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PURIFICATION AND PROPERTIES OF A STAPHYLOLYTIC FACTOR PRODUCED BY A STRAIN OF *STAPHYLOCOCCUS EPIDERMIDIS*[1]

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SUMMARY A cell wall lytic factor (ALE) which lyses intact cells of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Micrococcus lysodeikticus* was identified in the culture supernatant fluid of a strain (named EP-K1) of *Staphylococcus epidermidis* grown in Trypticase Soy Broth with aeration. It was purified 350 fold by precipitation with ammonium sulfate and acetone, and by DEAE-cellulose column fractionation. The lytic factor, named ALE, is non-dialysable, heat-labile, and is destroyed by digestion with crystalline trypsin. The lytic activity is inhibited completely by Cu^{2+} ions at a concentration of 0.0001 M and partially by the presence of 0.001 M Fe^{2+}, Ni^{2+} or Hg^{2+} ions. The pH optimum of the lytic activity of ALE was around 6.8 and NaCl had a definite potentiating effect at a concentration of 0.1 M.

Determination of free amino- and reducing groups liberated in the course of lysis of *S. aureus* and *M. lysodeikticus* cell walls by ALE has revealed that this lytic factor exerts its activity through amidase and/or endopeptidase action on a peptide moiety of the cell wall peptidoglycan (murein) of susceptible cells.

INTRODUCTION

During an investigation on the antistaphylococcal action of extracts of mouse spleen, a white colony which was surrounded by a clear zone which gradually increased in size was recognized as a contaminant on a nutrient agar plate

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which had been heavily inoculated with *Staphylococcus aureus* strain Newman 1, and incubated at 37°C. Later a coccus (strain EP-K1) isolated as a pure culture from this colony was shown to produce a factor with powerful lytic activity against the cell walls of *S. aureus*.

This paper reports the method of production and partial purification of the cell wall lytic factor of EP-K1 and the general properties of the active principle.

MATERIALS AND METHODS

1. *Preparation of a staphylolytic culture supernatant of strain EP-K1*

A loopful of EP-K1 which had been grown overnight on a slant of Trypticase Soy Agar (Baltimore Biological Laboratory, Baltimore, Md., U.S.A.) was inoculated into a flask containing 150 ml of Trypticase Soy Broth (B.B.L.). After shaking at 37°C for 24 hours, the contents of the flask were transfered to a 15 liter stainless steel tank containing 6 liters of Trypticase Soy Broth and 2 ml of Antifoam A (Dow-Corning Corp., Mich., U.S.A.). The inoculated tank was aerated vigorously at 37°C for 48 hours by passage of sterile air through a sintered glass sparger at the bottom of the tank. The culture supernatant fluid with staphylolytic activity was obtained by centrifuging off the cells at 10,000 *g* through a continuous flow rotor (No. 8 CS-60 F rotor, Tominaga Works, Tokyo) in the Tominaga Refrigerated Centrifuge (Model S-62).

2. *Test organisms and their cultures*

S. aureus strain Copenhagen and *S. epidermidis* ATCC 155 in nutrient broth supplemented with 0.5 per cent glucose were grown with aeration by shaking or bubbling air into the culture through a sintered glass sparger for 18 hours. *Micrococcus lysodeikticus* NCTC 2665 was cultivated in a nutrient broth containing 0.5 per cent dried extract of yeast (Daigo Nutritional Chemicals, Osaka) for 48 hours. *Streptococcus pyogenes* group A, type 12, strain SF 42 was grown in Todd-Hewitt Broth (Difco Laboratories, Mich., U.S.A.) for 48 hours. *Lactobacillus plantarum* ATCC 8014: a 24 to 27 hour old culture was grown in the medium described by ARCHIBALD, BADDILEY and BUCHANAN (1961). *Corynebacterium diphtheriae* substrain of Toronto-Harvard of Park-Williams No. 8: a 18 to 24 hour old culture was grown with shaking in modified Taylor's medium. *Bacillus megaterium* strain KM, *Escherichia coli* strain B, and *Proteus vulgaris* strain KS: overnight cultures were grown with shaking in a nutrient broth. All cultures were grown at 37°C. When necessary, bacteria were disinfected by treatment with 0.1 per cent formalin or 0.05 per cent sodium ethylmercurithiosalicylate (merthiolate sodium, Nakarai Chemicals, Kyoto).

