# Identification of lipoglycan antigens from the *Acholeplasma laidlawii* cell membrane in crossed immunoelectrophoresis

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Membranes from the wall-less prokaryote *Acholeplasma laidlawii* contain a component termed lipoglycan or lipopolysaccharide (LPS). The lipoglycan has extraction properties, which are similar to those of LPS of gram-negative bacteria, but it is chemically distinct from bacterial LPS. The membrane-bound lipoglycan of *A. laidlawii* did not seem to be particularly immunogenic and antibodies against it could not always be detected by rocket immunoelectrophoresis (RIE) or crossed immunoelectrophoresis (CIE) in hyperimmune sera raised against membranes. The immunoprecipitate corresponding to the lipoglycan, obtained by CIE of Tween 20-solubilized *A. laidlawii* membranes, has been identified and shown to be both a cathodically and anodically migrating component at pH 8.6. The shape of the immunoprecipitate in both RIE and CIE showed that the lipoglycan antigen is composed of at least two components, which are immunologically related.

Acholeplasma laidlawii *Crossed immunoelectrophoresis* *Growth inhibition test* *Membrane* *Mycoplasma* *Lipoglycan* *Lipopolysaccharide*

## 1. INTRODUCTION

*Acholeplasma laidlawii* belongs to a class of prokaryotic microorganisms generally called mycoplasmas (or Mollicutes), which are not surrounded by a cell wall [1]. The cell membrane of mycoplasmas has been extensively studied because it is the only permeability barrier of these cells, its lipid composition can be modified and it is of importance for pathogenicity and adherence [2]. Microbial membrane proteins are often highly immunogenic and crossed immunoelectrophoresis [3], which is often termed CIE, or other immunochemical analysis methods are therefore very useful techniques for studies of mycoplasmal membrane proteins [4]. For exchange and comparison of results from CIE experiments between laboratories, it is desirable to have a reference immunoprecipitation pattern available for the system being studied [5]. Such a reference immunoprecipitation pattern has been established for *Escherichia coli* membrane components [6] and is under construction for *A. laidlawii* membrane components [4]. LPS from several bacterial species has earlier been analyzed by CIE and shown to be heterogeneous [7]. It also proved possible to stain LPS as well as other amphiphilic antigens such as membrane proteins with Sudan black [7]. The immunoprecipitate corresponding to the lipoglycan (formerly called LPS) antigens [8,9] from *A. laidlawii* has now been identified.

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## 2. MATERIALS AND METHODS

### 2.1. *Preparation of membranes and lipoglycans*

*Acholeplasma laidlawii*, strain B(ju), was cultivated in a tryptose-containing medium supplemented with PPLO serum fraction and the



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membranes were prepared by osmotic shock [10]. Lipoglycans were prepared from lipid-depleted membranes by extraction with hot aqueous phenol [11].

### 2.2. *Electroimmunochemical analysis methods*

Polyspecific antisera against membrane components were raised in rabbits by immunization with membranes [4]. Membranes and the lipoglycan fraction were extracted with the neutral detergent Tween 20 (polyoxyethylene sorbitan monolaurate) and analyzed by RIE [12] or CIE [3] in the presence of 0.5% (w/v) of this detergent [13]. For detection of cathodically migrating antigens in CIE, antiserum containing agarose was cast on both sides of the antibody-free agarose gel slice from the first dimensional electrophoresis.

### 2.3. *SDS-PAGE*

SDS-PAGE was performed in linear gradient gels (6–30%) with a continuous buffer system [14] and stained with Coomassie blue.

### 2.4. *Growth inhibition tests*

Growth inhibition tests were performed by placing small filter paper disks soaked with the antisera on a lawn of *A. laidlawii* seeded on an agar plate [15]. The area of the clear (dark) zone around the filter paper reflects the degree of growth inhibition.

