Lysis of Streptococci by an Extracellular Lysin Produced by Competent Group H Streptococcus Strain CHALLIS

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INTRODUCTION

Competent cultures of the group H streptococcus strains CHALLIS and WICKY autolysed in a buffer at pH 9 containing a reducing agent whereas non-competent organisms did not (Ranhand, Leonard & Cole, 1971). Supernatant fluids of the autolysed competent cultures lysed non-competent WICKY organisms, but were inactive against *Hydrogenomonas eutropha*, *Micrococcus lysodeikticus* and a group A streptococcus (strain 60 × 15). Competence factor (CF) obtained by ammonium sulphate precipitation of supernatant fluids of strain CHALLIS grown in a defined medium (Leonard, Ranhand & Cole, 1970) was without lytic activity against non-competent WICKY organisms (Ranhand *et al.* 1971). Sterile supernatant fluids of CHALLIS cultures of declining competence grown for 3 h in a complex medium (brain-heart infusion with horse serum (BHI-HS) and neopeptone) have now been found to contain both CF and lytic activity against non-competent WICKY organisms. The lytic activity is selectively destroyed by heat (56 °C for 15 to 30 min).

The present report explores the range of lytic activity of crude filtered supernatant fluids of competent CHALLIS cultures grown in BHI-HS plus neopeptone as a preliminary step in defining the nature of the presumptive lysin. Some strains of streptococcal serogroups A, E, H, and K are lysed, whereas representatives of other streptococcal groups and cultures of *Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus lysodeikticus* are not.

METHODS

Bacteria. Table 1 lists the cultures used. Some of these were randomly selected from a collection in our laboratory. The group H streptococcus strain BLACKBURN was obtained from R. Pakula (School of Hygiene, University of Toronto, Ontario, Canada). Organisms designated as serological groups F (strain 57), M, N, P, Q, R and S were obtained from H. Slade (Northwestern University Medical School, Chicago, Illinois, U.S.A.). When possible, the strains were grouped in this laboratory with specific grouping antisera which were obtained from the Center for Disease Control, Atlanta, Georgia, U.S.A., except for that directed against group H organisms which was prepared in our laboratory. The groups designated with a question mark (?) were not confirmed here owing to a lack of specific antisera.

Culture. Cultures were grown overnight at 37 °C in 10 ml of brain-heart infusion (Difco Laboratories, Detroit, Michigan, U.S.A.) containing 2.5 % (v/v) heat inactivated horse serum (56 °C, 30 min; Microbiological Associates Inc., Bethesda, Maryland, U.S.A.; BHI-HS). All cultures were grown statically except for *Bacillus subtilis* which was grown with mild aeration. *Micrococcus lysodeikticus* was obtained from Difco and consisted of organisms

Table 1. *Bacterial cultures used*

Organism	Strains and/or type	*Lysis with the CHALLIS lysin
Streptococcal serological group	Type 1 (derived; M protein negative)	
A	59 × 161†	
A	Type 4 60 × 15†	+
A	Type 6 56 × 188†	+
B	54 × 548†	—
B	54 × 340†	—
B	9925‡	—
C	88	—
C	26RP66	—
D	F24P	—
D	55 × 132†	—
E	12390‡	+
F?	54 × 505†	—
F?	57	—
G	57 × 262	—
G	54 × 264†	—
H	WICKY	+
H	BLACKBURN	+
K?	66 × 6†	+
K?	60 × 256†	—
L?	D176B	—
L?	54 × 419†	—
M?	139	—
N?	C554	—
N?	9936‡	—
P?	54/583	—
Q?	E6844	—
R?	734	—
S?	11231	—
<i>Bacillus subtilis</i>	MARBURG	—
<i>Staphylococcus aureus</i>	6538P†	—
<i>Micrococcus lysodeikticus</i>	—	—

? = Those groups of organisms that were not specifically grouped for this report.

* Lysis was assessed as described in the Methods section.

† Our strain designations (Laboratory of Microbiology, NIAID, NIH).

‡ Those cultures that were originally obtained from the American Type Culture Collection.

killed with ultraviolet light. In general, 10 ml of an overnight culture was centrifuged for 20 min at 1000 to 2000g at 0 to 4°C and the pellet was resuspended in 10 ml of fresh BHI-HS. The overnight supernatant fluids were chilled and stored at -40°C to test for the presence of lytic activity against non-competent WICKY organisms. The resuspended pellet was diluted to 5% (v/v) in 50 ml of warmed BHI-HS in a 125 ml cotton-stoppered Erlenmeyer flask and incubated at 37°C until exponential growth was attained.

At appropriate times, 40 ml of culture was harvested by centrifuging (1000 to 2000g; 0 to 4°C; 20 min) and the pellet washed once with 5 ml of cold 0.85% NaCl. The washed pellet was suspended in 0.8 ml of cold deionized water. Two-tenths ml of this suspension was then added to the lysing buffer which was 0.5 M-TAPS buffer (tris (hydroxymethyl) methyl-amino-propane sulphonic acid; Calbiochem, Los Angeles, California, U.S.A.) containing 0.2 M-2-mercaptoethanol and 0.01 M-ethylenediaminetetraacetic acid, pH 9.0-9.3.

