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Autolysis of *Neisseria gonorrhoeae*

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Physiological conditions that would provide maximal rates of autolysis of *Neisseria gonorrhoeae* were examined. Autolysis was found to occur over a broad pH range with the optimum at pH 9.0 in 0.05 M tris(hydroxymethyl)aminomethane-maleate buffer. The temperature optimum was found to be 40 C. Potassium ions greatly stimulated autolysis at a concentration of 0.01 M. Exposure of growing *N. gonorrhoeae* cells to penicillin, vancomycin, or D-cycloserine influenced the susceptibility to the autolysin, whereas chloramphenicol afforded some protection against autolysis. The primary structure of the peptidoglycan is composed of muramic acid/glutamic acid/alanine/diaminopimelic acid/glucosamine in approximate molar ratios of 1:1:2:1:1, respectively. Exogenous radioactive diaminopimelic acid, D-glucosamine, and D-alanine were incorporated into peptidoglycan. During autolysis these radioactive fragments were released from cells.

The size and shape of a bacterial cell are determined by the cell wall, particularly by the rigid peptidoglycan component. In gram-positive bacteria the peptidoglycan accounts for as much as 40 to 90% of the dry weight of the cell wall. The cell walls of gram-negative bacteria are more complex, containing less peptidoglycan (5 to 20%) and large amounts of protein, lipid, and lipopolysaccharide (13-15). Hydrolysis of the peptidoglycan could facilitate enlargement of the cell surface during growth and cellular division (8, 13). Three classes of lytic enzymes have been described (5). Glycosidases such as endo-*N*-acetylglucosaminidases or endo-*N*-acetylmuramidases are responsible for the degradation of the carbohydrate backbone of the peptidoglycan. *N*-acetylmuramyl-L-alanine amidases hydrolyze the linkage between *N*-acetylmuramic acid and L-alanine. In addition there are many endopeptidases that cleave the peptide moiety of the peptidoglycan. Uncontrolled activity of any of these enzymes can lead to eventual autolysis of the bacterial cell. Altered biosynthesis of the peptidoglycan by specific inhibitors, such as penicillin or D-cycloserine, or the deprivation of required precursors, such as glucose, glucosamine, lysine, diaminopimelic acid, alanine or glutamic acid, have been shown to result in autolysis when the physiological conditions of pH, temperature, and salt concentrations are suitable (16). The autolytic activities of such enzymes have been well documented in both gram-positive and gram-negative organisms (4, 8, 12).

Autolytic enzymes may also perform an important function in the eradication of pathogenic organisms (19, 20). For instance, autolysis may facilitate the killing of organisms inhibited by penicillin, whereas the inhibition of growth by chloramphenicol frequently results in a reduction of the rate of autolysis in many model systems (6, 8). To understand the killing process by penicillin and other antibiotics, a study on the autolytic processes active in *Neisseria gonorrhoeae* was undertaken. Although several other investigators have made observations on the organism's lytic nature (1, 2), only one publication on autolysis appears in the literature (11). The present paper is an initial investigation into the physiological conditions necessary to induce autolysis. The effect of antibiotics on the rate of autolysis was examined, and methods were developed to aid in the determination of the nature of the autolytic system.

MATERIALS AND METHODS

Organisms. Three clinical isolate of *N. gonorrhoeae*, RUG37 types 2, 3, and 4, RUG38 type 4, and RUG12 type 4, were obtained from the Monroe County Venereal Disease Clinic. Strains RD_s type 4 and 2686 type 4 were obtained from cultures provided by F. J. Tyeryar (Naval Medical Reserve Institute, Bethesda, Md.). Strain F62 types 1 and 4 were obtained from D. S. Kellogg (Center for Disease Control, Atlanta, Ga.). The maintenance and properties of some of these strains and the diagnostic criteria for identification of *N. gonorrhoeae* strains were described previously (9).

Media. Cells were routinely cultured in Mueller-

Hinton broth supplemented (per liter) with 10 g of yeast extract, 22 mM glucose, and 5 mg $CaCl_2$ (standard growth medium, SGM). Gonococcal genetic medium (GGM) was used to maintain colony types 1 and 2 during growth (L. LaScolea, M. Dul, and F. Young, J. Clin. Microbiol., in press). All media used were preincubated at 37 C with 8 to 10% CO_2.