3. *Cell wall preparations*

A cell wall specimen of *S. aureus* was obtained by disrupting organisms grown as described above in a Braun Mechanical Cell Homogenizer (Model MSK, B. Braun Apparatesbau, Melsungen, West Germany) in the following way: a 20 ml aliquot of the thick bacterial suspension made by addition of an equal volume of distilled water to cells which had been harvested and washed by centrifugation, was placed in a 75 ml Duran flask together with 20 ml of 1 M NaCl and 30 g of glass beads (Glasperlen, Kat. Nr. 54140 (2883), 0.11–0.12 mm diameter, B. Braun Apparatesbau). The mixture in the stoppered flask was vigorously shaken by an elliptical shaking motion at 2,000 oscillations per minute for 20 minutes, under cooling by delivering a stream of CO_2 around the flask through a flexible capillary tube. Completion of the disruption was checked by gram stain. The suspension of disrupted cells was centrifuged at 300 g for 20 minutes to remove unbroken cells and coarse debris. This centrifugation at low speed was repeated 3 to 4 times until the supernatant fluid was practically free from unbroken cells. On centrifugation of the supernatant at 10,000 *g* for 30 minutes, a crude cell wall fraction was separated as a sediment. This was washed three times each with one fourth of the original volume of 1 M NaCl and with 0.01 M phosphate buffer of pH 7.0. The washed, crude cell wall fraction from 93 g dry weight of cells was suspended in 900 ml of 0.01 M phosphate buffer (pH 7.0) containing 180 mg of crystalline trypsin (Trypsillin, Mochida Pharmaceutical Co., Tokyo) and the suspension was incubated at 40°C for 2 hours. Then the preparation was redigested with 90 mg of Trypsillin. The trypsinized walls were thoroughly washed with distilled water and lyophilized. The yield of lyophilized cell walls from 93 g dry weight of *S. aureus* was 5.75 *g*.

Cell wall specimens of other organisms were pre-

pared using a Braun Cell Homogenizer for *S. epidermidis*, *Strep. pyogenes* and *L. plantarum* as described above, and by disruption with a Kubota 10 Kc/c Sonic Oscillator (Model KMS-100) for *M. lysodeikticus*, *B. megaterium* and *C. diphtheriae* by the method described previously (KOTANI, KITAURA *et al.*, 1959; KOTANI, HIRANO *et al.*, 1959), except that glass beads were added with *M. lysodeikticus*.

4. *Preparation of the peptidoglycan (murein) fraction from cell walls*

The peptidoglycan fraction was prepared by extraction of the cell walls of *S. aureus*, *Strep. pyogenes*, *L. plantarum* and *C. diphtheriae* with trichloroacetic acid (TCA) in the following way. A specimen of the wall was suspended in 5 per cent TCA solution at a rate of 20 mg/ml and the suspension was vigorously stirred in the cold for 24 hours. This extraction procedure was repeated until the extracts gave no appreciable turbidity on addition of 5 volumes of acetone. The insoluble residue which remained after TCA treatment was thoroughly washed with distilled water, lyophilized, and used as the peptidoglycan fraction.

5. *Assay of lytic activity*

The lytic activity of a test specimen was assayed by following the rate of decrease in turbidity of a suspension of intact cells or cell walls of the test organisms. To a 12×100 mm test tube were added 1.6 ml of a suspension of intact cells (0.5 mg/ml) or cell walls (1.0 mg/ml) and 1.6 ml of an appropriate dilution of test lytic specimens in 0.025 M phosphate buffer, pH 6.8, with 0.2 M NaCl, under cooling in ice-water. The lytic factor was ommitted from one of the control tubes and the suspension of intact cells or walls was ommitted from the other control. The optical densities of the test and control mixtures were immediately determined with a Shimadzu Bausch & Lomb Spectronic 20 Colorimeter (Shimadzu Seisakusho, Kyoto) at 550 mμ. Then, the mixtures were kept at 37°C and their change in optical density with time was followed. One unit of lytic activity was defined as the amount of test lytic specimen required to reduce by 50 per cent the optical density of a suspension of intact cells or cell walls of *S. aureus* (Copenhagen) on 60 minutes incubation under the standard conditions described above. The specific activity of a lytic specimen was defined as the number of lytic units per mg of protein. The latter was determined by the method of LOWRY *et al.* (1951), using bovine serum albumin (E. Merk AG, Darmstadt, West Germany) as a reference.

6. *Analytical procedures*

1) *Determination of free amino groups*

Free amino groups were measured by reaction of unhydrolysed test specimens with 2,4-dinitrofluorobenzene (DNFB) according to the description of GHUYSEN, TIPPER and STROMINGER (1966).

