteichoic acid from other lactobacilli, with a corresponding increase in the ability of G₃P₂ to inhibit the homologous reaction.

Earlier studies on *Lactobacillus fermenti* and *L. helveticus* suggested that, to varying extents, antibodies were combining with the glycerol phosphate units of the teichoic acids (Knox *et al.* 1970; Knox & Wicken, 1970). The conclusion was based partly on the cross-reaction of teichoic acids differing in sugar substitution, and the present results provide additional examples of such cross-reactions. More direct evidence is now provided by the observation that G₃P₂ is generally an effective inhibitor of the precipitin reaction. Thus glycerol teichoic acids differing in sugar substitution may cross-react owing to the reaction of antibodies with the common ‘backbone’ of 1 → 3 linked glycerol phosphate units.

The organisms tested represent most of the serological groups of lactobacilli, the groups being defined on the basis of the qualitative reaction of antisera to whole organisms with acid extracts of the different strains (Sharpe, 1955). This procedure depends on the production of suitable antisera and the presence of sufficient reactive material in the acid extract. For the strains of *Lactobacillus casei* examined injection of whole organisms does not give rise to a significant amount of antibody reacting with membrane teichoic acid (K. W. Knox & A. J. Wicken, unpublished observations), while only occasionally does injection of whole organisms of *L. plantarum* lead to the production of antibodies specific for membrane teichoic acid. In *L. fermenti* the membrane teichoic acid is the group antigen (Knox *et al.* 1970), while for *L. helveticus* both the wall and membrane teichoic acids react with group antisera (Knox & Wicken, 1970). In all these studies on the membrane lipoteichoic acids it has been observed that acid-extracted teichoic acid reacts only weakly with antisera in comparison to lipoteichoic acid. Thus the efficacy of the serological classification of the lactobacilli probably depends partly on the failure of strongly cross-reacting membrane teichoic acids to give rise to a significant level of antibody production, and partly on the membrane teichoic acid, present in only small amounts, being degraded by the acid extraction procedure. The minor cross-reactions between serological groups sometimes observed by Sharpe (1955) could have been due to reactions with membrane teichoic acids.

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REFERENCES