Growth conditions. Chocolate agar and gonococcal genetic agar were inoculated with various strains and incubated for 18 h at 37 C with 8 to 10% CO_2. A suspension of cells was prepared in liquid media and used to inoculate the growth media to an initial turbidity of 15 to 25 Klett units (Klett-Summerson colorimeter, filter no. 62 [620 nm]). Alternatively, cells were grown in liquid media directly from frozen cell stocks prepared as described by LaScolea and Young (9). Cultures were incubated at 37 C in a New Brunswick G76 water bath shaking at 160 rpm. The minimal inhibitory concentration (MIC) of each antibiotic was determined by serial dilution of antibiotics in SGM. The MIC was designated as the last dilution that showed turbidity.

Autolysis procedures. Cells were harvested by centrifugation (8,000 × *g*, 2 min), washed, and suspended in 0.05 M tris(hydroxymethyl)aminomethane (Tris)-maleate buffer (pH 7.0). The cells were then diluted into preincubated buffers to an initial turbidity of 120 to 180 Klett units. Autolysis was analyzed in Tris-maleate (0.05 M) and glycine-NaOH (0.05 M) buffer (7) at the various pH values shown in Results.

The decrease in optical density was monitored as a function of time in a Klett-Summerson colorimeter, filter no. 62 (620 nm). Rates of autolysis were determined as a function of the first-order rate constant $K = \log_{10} C_o/C_t \times 2.303 \times \text{min}$ where C_o is the initial turbidity and C_t is the turbidity after an incubation period.

Measurement of radioactivity. Cells labeled with radioactive compounds were harvested by centrifugation (8,000 × *g*, 2 min) and suspended in cold 5% trichloroacetic acid (20 min) to remove the intracellular pool of free radioactive compounds. Aqueous samples were suspended in 5 ml of Triton X-100 scintillation fluid (15.2 g of Omnifluor, 1 liter of Triton X-100, and 2.78 liters of toluene). Nonaqueous samples were suspended in 5 ml of toluene scintillation fluid (15.2 g of Omnifluor, 3.78 liters of toluene). The radioactivity was determined in a Beckman LS-230 liquid scintillation counter.

Analytical methods. Cells were fractionated according to a modified procedure of Martin et al. (10). Radioactive cells were harvested and washed by centrifugation (8,000 × *g*, 5 min at 20 C), and the intracellular pool was removed with cold 5% trichloroacetic acid (20 min). The acid-insoluble material was washed three times with glass-distilled water. The washed acid-insoluble material was suspended in 0.05 M Tris-maleate buffer (pH 7.5) containing 100 μg of Pronase per ml and incubated for 60 min at 37 C. This procedure was carried out twice. The sample was centrifuged in a Beckman L3-50 with a 50Ti rotor at 20 C for 35 min at 65,000 *g*. The pellet was washed three times with glass-distilled water under similar conditions. The washed pellet was then suspended in boiling 4% sodium dodecyl sulfate for 30 min, centrifuged, and washed as described above. The clear colorless gel obtained represented the peptidoglycan component.

RESULTS

Autolysis of various strains. The major aim of this study was to determine the physiological conditions under which *N. gonorrhoeae* will autolyze and to develop methods that would enable us to determine the mechanism of action of the autolytic system. A number of strains of *N. gonorrhoeae* were screened for the ability to autolyze. The cells, grown in either SGM or gonococcal genetic medium, were harvested in late logarithmic phase of growth and suspended in 0.05 M Tris-maleate buffer (pH 5.5 to 9.0) to a density of 130 to 180 Klett units. The rate of autolysis at each pH was then determined. The maximum rate of autolysis occurred in each strain at pH 8.5 to 9.0. The time required for a 50% decrease in optical density ($t_{½}$) and the first-order rate constant are shown in Table 1. Type 3 and type 4 variants of the strains studied in this investigation lyse rapidly ($t_{½}$ 25 to 35 min). The rate of autolysis of type 1 and type 2 colony variants of the same strains is slower ($t_{½}$ 80 to 90 min); however, due to the high degree of clumping of the cells, no firm conclusions can be made at this time. Strain RD_5, which underwent the most rapid autolysis, can be readily propagated in our laboratory and thus was selected as the organism of choice for the rest of this study.