To 20 μl of a test specimen estimated to contain 20 to 80 mμmoles of free amino groups were added 80 μl of 1 per cent $Na_2B_4O_7 \cdot 10H_2O$ solution and 10 μl of 0.1 M DNFB solution in ethanol. The contents of the tubes were immediately mixed with a Thermo-Mixer (Model TM-100, Thermonics Co., Tokyo) and placed in a water bath at 60°C for 30 minutes. After addition of 400 μl of 2 N HCl, the optical density at 420 mμ of the reaction mixture was read with a Hitachi Perkin-Elmer Spectrophotometer (Model 139 UV-VIS, Hitachi Ltd., Tokyo). L-Glutamic acid was used as a reference. If a test specimen contained insoluble material, as in the case of a partially lysed cell suspension, 50 μl of conc. HCl were added in place of 2 N HCl, and the mixture in a sealed tube was hydrolyzed in boiling water for 8 hours. The hydrolyzed mixture was diluted with 300 μl of distilled water and the optical density at 420 mμ was determined.

2) *Determination of free reducing groups*

The method of SOMOGYI (1952) was used on a microscale: 40 μl of a test specimen estimated to contain 20 to 120 mμmoles of reducing groups was mixed with 40 μl of the freshly prepared copper reagent. After heating for 20 minutes in boiling water, the mixture was cooled in cold water and then mixed with 40 μl of arsenomolybdate reagent and 500 μl of distilled water. After centrifugation at 1,400 *g* for 10 minutes, the optical density of the supernatant was read with a Hitachi Perkin-Elmer Spectrophotometer at 500 mμ.

RESULTS

1. *Bacteriological characteristics of a staphylolytic coccus, EP-K1*

The organism grows luxuriantly on a variety of solid and liquid media. The colonies grown on nutrient agar at 37°C closely resemble those of the Genus *Staphylococcus* in characters such

as appearance, size, colour, transparency and consistency. The gram stain reveals that the colonies consist of clusters of gram-positive cocci, of about 1 μ diameter. No hemolysis was recognized around colonies grown on a nutrient agar plate with rabbit or ox blood.

The organisms produced acid but not gas from glucose, sucrose, maltose and glycerol during growth in stab cultures in nutrient agar containing these carbohydrates. Acid production from mannitol was positive in stab cultures, but negative on surface cultures of organisms. No acid was produced from galctose, mannose, xylose, lactose, starch or salicin. Nitrate was reduced to nitrite. The Voges-Proskauer reaction was negative. Organisms could not utilize $NH_4H_2PO_4$ as a sole source of nitrogen. Production of free and bound coagulase was found to be negative by conventional tests.

The description mentioned above indicates that strain EP-K1 should be classified as a member of *S. epidermidis*.

2. *Concentration and partial purification of the lytic factor produced by EP-K1*

Preliminary experiments demonstrated that Trypticase Soy Broth is the best medium for production of the staphylolytic factor (named ALE) by EP-K1 and that the maximum yield of the factor is obtained after 36 to 48 hours cultivation. Thus the culture supernatant fluid obtained as described in Materials and Methods was used as a starting material for concentration and purification of ALE. The culture supernatants were found to contain about 2 lytic units/ml when tested on intact *S. aureus* cells.

Step 1. Precipitation with ammonium sulfate

Unless otherwise stated, this and all subsequent steps were carried out in a cold room at about 4°C. A 6 liter aliquot of the culture supernatant was brought to 20 per cent saturation of $(NH_4)_2SO_4$ (with 850 g of powdered salt) with vigorous stirring. After standing for 2 hours, the slightly turbid mixture was clarified by passage through a pad of 30 g of Celite 535 (Johns-Manville Products, U.S.A.) and a filter paper on a Buchner funnel. The filtrate was mixed with 2,700 g of powdered $(NH_4)_2SO_4$ with stirring to give 85 per cent saturation. The mixture was stored overnight and then filtered through a filter paper with the aid of 30 g of Celite 535. The filter cake was extracted with 600 ml of cold 0.01 M phosphate buffer-0.2 M NaCl (pH 7.8). The extracts were dialyzed against two changes of 20 liters of buffered saline for 24 hours each. Almost all the lytic activity measured with intact cells of *S. aureus* in the culture supernatants was recovered in this dialyzed fraction (Table 1). This fraction of 1,000 ml was then brought to 50 per cent saturation of $(NH_4)_2SO_4$ by addition of 350 g of salt. After standing overnight, the mixture was centrifuged at 10,000 *g* for 30 minutes. A clear amber-coloured supernantant fluid containing no measurable lytic activity was discarded and the precipitate was taken up in a minimum of 0.05 M Tris-HCl buffer-0.2 M NaCl of pH 7.8. The solution was dialyzed against 10 liter portions of the same buffered saline for 24 hours. As shown in Table 1, this second $(NH_4)_2SO_4$ precipitation resulted in 24-fold increase in specific activity with 226 per cent recovery on the basis of the activity and protein content of the first $(NH_4)_2SO_4$ fraction. The apparent recovery of over 100 per cent activity might be due to removal of some inhibitor(s) of the lytic factor.