Lysis. In general, 0.54 ml of lysing buffer, 0.2 ml of the suspended organisms, and 0.16 ml of either the CHALLIS lysin, or BHI-HS plus 1% (w/v), neopeptone (Difco) or the overnight

homologous supernatant fluid, or deionized water were mixed together in glass cuvettes and kept at 0 °C. The last three cuvettes were controls. The extinction (*E*) was then read against a zero of water at 750 nm. with a Beckman Model B spectrophotometer. At this wavelength there was little or no absorption by BHI-HS plus neopeptone. After all zero-time extinctions were obtained, the tubes were incubated at 37 °C and readings were made every 5 min for 1 h. In most instances, the tubes were incubated overnight and *E* was again read after 18 h.

Electron microscopy. Lysis was also monitored by examining organisms in the electron microscope after incubation for 18 h with the CHALLIS lysin. Negative staining was used as already described (Ranhand *et al.* 1971).

CHALLIS lysin. The CHALLIS lysin was most active in culture supernatant fluids derived from organisms that had passed their peak of competence. CHALLIS organisms were grown overnight at 37 °C in 10 ml of BHI-HS, then diluted to 5% (v/v) into 100 ml of BHI-HS plus 1% (w/v) neopeptone in a 250 ml cotton-stoppered Erlenmeyer flask. The bacteria were incubated at 37 °C for 3 h then centrifuged (1000 to 2000g; 0 to 4 °C; 20 min) and the supernatant fluids were filtered twice. The first filtration was through a 0.8 µm membrane (type GA-4; Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) for further clarification of the supernatant fluid, and the second filtration was for sterilization (0.22 µm membrane; no. 7103, Falcon Plastics, Los Angeles, California, U.S.A.). The sterile crude lysin (0.3 ml) was stored at -40 °C.

RESULTS

Initial lysis experiments employed overnight cultures of the group H organisms as substrate for the lysin, but lysis was slow. With organisms harvested in the exponential phase of growth as substrate, lysis was relatively rapid. Table 1 gives the lytic response of all cultures tested.

Lysis, which was not observed without the addition of the CHALLIS supernatant fluid, was manifested as a decrease in *E* and an increase in viscosity. In all controls there was a slight decrease in *E* that never exceeded 40% (after overnight incubation) without visible increase in viscosity. The reason for the decrease of *E* is not clear but may be due to a number of factors such as pH, ionic strength, osmolality, molarity, or the chemical composition of the suspending solution. Autolysis was not apparent with any of these cultures except for competent CHALLIS and SBE I/II organisms.

The rate and extent of lysis for all three group A strains (59 × 161, 60 × 15, 56 × 188) was about equal. In 60 min, the *E* decreased by about 60% and reached 25% of the starting *E* during the overnight incubation period. The single group E streptococcal strain (12390) also lysed. By 60 min, the *E* decreased 75%. Of the two strains of group K organisms tested, only one lysed (66 × 6). At 60 min, there was a 45% decrease in *E* which upon overnight incubation had become a 72% decrease. Another group K strain (60 × 256) was refractory to the action of the lysin. The fact that some strains of a given group are lysed by the enzyme and others are not, may reflect group heterogeneity in their cell-wall composition as alluded to by Colman & Williams (1965).

Both group H strains, WICKY and BLACKBURN, lysed with the CHALLIS enzyme. WICKY organisms however, were more sensitive. In 60 min, these cultures lost 80% of their initial *E* and more than 90% in 18 h. The BLACKBURN organisms decreased 60% in 60 min and about 70% in 18 h. The other organisms listed in Table 1 were not lysed by the CHALLIS lysin.

None of the organisms tested lysed when incubated in lysing buffer containing a sample (0.16 ml) of their respective overnight supernatant fluids. However, lytic activity against

non-competent WICKY organisms was present in overnight culture fluids derived from CHALLIS, SBE I/II, and from the group D streptococcus strain 55 × 132.

DISCUSSION

In a previous report (Ranhand *et al.* 1971) we showed that only competent group H strains autolysed in a buffer at pH 9 containing 2-mercaptoethanol. Recent assays of sterile competence factor-containing solutions prepared in complex media, as well as overnight culture fluids from CHALLIS organisms that lacked competence factor activity, revealed that these contained lytic activity against non-competent WICKY organisms. Since lysis of streptococcal organisms has always been a topic of interest to us and a number of other investigators (Barkulis, Smith, Boltralik & Heymann, 1964; Shockman, Pooley & Thompson, 1967; Coleman, van de Rijn & Bleiweis, 1970), and, since an extracellular lysin derived from group H organisms has never been before described, we undertook the survey to see whether the lysin was group or genus specific.

The data presented in this report, although limited to some few strains from 16 serological groups of *Streptococcus* and three strains from other Gram-positive genera, show that the lysin is active only on bacteria from serological groups A, E, H, and K. Although we do not know the nature of the substrate for the lysin, we can tentatively suggest that it is limited to the cross-wall region (Ranhand *et al.* 1971). In susceptible organisms, the CHALLIS lysin, as well as the autolysin of competent organisms (Ranhand *et al.* 1971), does not cause the complete dissolution of the cell wall but appears to act only at a limited site of cross-wall formation. Kinetic data (to be presented in another communication) suggest that the CHALLIS lysin is the autolysin of competent organisms. That they are one and the same enzyme is also indicated by the conditions in which they are active – high pH value in the presence of a reducing agent.

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