Effect of pH, temperature, ions, and stage of growth on autolysis. The effect of pH on autolysis is shown in Fig. 1. Harvested cells were suspended in 0.05 M Tris-maleate buffer preincubated at 37 C. Lysis was then followed turbidimetrically as a function of time. Autolysis was observed at all pH values tested, with the maximum rate occurring at pH 8.5 to 9.0. At

TABLE 1. *Autolysis of N. gonorrhoeae strains*[a]

Strain	Autolysis	
	$t_{½}$	$10^{-3}k$
RD_5	23	30.1
RUG37	25	27.7
RUG38	27	25.7
F_{62}	30	23.1
2686	33	21.0
RUG12	35	19.8

[a] Harvested logarithmic-phase cells grown in standard growth medium or gonococcal genetic medium were suspended in 0.05 M Tris-maleate buffer (pH 8.5) at a density of 130 to 180 Klett units.

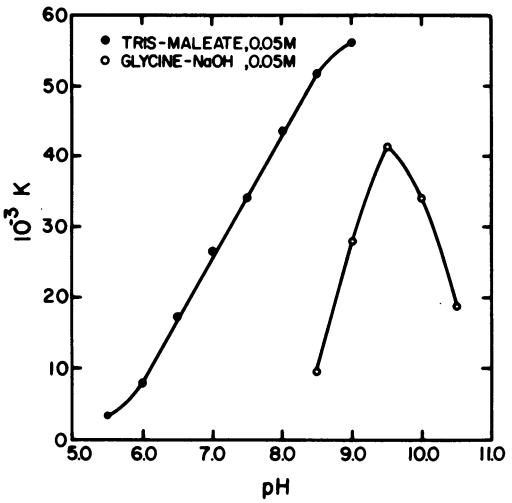

FIG. 1. *Effect of pH on the rate of autolysis. Harvested cells, grown in standard growth medium, were suspended in 0.05 M Tris-maleate (●) or glycine-NaOH (○) buffer at various pH values. The buffers were preincubated at 37 C before the addition of cells. The samples were incubated at 37 C, and the rate of autolysis was determined.*

pH 8.5 to 9.0, 75 to 80% reduction in turbidity was commonly observed. Microscope examination under phase contrast demonstrated few unlysed cells compared to the number of "ghosts." A control utilizing heat-killed cells (80 C, 10 min) displayed no autolytic activity or solubilization up through pH 10.5.

The effect of temperature on the rate of autolysis was measured by suspending harvested cells in 0.05 M Tris-maleate buffer (pH 8.5) preincubated at the various temperatures. The results indicate (Fig. 2) that the rate of autolysis is dependent upon temperature, with the maximum rate occurring at 40 C. The decrease in autolytic activity at 50 C is probably due to the inactivation of the autolytic enzyme system. At 60 C no autolysis was observed.

Autolytic activity was markedly influenced by ionic strength (Fig. 3). Potassium ions greatly stimulated the rate of autolysis at a concentration of 0.1 M, whereas sodium and ammonium ions had no stimulatory effect.

To determine the effect of growth phase on the rate of autolysis, cells were harvested at early, mid-, and late logarithmic phase and at early stationary phase, suspended in 0.05 M Tris-maleate buffer (pH 8.5), and incubated at 37 C. The results of this experiment (not shown) indicated there was no phase of growth in which the cells were more susceptible to autolysis.

Effect of antibiotics on autolysis. The effect of various antibiotics on the rate of autolysis was examined. Cells were incubated in SGM until the mid-logarithmic phase of growth. At that point (Klett = 80), the antibiotic was added and the cells were harvested after 45 min of incubation. The harvested cells were then suspended in 0.05 M Tris-maleate (pH 8.5), and the rate of autolysis was determined. Penicillin-, vancomycin-, and D-cycloserine-treated cells underwent lysis much more rapidly than the control, whereas chloramphenicol-treated cells lysed slower than untreated cells (Fig. 4). To examine these results further, cells were grown to mid-logarithmic phase in SGM and antibiotic was added for varying time periods before harvesting and suspension in 0.05 M Tris-maleate buffer (pH 8.5). Figure 5 shows the change in the rates of autolysis when growing cells are exposed to antibiotics for varying time periods. The autolytic rates in this figure have been normalized to the control autolytic rate ($K \times 10^{-3} = 30.0$). The data show that after exposure of cells for 5 min to 10 μg of penicillin per ml the rate of autolysis was greatly increased. The longer the exposure the greater was the susceptibility to autolysis. At the MIC