Step 2. Precipitation with acetone

To 300 ml of the second $(NH_4)_2SO_4$ fraction were added dropwise with stirring 240 ml of acetone at -20°C. After standing the mixture at -5°C for 10 minutes, the resulting slightly turbid suspension was centrifuged at 10,000 *g* for 10 minute. Three hundred ml of chilled acetone was added to the supernatant. After standing at -5°C for 10 minutes, a flocculent precipitate was collected by centrifugation at 10,000 *g* for 10 minutes, and it was immediately dissolved in 60 ml of 0.01 M Tris-HCl buffer, pH 7.8. The increase in specific activity at

TABLE 1 *Summary of concentration and purification procedure of ALE*

Preparation	Volume (ml)	Lytic activity* (units/ml)	Total lytic activity* (units)	Protein (μg/ml)	Total protein (mg)	Specific activity (units/mg)	Purification (×)	Recovery (%)
Culture supernatant	6,000	2.75	16,500	—	—	—	—	—
Step 1								
1st $(NH_4)_2SO_4$ fraction	1,000	15.4	15,400	8,400	8,400	1.83	1.0	100
2nd $(NH_4)_2SO_4$ fraction	300	116	34,800	2,620	786	44.3	24.2	226
Step 2	60	538	32,300	5,470	328	98.5	53.8	210
Step 3	300	77.5	23,300	122	36.6	635	347	151

* Lytic units against intact cells of *S. aureus*.

this stage was 54-fold and the recovery was 210 per cent of the activity of the first $(NH_4)_2SO_4$ fraction (Table 1).

Step 3. Separation of the lytic factor by DEAE-cellulose column chromatography.

The active fraction obtained by acetone precipitation was applied to a column (20×2.5 cm) of DEAE-cellulose (Pharmacia, Uppsala, Sweden) equilibrated with 0.01 M Tris-HCl buffer of pH 7.8. After washing the column with 500 ml of the same buffer, the lytic factor was eluted with 0.01 M Tris-HCl buffer, pH 7.8, containing 0.2 M NaCl. Fractions (about 10 ml each) with lytic activity were combined (total, about 80 ml) and stored in a deep freezer at −20°C until use. As shown in Table 1, this purification procedure resulted in 350-fold purification with 150 per cent overall recovery of lytic activity. In the calculations the initial values are those of the first $(NH_4)_2SO_4$ fraction.

3. *Enzymatic properties of the lytic factor (ALE)*

In the following experiments specimens of ALE purified to the final step were used.

1) Effect of pH on the lytic activity of ALE

The effect of pH on lysis of *S. aureus* cell walls by ALE was studied by varying the pH of the reaction mixture. 0.05 M Tris-maleate buffers of pH 5.8 to 9.2, containing 0.1 M NaCl were used in place of the 0.0125 M phosphate buffer-0.1 M NaCl, pH 6.8, mixture in the standard assay system. Fig. 1 shows the extent of cell wall lysis at various pH values after 30 minutes incubation with an appropriate amount of ALE. The optimum pH of the lytic activity was about pH 6.8.

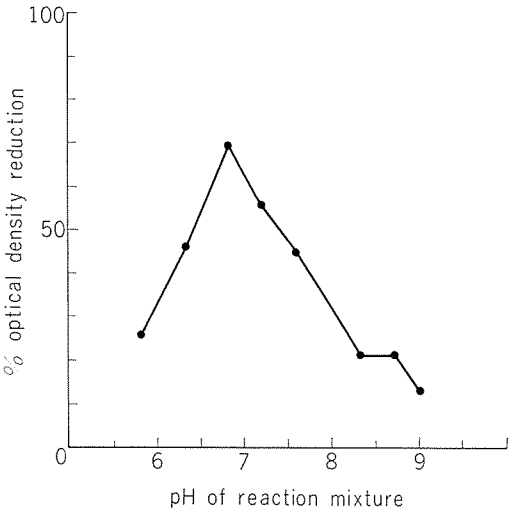

FIGURE 1 The pH optimum for lytic activity of ALE against *S. aureus* cell walls.
Buffer: 0.0125 M Tris-maleate-0.1 M NaCl.

