Lysis of the Limiting Membrane of *Mycoplasma gallisepticum* by Chemical Agents

By H. ZOLA

Wellcome Research Laboratories (Biological Division), Beckenham, Kent

(Accepted for publication 6 March 1968)

SUMMARY

Lysis of *Mycoplasma gallisepticum* by various chemical reagents and enzymes was examined. Lysis was measured by determining the release of intracellular constituents, and compared with the results obtained by the turbidimetric method. The ultracentrifuge was used to compare solutions of isolated membrane in 2-chlorethanol, phenol + acetic acid + water mixture, aqueous urea, and aqueous sodium lauryl sulphate, all of which readily dissolved membranes and whole organisms.

INTRODUCTION

Chemical lysis of the limiting membranes of several *Mycoplasma* species has been studied by Rodwell (1956), Smith & Rothblat (1960), Razin (1963), Razin & Argaman (1962, 1963) and Razin, Morowitz & Terry (1965). These membranes, which are thought to be three-layered lipoprotein membranes (see, for instance, Razin, 1963), were readily lysed by surface-active agents, alcohols and a lipase.

As a preliminary to studies of the macromolecules of *Mycoplasma gallisepticum*, lysis of its enveloping membranes by several chemical reagents was examined. An attempt was made to compare the turbidimetric methods of following lysis used by previous workers with a method which measures the release of intracellular constituents. The ultraviolet spectrum of the cell contents shows an absorption maximum at 260 m μ , due principally to nucleotide material; this provides a convenient method of determining the cell contents released on lysis. A quantitative index of lysis was sought by using extinction measurements at 260 m μ . Some mild reagents which lyse the organisms were used to dissolve isolated membranes, and membrane solutions were examined in the ultracentrifuge.

METHODS

Organism. *Mycoplasma gallisepticum* strain X95 (PG 31) was grown on Difco PPLO broth supplemented with 20% normal horse serum and 10% (w/v) yeast extract; penicillin and thallium acetate were added to inhibit the growth of other organisms. After incubation at 37° for 3 days the cultures were concentrated by using a continuous-flow M.S.E. centrifuge and the concentrates sedimented at 6000 g for 30 min. at 2° in a refrigerated centrifuge (M.S.E. Ltd., London). The sediments were resuspended in sterile phosphate-buffered saline (0.15 M-phosphate, 0.85% (w/v) sodium chloride; pH 7.2; autoclaved) and recentrifuged three times. The final washed product was a thick paste of organisms containing 17–18% dry solids.

Lysis. For the turbidity and ultraviolet absorption experiments organisms were taken up in M-sucrose solution containing sodium chloride (0.05 M) and the suspensions shaken vigorously to break up aggregates. In a typical turbidity experiment a suspension of 8 mg. wet organism/ml. was made and 0.3 ml. of solution of lytic agent was added to each 3 ml. sample of Mycoplasma suspension. Extinctions were read at 500 m μ against appropriate controls after 1 hr, the suspensions being shaken before reading. For ultraviolet absorption experiments the concentration of organism was 2–3 mg. wet/ml. After standing with the reagents for 1 hr at 4°, suspensions were sedimented at 9000 g for 30 min. at 4° and extinctions read at 260 m μ . Proteolysis by pepsin (1 mg./ml.) was done in M-sucrose in 0.2 M-acetate buffer (pH 3.0) for 24 hr at 37° and the suspensions were then treated as above.

Isolation of cell membranes. Organisms were suspended in de-ionized water and disrupted ultrasonically at 20 Kc./sec. (60 W M.S.E.—Mullard ultrasonic oscillator), the disruption being done in an ice water bath. Ultrasonic treatment was followed by centrifugation at 6000 g for 30 min. at 2° to remove unbroken organisms; the membranes in the supernatant fluid were then collected by ultracentrifugation at 30000 g.