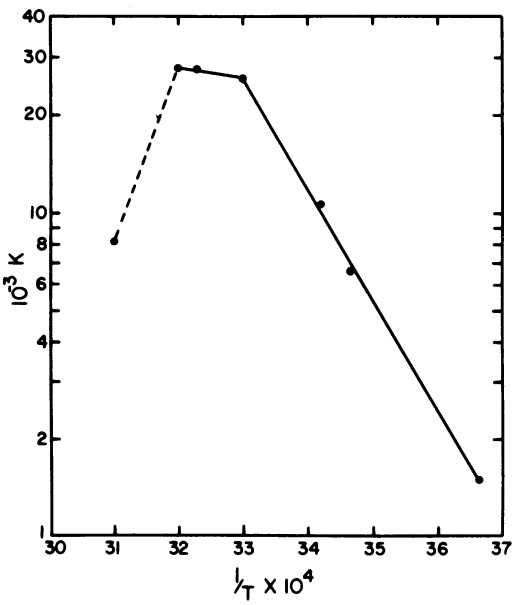

FIG. 2. *Effect of temperature on the rate of autolysis. Harvested cells, grown in standard growth medium, were added to 0.05 M solutions of Tris-maleate buffer (pH 8.5) and incubated at various temperatures. Buffer samples were preincubated at the various temperatures before the addition of cells. The rate of autolysis was then determined. The reciprocal of absolute temperature is plotted on the abscissa and the rate constant is plotted on the ordinate.*

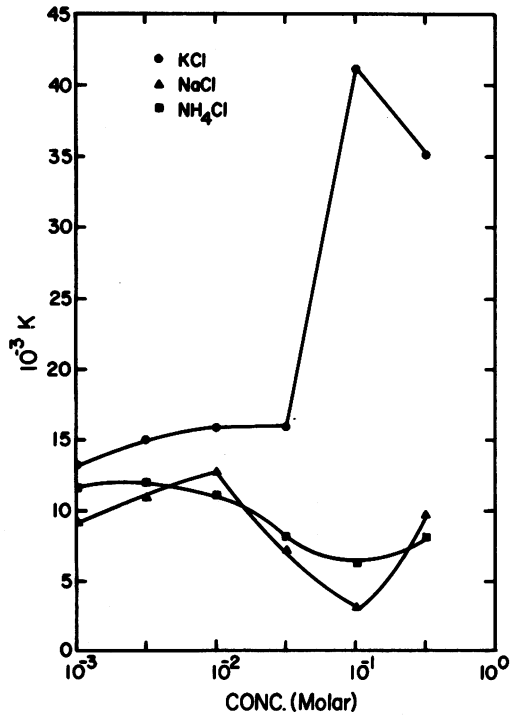

FIG. 3. *Effect of ionic strength on autolysis. Harvested cells, grown in standard growth medium, were added to .01 M Tris-maleate buffer (pH 8.5) containing varying concentrations of KCl (●), NaCl (▲), and NH_4Cl (■). The samples were incubated at 37 C, and the rate of autolysis was then determined.*

level for penicillin (0.01 μg/ml), the increase in the rate of autolysis is linear relative to the time of antibiotic treatment. At twice the MIC level for vancomycin (MIC = 0.5 μg/ml), no effect on the rate of autolysis is observed. At a 100-fold increase of the MIC level, the autolysis rate remained constant after 10 min of antibiotic treatment, and then a linear rise in the rate of lysis occurred. Chloramphenicol (MIC = 0.5 μg/ml), on the other hand, showed a rapid inhibition of autolysis within the first 5 min of antibiotic treatment, with a gradual linear decrease in the rate of autolysis until 20 min of treatment, after which the rate of autolysis remained constant.

Analysis of products released during autolysis. Before extensive analysis on the mechanisms of enzymatic cleavage can be undertaken, it is necessary to devise some means of identification of end products. One approach would be to radioactively label the peptidoglycan and then examine the amount and structure of the peptidoglycan fragments released during autolysis. Logarithmic-phase cells, grown in SGM, were labeled with various compounds for