2) Effect of the concentration of NaCl

Lysis of *S. aureus* cell walls by ALE was markedly influenced by the concentration of NaCl added to the reaction mixture containing 0.05 M phosphate buffer of pH 6.8. As indi-

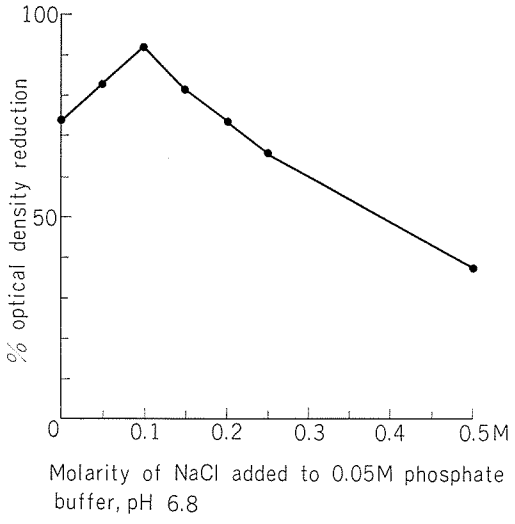

FIGURE 2 Effect of NaCl concentration on lytic activity of ALE against *S. aureus* cell walls.

cated in Fig. 2, the per cent reduction of optical density of the standard reaction mixture was increased with increase in the concentration of NaCl until 0.1 M NaCl and decreased with increasing concentration above 0.15 M NaCl.

3) Effect of divalent cations

The possible activating or inhibiting effects of divalent cations (Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ba^{2+} and Hg^{2+}) on the lytic activity of ALE on *S. aureus* cell walls were examined at final cation concentrations of 0.00008 to 0.001 M. All the cations were tested as chlorides using 0.0125 M Tris-HCl buffer-0.1 M NaCl (pH 6.8) instead of phosphate buffer to prevent formation of insoluble cation phosphate compounds. It can be seen from Fig. 3 that Cu^{2+} completely inhibited the lytic activity of ALE at a concentration of 0.0001 M and exhibited a definite inhibitory effect even at 0.00008 M. Fe^{2+}, Ni^{2+} and

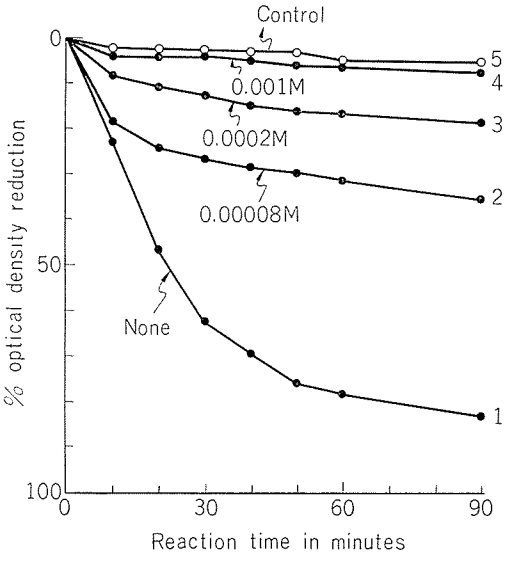

FIGURE 3 Inhibitory effect of Cu^{2+} ions on lytic activity of ALE against *S. aureus* cell walls.

1. None
2. 0.00008 M
3. 0.0002 M
4. 0.001 M
5. Control without enzyme

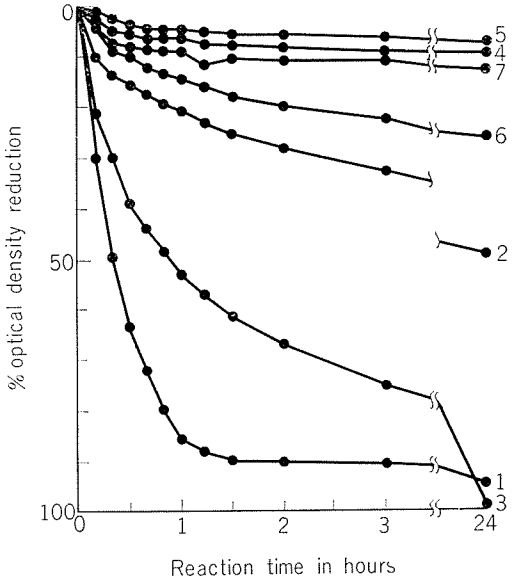

FIGURE 4 Lytic activity of ALE on cell walls of various bacterial species.

1. *S. aureus*, 2. *S. edidermidis*, 3. *M. lysodeikticus*, 4. *Strep. pyogenes*, 5. *L. plantarum*, 6. *B. megaterium*, 7. *C. diphtheriae*

There were no significant changes in optical density in the controls without ALE.