Ultracentrifugation. This was done with schlieren optics in a Beckman Spinco Model E ultracentrifuge. Double sector optical cells were used to obtain a baseline and thus establish whether curvature was due to the solute or the solvent.

RESULTS

Qualitative examination of lytic reagents

Several reagents cleared suspensions of organisms rapidly, requiring no more than visual observation. Thus a suspension of organisms (5 mg. wt/ml.) in phosphate-buffered saline was cleared rapidly at room temperature by 1–1.5 vol. of phenol + acetic acid + water (2 + 1 + 0.5, w/v/v; Rottem & Razin, 1967), or by 0.5–1 vol. of 2-chlorethanol or 1 vol. of 16 M-urea in water. Although when these reagents were used occasional clumps of organisms remained unless the suspension had been very thoroughly shaken before adding the reagent, sodium lauryl sulphate caused complete lysis rapidly and consistently.

Reagents which did not cause rapid or complete lysis were examined with the help of the turbidimetric and ultraviolet absorption methods for following the lysis. The results obtained by the two methods were in agreement qualitatively. Sodium deoxycholate, saponin, digitonin, and a toxin from *Staphylococcus aureus* (known to possess lipase activity) all resulted in some degree of lysis. Pepsin in acetate buffer (pH 3.0) caused no more lysis during 24 hr at 37° than a control solution containing buffer but no enzyme. Aggregation and settling limited the reliability of the turbidimetric method of following lysis, but the ultraviolet absorption method did not suffer from these drawbacks, required only one third as much material, and was more sensitive to small degrees of lysis.

Quantitative comparison of lytic agents

The ultraviolet adsorption method was used for quantitative studies. Degree of lysis (%) was expressed in two ways:

$$(A) \quad (E_x/E_{sLS}) \times 100; \quad (B) \quad 100 \times (E_x - E_c)/(E_{sLS} - E_c),$$

where E denotes the extinction at $260\text{ m}\mu$, and the suffix X represents the test solution, C the untreated control and SLS the suspension cleared completely by using sodium lauryl sulphate.

Expression (A) is analogous to that used by Razin (1963) in treating turbidimetric results. Expression (B) allows an estimate of the lysis by the reagent after correction for lysis by autolysis or physical handling. The amounts of lysis caused by some chemical reagents are set out in Table 1.

Table 1. *Extent of lysis of Mycoplasma gallisepticum, estimated by extinction at 260 mμ*

The concentrations refer to the reaction mixture. $A = (E_X/E_{SLS}) \times 100$ and $B = 100 \times (E_X - E_C)/(E_{SLS} - E_C)$, where E is the extinction at $260\text{ m}\mu$ and the suffixes X , C and SLS refer to test solution, untreated control, and sodium lauryl sulphate-treated sample respectively.

Reagent	A	B
Sodium lauryl sulphate 0.1 M	100	100
Control	15	0
Sodium dextran sulfate 0.1 M	80	75
<i>n</i> -Butanol 3 %	30	10
Cetyltrimethylammonium bromide	20	7
Saponin 50 μg./ml.	35	25
Digitonin 25 μg./ml.	35	25
EDTA 0.1 %	15	0
Magnesium chloride 0.2 M	15	0

Preparation of isolated membranes

To determine the optimum period for ultrasonic treatment, equal samples of a suspension of organisms in de-ionized water were treated ultrasonically for periods from 10 sec. to 30 min. An untreated control was used as well as a sample which was completely lysed with sodium lauryl sulphate. The ultraviolet absorptions of the preparations were examined after centrifugation. The results indicated that 20–30 min. ultrasonic treatment was necessary to approach complete lysis. Examination in the electron microscope of a centrifuge pellet (30,000 g, 1 hr) obtained after 30 min. ultrasonic treatment indicated that it consisted of membranes which did not appear to be very extensively degraded.