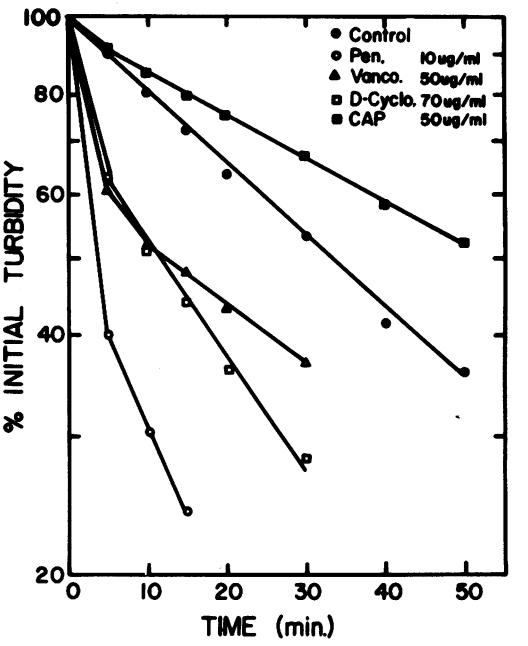

FIG. 4. *Effect of antibiotics on autolysis. Logarithmic-phase cells grown in the presence of antibiotics for 45 min were harvested. Harvested cells were added to 0.05 M Tris-maleate (pH 8.5) buffer. Autolysis was followed by measuring the percent decrease in turbidity as measured in Klett units.*

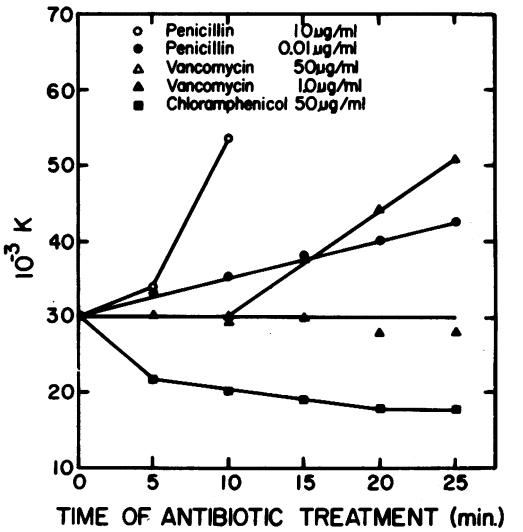

FIG. 5. *Effect of antibiotic exposure on the rate of autolysis. Logarithmic-phase cells grown for various times in the presence of antibiotics were harvested and suspended in 0.05 M Tris-maleate buffer (pH 8.5). The samples were incubated at 37 C, and the rate of autolysis was then determined.*

a period of two generations, harvested, washed, suspended in cold 5% trichloroacetic acid, and fractionated. The results are shown in Table 2; the left side of the table indicates the total counts per minute in the cold 5% trichloroacetic acid before fractionation, the total counts per minute recovered after fractionation, and the percentage recovered. The right side of the table indicates the total counts per minute solubilized after Pronase digestion (100 μg/ml, 60 min, 37 C, repeated two times), after boiling 4% sodium dodecyl sulfate digestion (100 C, 20 min), and in the peptidoglycan fraction. All fractions were washed three times, and any radioactivity recovered was added to that fraction. Pronase digestion released 93% of the lysine, 99% of the phenylalanine, and 87% of the glycine incorporated into cold 5% trichloroacetic acid-insoluble material. Diaminopimelic acid, D-glucosamine, and D-alanine were also solubilized but to a lesser degree. Subsequent sodium dodecyl sulfate digestion released the remaining lysine, phenylalanine, and glycine. The clear gel that was recovered after Pronase and then sodium dodecyl sulfate digestion contained only those amino acids usually found in the peptidoglycan. These amino acids contained a high percentage of the radioactive isotopes incorporated into the cell: diaminopimelic acid, 41%; D-glucosamine, 51.7%; and D-alanine, 27.4%. Virtually no lysine, phenylalanine, or glycine was in this fraction. The clear gel was completely solubilized by lysozyme (100 μg/ml, 37 C) and represents the peptidoglycan fraction. Preliminary amino acid analysis indicates an almost pure peptidoglycan with molar ratios of 0.76:1:1.77:1:0.96 for muramic acid, glutamic acid, alanine, diaminopimelic acid, and glucosamine, respectively. No correction was made for degradation of amino sugars during hydrolysis at 105 C for 14 h in 4 N HCl.