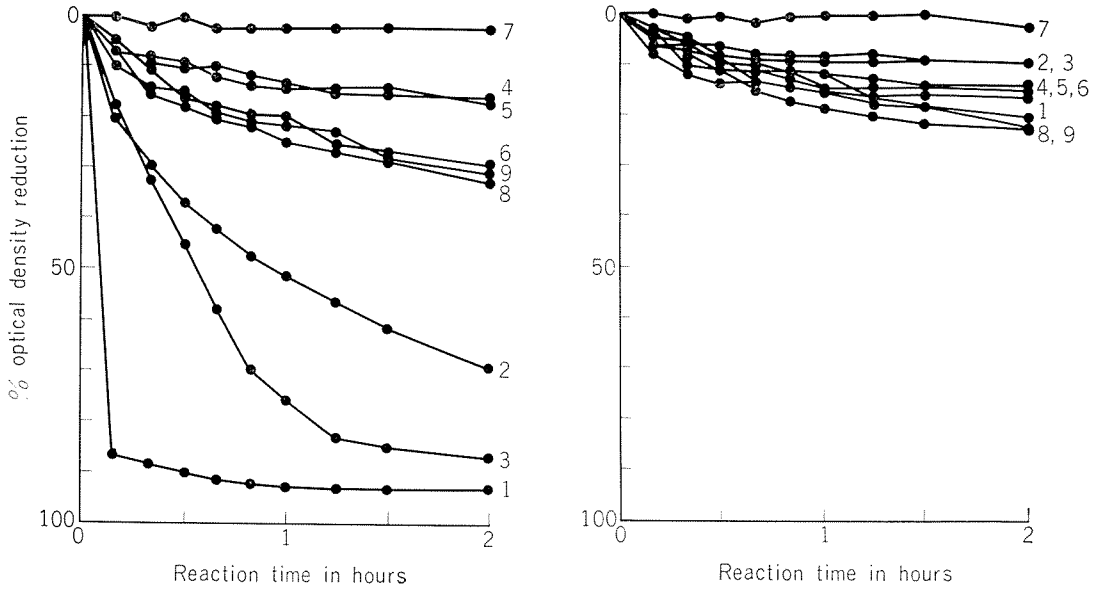

FIGURE 5 Lytic activity of ALE on intact cells of various bacterial species.
8. *E. coli*, 9. *P. vulgaris*. Other numbers as in Fig. 4.

Hg^{2+} ions were shown in another experiment to exert some inhibitory effect on ALE at a concentration of 0.001 M. The other divalent cations tested had no effect.

4) Specificity of the lytic activity of ALE

To test the specificity of ALE, the lytic actions of specimens of ALE on the cell walls and intact cells of various bacterial species were tested. Among the cell walls tested, those of *S. aureus*, *S. epidermidis* and *M. lysodeikticus* were found to be susceptible to 4 cell wall lytic units of ALE, while the walls of *Strep. pyogenes*, *L. plantarum*, *B. megaterium* and *C. diphtheriae* were quite resistant to ALE, as shown in Fig. 4. This figure also indicates that the susceptibility of the walls of *S. epidermidis* and *M. lysodeikticus* to ALE was definitely less than that of *S. aureus* walls, and that in the case of *S. epidermidis* walls, complete clarification of the suspension was never obtained and the reduction in optical density did not exceed 50 per cent at the maximum lysis. The susceptibilities of intact cells of different organisms were essentially similar to those of their cell walls*. Moreover, no significant lysis of the intact cells of *E. coli* or *P. vulgaris* was observed. The walls of these organisms have not yet been studied (Fig. 5). Six intact cell lytic units of ALE were used in this experiment. It should be added here that one cell wall lytic unit corresponded to an average of 18 intact cell lytic units when *S. aureus* was employed as test organism.

Fig. 6 indicates the action of ALE (6 cell wall lytic units) on the peptidoglycans of *S. aureus*, *Strep. pyogenes*, *L. plantarum* and *C. diphtheriae*. It can be seen from this figure that on removal of the ' special structures ' of the walls by TCA extraction, both the cell

* In a separate paper (SAKAGUCHI *et al.*, 1966), it was reported that intact cells of *Pediococcus pentasaceus* strain 82 and *P. halophilus* strain Mees are susceptible to the lytic action of ALE.

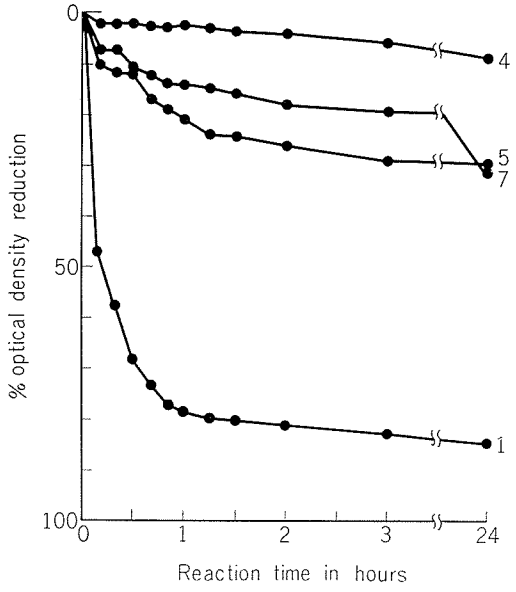

FIGURE 6 Suceptibilities of cell wall peptidoglycan (murein) fractions of various microorganisms to ALE.
Legend as for Fig. 4.
There were no significant changes in optical density in the control without ALE.

walls of *L. plantarum* and those of *C. diphtheriae* gained a definite susceptibility to ALE, as indicated by some 30 per cent reduction in the optical density after 24 hours incubation. However, no significant change in the susceptibility was recognized with the peptidoglycan of *Strep. pyogenes*.