## 3. RESULTS AND DISCUSSION

### 3.1. *Analysis by SDS-PAGE*

Fig.1 shows the result of an SDS-PAGE of membranes and the lipoglycan fraction solubilized with Tween 20. Only one very faint band (indicated with an arrow) could be detected in the lipoglycan fraction. The lipoglycan fraction could not be completely solubilized with Tween 20 and it was therefore further extracted with 5% (w/v) of sodium deoxycholate in 0.1 M glycine-NaOH buffer (pH 9.1) and 0.2 M SDS in 0.1 M Tris-HCl buffer (pH 8.0). These fractions contained even less stainable material than the Tween extract. The substance, which was detected in the Tween extract of the lipoglycan preparation was of low molecular mass and present only in minute amounts. Furthermore, a corresponding component could not be detected in the Tween extract of the membranes

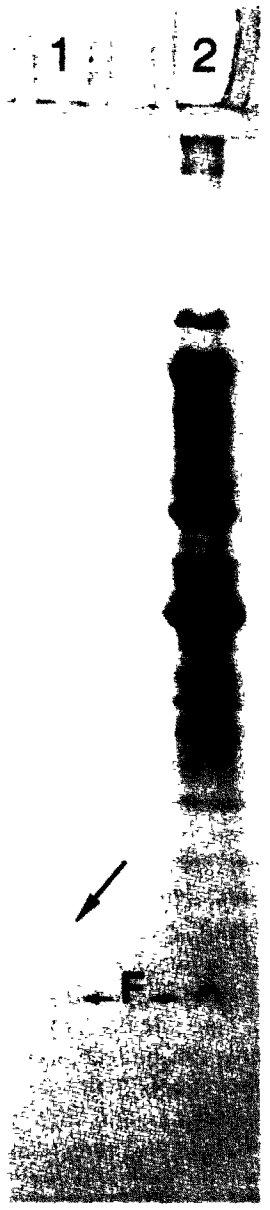


Fig.1. SDS-PAGE of 0.05 mg of Tween 20-solubilized lipoglycan (1) and 0.1 mg of membranes (2) of *A. laidlawii*. The position of the front is marked F.

(sample lane 2) and it is, therefore, not very likely that this protein will give an immunoprecipitate in the electroimmunochemical analysis experiments described below.

### 3.2. *Analysis by RIE*

Fig.2 shows the results of RIE of Tween

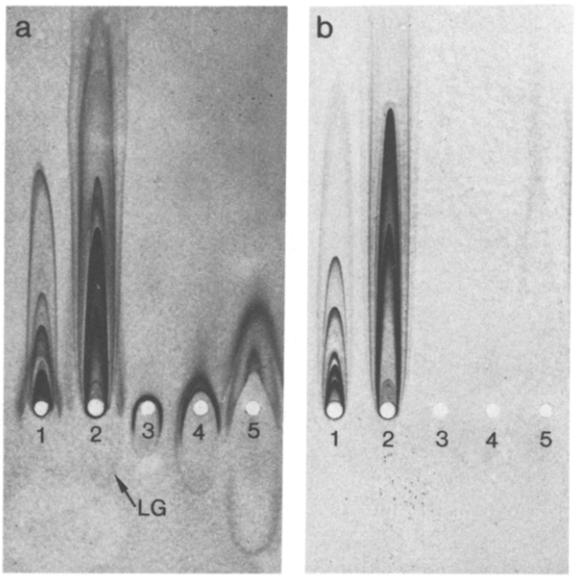


Fig.2. Rocket immunoelectrophoresis of Tween 20-solubilized membranes (wells 1 and 2) and lipoglycan (wells 3–5) at different dilutions from *A. laidlawii*. (a) Antiserum a.s.a, 23 $\mu$l/cm$^2$. (b) Antiserum a.s.b, 11 $\mu$l/cm$^2$.

20-solubilized membranes and lipoglycan fraction with antisera from two different rabbits (a,b). Only one of the antisera (see fig.2a) gave immunoprecipitates with the lipoglycan fraction and the antigen is mainly cathodically migrating (well 3). When more material was applied (wells 4 and 5), the shapes of the immunoprecipitates indicated heterogeneity; at least two distinct immunoprecipitates can be seen, which confirms the observations of Smith [16]. In the membrane samples (wells 1 and 2), it is also possible to see a very faint immunoprecipitate (denoted LG) with cathodic migration. Antibodies against cathodically migrating components have only been observed in one out of four anti-membrane hyperimmune sera, indicating that the lipoglycan is a comparatively poor immunogen or present at too low a concentration in the membrane to stimulate an immune response. The poor antibody response is