Chromatographic analysis of radioactively labeled peptidoglycan. Since there is the possibility of conversion of diaminopimelic acid to lysine, cells were labeled with [^{3}H]diaminopimelic acid and D-[^{14}C]glucosamine, and the peptidoglycan component was isolated. The labeled peptidoglycan was hydrolyzed in 4 N HCl at 105 C for 14 h and chromatographed on SG81 paper developed in 95% ethanol-water (70:30, vol/vol). This system was chosen for its great separation of lysine, diaminopimelic acid, and D-glucosamine (Fig. 6). Controls employed are numbered: L-alanine = 1; L-glutamic acid = 2; glycine = 3; D-glucosamine = 4; diaminopimelic acid = 5; L-lysine = 6; and muramic acid = 7. Two samples of the hydrolyzed labeled peptidoglycan were spotted. Sample A was cut into 1-cm strips after chromatography and assayed for radioactivity in toluene scintillation fluid. The controls and sample B were sprayed with 0.5% ninhydrin in 95% acetone and 5% 2,4-lutidine, and developed at room temperature. It is clearly shown in the figure that the ^{3}H counts incorporated remained as diaminopimelic and were not randomized to lysine. The ^{14}C counts appeared to be in both D-glucosamine and muramic acid.

TABLE 2. *Fractionation of cold 5% trichloroacetic acid-treated cells*[a]

Radioactive label[b]	Acid-insoluble counts/min			Total counts/min recovered in fraction (%)		
	Acid precipitated	Recovered	% Recovered	Pronase solubilized	Boiling sodium dodecyl sulfate solubilized	Peptidoglycan fraction
[^{3}H]diaminopimelic acid[c]	70,394	68,246	97	29,096 (42.7)	11,070 (16.2)	28,080 (41.1)
[^{14}C]lysine[c]	51,873	43,650	84	40,698 (93.2)	2,952 (6.8)	0 (0.0)
[^{14}C]phenylalanine	291,356	225,065	77	222,003 (98.7)	2,772 (1.2)	290 (0.1)
[^{3}H]glycine	79,549	86,762	109	75,168 (86.7)	11,394 (13.1)	200 (0.2)
D-[^{14}C]glucosamine	31,679	36,602	116	7,344 (20.1)	10,368 (28.3)	18,890 (51.6)
D-[^{14}C]alanine[c]	42,318	41,966	99	19,746 (47.1)	10,710 (25.5)	11,510 (27.4)

[a] Culture (20 ml) grown for two generations in the presence of label was collected and suspended in cold 5% trichloroacetic acid. The acid-insoluble precipitate was then fractionated.

[b] Final concentrations (microcuries per milliliter) of radioactive labels were: [^{14}C]phenylalanine, [^{3}H]glycine, D-[^{14}C]glucosamine, 0.45; D-[^{14}C]alanine, 0.41; [^{14}C]lysine, 0.09; [^{3}H]diaminopimelic acid, 4.50. Specific activities (microcuries per micromole) were: D-[^{14}C]phenylalanine, 464; D-[^{14}C]glucosamine, 10; D-[^{14}C]alanine, 36; [^{14}C]lysine, 260; [^{3}H]diaminopimelic acid, 300; [^{3}H]glycine, 6,800.

[c] [^{3}H]diaminopimelic acid, [^{14}C]lysine, and D-[^{14}C]alanine was added separately to cultures supplemented with 10^{-3} M lysine, diaminopimelic acid, and L-alanine, respectively. The presence of radioactivity in Pronase-solubilized and SDS fractions in the culture labeled with diaminopimelic acid is due to the conversion of radioactive diaminopimelic acid to radioactive lysine. The radioactivity in the peptidoglycan fraction is only in diaminopimelic acid.