4. *Physicochemical properties of ALE*

The effects of heating and trypsin digestion on the activity of ALE were studied. In the presence of 0.05 M phosphate buffer-0.1 M NaCl (pH 6.8), the lytic activity of ALE was completely destroyed by heating at 60°C for 30 minutes. ALE also lost all its lytic activity when digested with crystalline trypsin (Trypsillin).

Incidentally, ALE specimens at purification step 2 (in 0.01 M Tris-HCl buffer of pH 7.8) tended to lose activity with appearance of a flocculent precipitate on standing at 0°C for one hour or even on storage in the deep-freezer at −20°C. The ALE preparation at the final stage, on the other hand, can be stored for several months in a deep-freezer, without any significant loss of lytic activity, if care is taken to avoid repeated freezing-thawing.

The properties of ALE described above indicate that ALE is an enzyme (or enzymes).

5. *Mode of lytic action of ALE*

In this experiment, a specimen of *S. aureus* cell walls was used after removal of alkali-labile alanine, linked as an ester to a teichoic acid polymer in the walls, since the presence of the alkali-labile alanine complicated interpretation of results. A specimen of cell walls was suspended in 0.01 M pyrophosphate buffer of pH 9.0 and heated at 40°C for 4 hours. After washing thoroughly with distilled water, the treated cell walls were lyophilized and used as the substrate.

Twenty mg of these walls were incubated with 10 cell wall lytic units of ALE in a total volume of 4 ml of 0.0125 M phosphate buffer-0.1 M NaCl, pH 6.8. At intervals during incubation at 37°C, samples were removed to

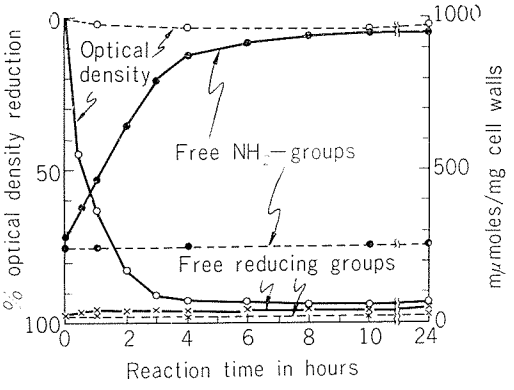

FIGURE 7 Liberation of free amino groups on lysis of *S. aureus* cell walls with ALE.
Solid line—test mixture with ALE; dotted line—control mixture without ALE.
○: Optical density; ●: Free NH_2-groups; ×: Free reducing groups.

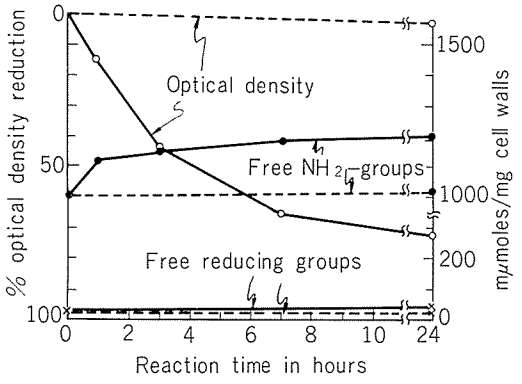

FIGURE 8 Liberation of free amino groups from *M. lysodeikticus* cell walls on lysis with ALE.
Solid line—test mixture with ALE; dotted line—control mixture without ALE.
○: Optical density; ●: Free NH_2-groups; ×: Free reducing groups.

measure the optical density at 550 mμ, and release of free amino- and reducing groups. As shown in Fig. 7, in parallel with decrease in optical density, amino groups were liberated rapidly and steadily for 3 hours, and then more gradually for several hours. A maximum of 650 mμmoles of free amino groups were liberated from one mg of walls under these experimental conditions. No significant release of free reducing groups was recognized during the entire period of lysis. Essentially similar results were obtained with *M. lysodeikticus* cell walls when incubated with ALE (1.5 cell wall lytic units/mg walls) except that at 0 time the concentration of free amino groups in these walls was very high (about 1,000 mμmoles/mg) and a maximum release of only 200 mμmoles was obtained (Fig. 8).