Dissolution of isolated membranes

Membranes dissolved readily in 0.01 M-sodium lauryl sulphate in water on shaking. When a solution of approximately 10 mg. wet wt membrane/ml. was examined in the ultracentrifuge a single peak was obtained, resembling that obtained by Razin (1967), but the sedimentation coefficient of 2.5 S was somewhat lower than the value of 3.0 S obtained by Razin.

Solutions of membranes in 2-chlorethanol or 8 M-urea in water showed no sedimenting components at rotor speeds up to 59,780 rev./min. Strongly curving base-lines were however observed. A solution in phenol+acetic acid+water (2+1+0.5, w/v/v) showed several minor fast-moving components, and a strongly curving base-line, at rotor speeds of 40,000 rev./min. Membrane suspensions did not clear on extensive dialysis against de-ionized water.

DISCUSSION

Although results obtained by turbidimetry were in qualitative agreement with those obtained by measuring the release of cell contents from the organisms, the latter technique was preferable for the quantitative measurement of lysis. Razin (1964) used an ultraviolet absorption technique in measuring osmotic lysis, which is not accompanied by much membrane dissolution. Lysis by some chemical agents may also occur without dissolution of the membranes; in such cases the turbidimetric method might be misleading.

The bulk of the work done previously on the chemical lysis of *Mycoplasma* has been concerned with the saprophytic *Mycoplasma laidlawii*. The present results indicate that the membrane of the parasitic *M. gallisepticum* is qualitatively similar to that of *M. laidlawii* in its behaviour towards various chemical reagents. Membranes dissolved in 2-chlorethanol, urea or phenol+acetic acid+water give patterns with base-lines which show strong curvature at the meniscus and cell bottom, indicating the presence of compounds of low molecular weight. These solvents thus do not appear to produce the same subunits as those obtained using sodium lauryl sulphate, suggesting that the situation may be more complex than that envisaged either by Razin *et al.* (1965) or by Engelman, Terry & Morowitz (1967).

The author thanks Mr W. Baxendale (Department of Virology) for growing the *Mycoplasma gallisepticum*, and Mr P. Laine and Miss L. Sayer for skilled technical assistance.

REFERENCES

- ENGELMAN, D. M., TERRY, T. M. & MOROWITZ, H. J. (1967). Characterization of the plasma membrane of *Mycoplasma laidlawii*. *Biochim. biophys. Acta* **135**, 381.
- RAZIN, S. (1963). Structure, composition and properties of the PPLO cell envelope. *Recent Progress in Microbiology*. Ed. by N. E. Gibbons, *Symp. Int. Congr. Microbiol.* **8**, 526. Toronto: University Press.
- RAZIN, S. (1964). Factors influencing osmotic fragility of *Mycoplasma*. *J. gen. Microbiol.* **36**, 451.
- RAZIN, S. (1967). The cell membrane of *Mycoplasma*. *Ann. N.Y. Acad. Sci.* **143**, 115.
- RAZIN, S. & ARGAMAN, M. (1962). Susceptibility of *Mycoplasma* (PPLO) and bacteria protoplasts to lysis by various agents. *Nature, Lond.* **193**, 502.
- RAZIN, S. & ARGAMAN, M. (1963). Lysis of *Mycoplasma*, bacterial protoplasts, spheroplasts, and L-forms by various agents. *J. gen. Microbiol.* **30**, 155.
- RAZIN, S., MOROWITZ, H. J. & TERRY, T. M. (1965). Membrane units of *Mycoplasma laidlawii* and their assembly to membranelike structures. *Proc. natn. Acad. Sci., U.S.* **54**, 219.
- RODWELL, A. W. (1956). The role of serum in the nutrition of *Asterococcus mycoides*. *Aust. J. Bio Sci.* **9**, 105.
- ROTTEM, S. & RAZIN, S. (1967). Electrophoretic patterns of membrane proteins of *Mycoplasma*. *J. Bact.* **94**, 359.
- SMITH, P. F. & ROTHBLAT, G. H. (1960). Incorporation of cholesterol by PPLO. *J. Bact.* **80**, 847.