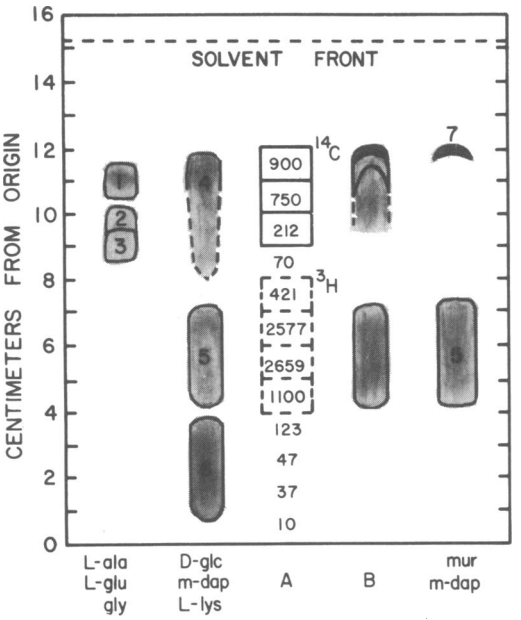

FIG. 6. *Chromatographic separation of radioactively labeled peptidoglycan. Early logarithmic-phase cells were grown in standard growth medium containing 5 μCi of [³H]diaminopimelic acid and 0.5 μCi of D-[¹⁴C]glucosamine per ml for two generations. Harvested cells were washed, suspended in cold 5% trichloroacetic acid, and fractionated. The peptidoglycan fraction was hydrolyzed in 4 N HCl at 105 C for 14 h and spotted on SG81 paper developed in 95% ethanol-water (70:30, vol/vol). Sample A was cut into 1-cm strips and counted in toluene scintillation fluid. The rest of the chromatogram was developed by ninhydrin. Specific activity (microcuries per micromole): [³H]diaminopimelic acid, 300; D-[¹⁴C]glucosamine, 10. Controls used: 0.01 M L-alanine (1), L-glutamic acid (2), glycine (3), D-glucosamine (4), diaminopimelic acid(s), L-lysine (6), muramic acid (7).*

Release of radioactively labeled fragments during autolysis. Assuming that the autolytic enzyme system responsible for autolysis is hydrolyzing linkages in the peptidoglycan component of the cell wall, it would be expected that fragments would be released from the cell into the lysis buffer. To test this, logarithmic-phase cells grown in SGM were labeled for two generations with [³H]diaminopimelic acid or D-[¹⁴C]glucosamine, harvested, then suspended in fresh medium containing 1 mM unlabeled diaminopimelic acid or D-glucosamine for 10 min to remove any labeled pool material, washed, and finally suspended in 0.05 M Tris-maleate buffer (pH 8.5). The release of counts from the cells into the supernatant was examined as a function of time. Figure 7 shows the increase of radioactive counts into the supernatant fraction and the corresponding decrease in the cell pellet. Over 25% of the total D-[¹⁴C]glucosamine incorporated by the cells was released after 60 min of autolysis, whereas 45% of the total [³H]diaminopimelic acid incorporated was released. This indicates that peptidoglycan fragments are released during autolysis and could provide a method to aid in the determination of the mechanism of autolysis.

DISCUSSION

Autolysins were first observed by Dubos (3) and were initially thought to be suicidase(s). More recently it has been suggested that these enzymes play an important role in deoxyribonucleic acid-mediated transformation (18, 21), sporulation, excretion of macromolecules such as toxins or endotoxins, and cellular growth and division (8). The autolytic processes active in *N. gonorrhoeae* are unknown, although several investigators have made observations on its lytic nature (1, 2). Morse and Bartenstein (11) observed that glucose-limited cultures were susceptible to autolysis, but cultures limited by nitrogen or by the addition of chloramphenicol or rifampin were not susceptible to lysis when kept in the growth medium (pH 7.3 to 6.9). The present study examined the physiological conditions that would provide maximal rate of autolysis. It was found that the pH optimum of the autolysin is broad, with a maximum at pH 9.0. The pH optimum of lysozyme and most other β-*N*-acetylhexosaminidases is below pH 7.0. The pH optimum of *Bacillus subtilis* 168 *N*-acetylmuramyl-L-alanine amidase, one of the more extensively studied autolysins, is pH 9.5 (22). The rate of autolysis was found to be dependent on temperature, with the maximum rate occurring at 40 C. The decline in the rate of autolysis above 40 C is probably attributable to the inactivation of the autolysin.

Potassium ions markedly influenced the rate of autolysis at a concentration of 0.1 M. This effect was also reported in the *B. subtilis* amidase system (22). The presence of high autolytic activity during all stages of growth suggests that the enzyme may have a physiological function involved in both growth of the cell and in turnover of the peptidoglycan. It was shown that radioactively labeled peptidoglycan fragments are released from the cell into the supernatant. Analysis of fragments released during autolysis should provide the mechanism of action of the autolysin. Up to 45% of the incorporated [³H]diaminopimelic acid was released during 60 min of autolysis, suggesting that the autolysin is active over a large portion of the cell surface.