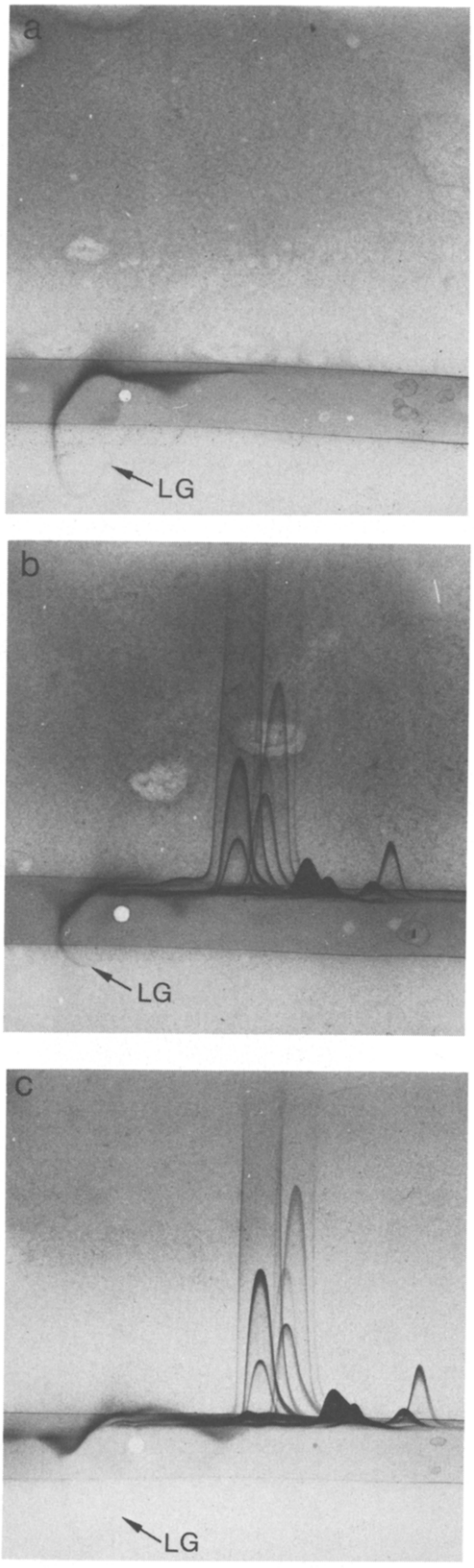


Fig.3. Crossed immunoelectrophoresis of Tween 20-solubilized: (a) 5 $\mu$g lipoglycan; (b) 25 $\mu$g membranes; and (c) a mixture of lipoglycan and membranes from *A. laidlawii* with antiserum a.s.a, 23 $\mu$l/cm$^2$.

expected since the major component of the lipoglycan fraction of *A. laidlawii* contains mostly glucose and very little amino sugar. This effect is seen with lipoglycans from *A. oculi* and *Ureaplasma* which are devoid of amino sugars.

### 3.3. *Analysis by CIE*

Fig.3a shows a CIE of the lipoglycan and the main part of the material is cathodically migrating. In this experiment, the cathodically and the anodically migrating components form a continuous immunoprecipitate (LG), which indicates at least partial immunological identity. The cathodically migrating antigen can also be detected in membranes (fig.3b) and the area subtended by the corresponding antigen increases upon co-electrophoresis with the lipoglycan fraction (fig.3c). The lipoglycan immunoprecipitate was very faint and, therefore, the photos had to be overexposed. LPS from the outer membrane of *E. coli* has also been shown to be cathodically migrating in CIE under conditions similar to those used in this work [6].

### 3.4. *Growth inhibition tests*

Fig.4 shows the result of a growth inhibition experiment with the two antisera used in the RIE shown in fig.2. As can be seen, the antiserum (a.s.b) used in fig.2b is much more efficient in the growth inhibition test than the antiserum (a.s.a) used in fig.2a. Interestingly, a.s.b has a higher titre of antibodies against membrane proteins than a.s.a has, which shows that antibodies against membrane proteins are more important than antibodies against the lipoglycan in growth inhibition of *A. laidlawii*. It has in fact been shown that monospecific antisera against membrane proteins of *A. laidlawii* are also efficient in growth inhibition [17].

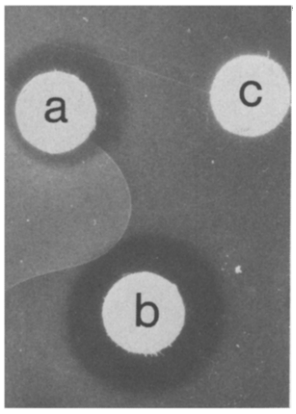


Fig.4. Growth inhibition test of *A. laidlawii* with 25 μl antisera a.s.a and a.s.b applied on filter papers a and b, respectively. A control antiserum raised against a protein, which is not related to any mycoplasmal protein, was applied on filter disk c.

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