DISCUSSION

The morphological characteristics and biochemical activities of the staphylolytic coccus (EP-K1) reported here indicate that this organism should be identified as a strain of *S. epidermidis*, with reference to Bergey's Manual of Determinative Bacteriology (7th Edition, 1957). Although EP-K1 somewhat resembles strain K-6-W1, which was reported to produce a powerful staphylolytic enzyme, Lysostaphin (SCHINDLER and SCHUHARDT, 1960, 1961, 1964, 1965; SHINDLER, 1965; BROWDER, ZYGMUNT, YOUNG and TAVORMINA, 1965), there are distinct differences between the biochemical characteristics of these two organisms, as shown in Table 2.

TAELB 2 *Comparison of the biochemical activities of EP-K1 and K-6-W1*

	Tests	EP-K1	K-6-W1
Fermentation	Glucose	+*	+
	Galactose	−	+
	Mannose	−	+
	Xylose	−	−
	Sucrose	+	+
	Lactose	−	+
	Maltose	+	−
	Starch	−	−
	Glycerol	+	−
	Mannitol	(+)**	−
	Salicin	−	−
Voges-Proskauer test		−	−
Nitrate reduction		red.***	red.
Utilization of $NH_4H_2PO_4$		−	−

* +: production of acid, but not gas, from the carbohydrate.
** (+): acid production positive in stab cultures, but negative on surface cultures.
*** Nitrates reduced to nitrites.

The insensitivity of *S. aureus* cell walls to egg white lysozyme has stimulated research on staphylolytic enzymes of bacterial origin. Since the first report of GHUYSEN (1957) on *Streptomyces albus* enzymes, there have been a number of studies on extracellular staphylolytic enzymes produced by a variety of microorganisms: *Streptomyces albus* G (GHUYSEN and STROMINGER, 1963 a, 1963 b; GHUYSEN, TIPPER and STROMINGER, 1965; GHUYSEN, DIERICKX *et al.*, 1965; GUHYSEN, TIPPER *et al.*, 1965; GHUYSEN, PETIT, MUÑOZ and KATO 1966), *Flavobacterium* sp. (KOTANI *et al.*, 1959; KATO *et al.*, 1960, 1962; GHUYSEN,

PETIT, MUÑOZ and KATO, 1966), *Micrococcus* sp. (SCHINDLER and SCHUHARDT, as mentioned above), *Chalaropsis* sp. (HASH, 1962, 1963, HASH *et al.*, 1964), *Myxobacter* sp. (ENSIGN and WOLFE, 1964, 1965, 1966; TIPPER, STROMINGER and GHUYSEN, 1964; TIPPER, STROMINGER and ENSIGN, 1967), *Pseudomonas aeruginosa* (ZYSKIND and PATTEE, 1965, BURKE and PATTEE, 1967), *Aeromonas* sp. (COLES and GILBO, 1967) and bacteriophage-induced cell wall peptidase (DOUGHTY and MANN, 1967).

Of the staphylolytic enzymes produced by these microorganisms, the *Flavobacterium* L-11 enzyme, the SA endopeptidase from *Streptomyces albus G*, Lysostaphin, and the *Cytophaga* and *Pseudomonas* enzymes and phage-induced peptidase exert their lytic activities by splitting the bond(s) of amino groups of the peptide moiety of the cell wall peptidoglycan through an amidase and/or endopeptidase action. The other enzymes, the 32 and F_1 enzymes from *Streptomyces albus G*, the B enzyme from *Chalaropsis* sp. and glycosidase in Lysostaphin, cleave the glycosidic linkages of N-acetylmuramic acid or N,O-diacetylmuramic acid and N-acetylglucosamine in a glycan portion of the *S. aureus* peptidoglycan (cf. review of GHUYSEN, TIPPER and STROMINGER, 1966). No information is yet available on the mode of lytic action of *Aeromonas* enzyme.

An analysis of the free amino- and reducing groups liberated by lysis of *S. aureus* cell walls by ALE has demonstrated that the action of ALE is like that of the first group of enzymes mentioned above. Thus ALE exerts its lytic activity against *S. aureus* and *M. lysodeikticus* cell walls by an amidase and/or endopeptidase action. The similarity and dissimilarity in the mode of action of ALE and that of the staphylolytic enzymes described previously, especially the L-11 enzyme and Lysostaphin, will be discussed in a subsequent paper giving the results on qualitative and quantitative end-group analyses of amino- and carboxyl terminal groups liberated from *S. aureus* walls by ALE. The paper will also report the characterization of the chemical entity of low molecular peptides isolated from ALE-digests of walls.

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