Antibiotics added to logarithmically growing cells markedly influenced the rate of autolysis. Penicillin (10 μg/ml), D-cycloserine (70 μg/ml),

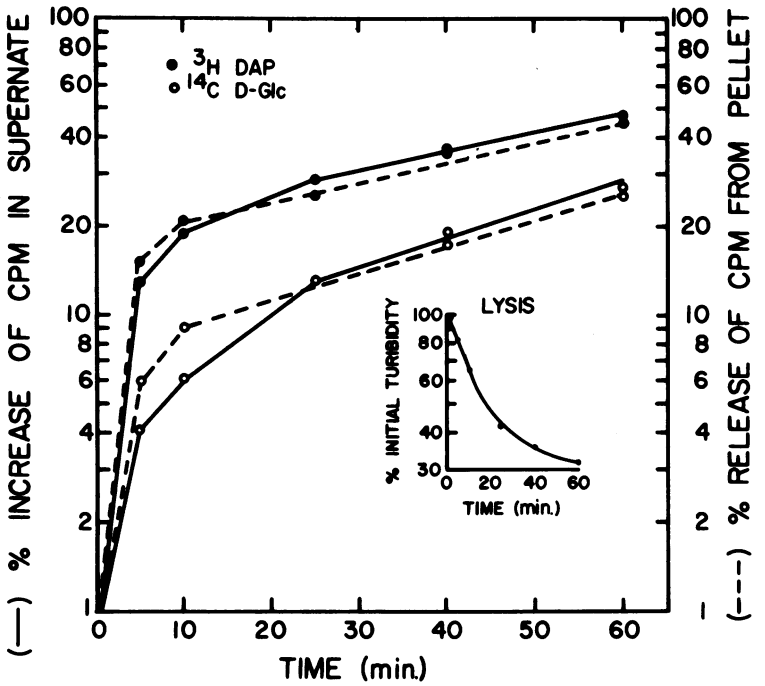

FIG. 7. *Release of labeled fragments during autolysis. Early logarithmic-phase cells were grown in standard growth medium containing 5 μCi of [^{3}H]diaminopimelic acid per ml plus 10^{-3} M lysine, or 2 μCi of D-[^{14}C]glucosamine per ml, for two generations. Cells were harvested, washed, and suspended in fresh growth medium containing 10^{-3} M lysine plus 10^{-3} M diaminopimelic acid, or 10^{-3} M D-glucosamine, for 10 min. The cells were again harvested, washed, and suspended in 0.05 Tris-maleate buffer (pH 8.5). The release of radioactivity from the pellet into the supernatant was then determined.*

and vancomycin (50 μg/ml) added to a growing culture for 45 min greatly increased the sensitivity to lysis. At the MIC level of penicillin (0.01 μg/ml), a linear increase in sensitivity to lysis as a function of time exposure to antibiotics was observed. At twice the MIC level of vancomycin (MIC = 0.5 μg/ml) no increase in sensitivity to lysis was observed. Sensitivity to lysis was seen, however, when 50 μg of vancomycin per ml was used. The high level of vancomycin needed to significantly increase cellular sensitivity to autolysis may explain the effectiveness of this antibiotic in Thayer-Martin medium (17). Thayer-Martin medium is routinely used to isolate and cultivate *N. gonorrhoeae* from clinical isolates. It contains 3 μg of vancomycin per ml to prevent the growth of the gram-positive organisms. Chloramphenicol, on the other hand, rapidly inhibited the rate of autolysis after a 5-min exposure. This effect was also noted by Morse and Bartenstein (11) when growing cultures of *N. gonorrhoeae* were incubated in the presence of chloramphenicol over several hours, and had also been previously observed in *Streptococcus faecalis* and *Staphylococcus aureus* H (6, 8). It has been suggested that chloramphenicol liberates a low-molecular-weight regulator that prevents an increase in a latent form of the lytic enzyme which is, in turn, a precursor of the active form (8).

It was also shown that the primary structure of the peptidoglycan consists of muramic acid, glutamic acid, alanine, diaminopimelic acid, and glucosamine in an approximate molar ratio of 1:1:2:1:1 and can be radioactively labeled by alanine, D-glucosamine, and diaminopimelic acid. The presence of diaminopimelic acid instead of lysine is similar to all other gram-negative organisms thus far examined (10, 12). The ability to label and subsequently isolate the peptidoglycan component will be an invaluable aid in the determination of the nature of the autolytic enzyme.

ACKNOWLEDGMENTS

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