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Autolysis of *Escherichia coli*

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Autolysis of unwashed exponential-phase *Escherichia coli* cells was efficiently promoted by first submitting them to a quick downshock with distilled water before an upshock with 0.5 M sodium acetate, pH 6.5. The association of these two osmotic shocks had a remarkable synergistic effect and led to significant decreases in turbidity and viability. Different factors influencing the rate of cell lysis were examined. A close correlation was established between autolysis and the degradation of peptidoglycan. Both phenomena were induced by the same shock treatment, followed similar kinetics, and were efficiently blocked by addition of divalent cations. Cell lysis was also inducible by a shock treatment with 10^{-3} M ethylenediaminetetraacetic acid or ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid and blocked by the addition of divalent cations.

Surprisingly, the conditions leading to the autolysis of *Escherichia coli* cells have not been extensively studied. Among the different factors influencing the triggering of bacterial autolysis, growth conditions, the osmotic environment, and pH have been recognized as being particularly important (30). By treating exponentially grown cells of *E. coli* B with sodium bicarbonate and sodium acetate, Mohan et al. (25) were able to bring about cell lysis. The rupture of the cell envelope, accompanied by the clearance of the turbid bacterial suspension and the release of intracellular material, is a late step in the presumably multistep autolytic process. It is generally assumed that peptidoglycan, by conferring mechanical resistance to the cell envelope, plays a key role in the osmotic protection of bacteria (9, 10, 28) and that one of the main steps in autolysis is its degradation by specific endogenous hydrolases (15, 30, 34, 36). In *E. coli,* no direct correlation between autolysis and the breakdown of peptidoglycan has yet been established. However, the formation of spheroplasts in osmotically stabilized systems (20, 23, 29) clearly suggested that the degradation of peptidoglycan is a required intermediate step in the autolytic process and that endogenous peptidoglycan hydrolases are an important part of the autolytic system.

Although a set of enzymes hydrolyzing peptidoglycan linkages has been described in *E. coli* (see 33 for references), neither their exact in vivo function nor their participation in autolysis has been defined. Investigation of the autolysis of *E. coli* could help to determine which peptidoglycan hydrolases are involved in the early phases of cell lysis and to understand how changes in environmental conditions trigger the action of these enzymes. Study of their abnormal behavior might provide some insight into the nature of their normal physiological functions and that of the regulatory mechanisms involved in their control. For these reasons, we have undertaken to examine more thoroughly the experimental conditions which promote autolysis in *E. coli* cells and, in particular, the effects of sudden changes in the osmotic environment. Such decreases and increases of osmolality have been referred to as downshocks and upshocks, respectively (2). The results reported here concern the induction of autolysis of exponential-phase cells of *E. coli* by combined downshock and upshock treatments, its control by divalent cations, and its correlation with the degradation of peptidoglycan. Different factors influencing the rate of cell lysis were examined.

MATERIALS AND METHODS

Bacterial strains. Most of the experiments were carried out with *E. coli* K-12 HfrH, which was a gift from J. C. Patte (Institut de Microbiologie, Orsay, France). The other strains of *E. coli* K-12 used were: K335 (DAP^-) and D-09-M-10-20 (DAP^- Lys^-) (kindly provided by J. C. Patte); CE1042 (kindly provided by B Lugtenberg, Department of Microbiology, Utrecht, The Netherlands); and PA3092 and three temperature-sensitive strains derived therefrom (337, 4655, and 4789, kindly provided by Y. Hirota, National Institute of Genetics, Shizuoka-ken, Japan). *E. coli* W7 (DAP^- Lys^-) was a gift from C. Lazdunski (C.B.B.M., Centre National de la Recherche Scientifique, Marseilles, France). *E. coli* B and *E. coli* K235 were American Type Culture Collection strains.

Growth conditions. All the strains used were grown in a rich medium containing, per liter, 10 g of Pastone (Institut Pasteur Production, Paris), 10 g of yeast extract (Institut Pasteur Production), 5.6 g of KH_2PO_4, and 29 g of K_2HPO_4 supplemented with thiamine (0.5 mg/liter), or in minimum medium M63 (24), supplemented with 0.5% glucose and thiamine.

When necessary, 10^{-4} M *meso*-α,α'-diaminopimelic acid (DAP) and 10^{-4} M L-lysine were added. All liquid cultures (40 ml of medium in 200-ml flasks) were incubated at the appropriate temperature in a shaking water bath. Growth was followed by measuring the optical density at 600 nm (OD_{600}) in a Gilford spectrophotometer (model 240). A correlation between the OD values and cell counts was made with exponential-phase cells of *E. coli* K-12 HfrH grown in rich medium. At different intervals of time during growth, 0.05-ml culture samples were added to 10 ml of a 2.5% formaldehyde solution containing 0.85% sodium chloride. After thorough agitation and appropriate dilutions, cells were counted in a Coulter Counter model Fn (Coultronics, Margency 95, France) equipped with a 30-μm-diameter orifice. An OD of 0.4 corresponded to 2.2×10^8 cells/ml.

Autolysis procedures. Cells from a 40-ml culture with an OD of 0.4 were harvested by centrifugation at room temperature (25°C) for 5 min at 12,000 × *g*. After the supernatant fluid was discarded, the inside of the centrifuge tube was wiped carefully with cotton tips to remove as much growth medium as possible. The unwashed pellet was immediately suspended by vigorous agitation at room temperature in 20 ml of distilled water or of the appropriate buffer. When autolysis was induced by combining treatments with water and buffer, cells were first suspended quickly in 10 ml of distilled water, and 15 s later 10 ml of buffer was added. After absorbancy measurements at zero time, the suspension was transferred to a 200-ml flask and placed at the appropriate temperature in a water bath without shaking. Cell lysis was followed by measuring the decrease of turbidity at 600 nm with time. The first-order rate constant of autolysis was calculated as $k = 2.3 \log_{10} (C_0/C_1) \times \min^{-1}$, where C_0 and C_1 were the turbidities at t_0 and t_1.

Viability counts. The number of viable cells was determined by the standard procedure for counting colonies (in triplicate) on agar plates. Each sample (1 ml) taken from autolyzing cells was homogenized in a 2-ml Potter tissue homogenizer, and 0.1-ml portions of appropriate dilutions with M63 medium were spread on agar plates containing 2.5% antibiotic medium M3 (Difco Laboratories, Detroit, Mich.). Plates were incubated for 48 h at 37°C before counting colonies. For an exponentially growing culture of *E. coli* K-12 HfrH (OD = 0.4), viability counts (2.5×10^8/ml) correlated well with the Coulter counts (2.2×10^8/ml).

Measurements of peptidoglycan hydrolysis. Cells of *E. coli* W7 grown at 37°C in 40 ml of rich medium containing 10^{-4} M [^{3}H]DAP (specific activity, 60 mCi/mmol; C.E.A., Saclay, France) were harvested at the outset of the exponential phase (OD = 0.4) and washed once with culture medium containing unlabeled 10^{-4}M DAP. They were then submitted to the double-shock treatment as described before and incubated at 37°C. At different time intervals, 0.5 ml of autolyzing [^{3}H]DAP-labeled cells was treated in one of three ways. (i) Cells were suspended in 0.5 ml of 10% trichloroacetic acid in ice for 1 h. Then 150-μl samples, in triplicate, were centrifuged at 130,000 × *g* for 30 min in a Beckman Airfuge air-driven ultracentrifuge. The pellets were washed once with 100 μl of distilled water. Tritium in pellets and supernatants was converted into tritiated water by combustion in an Oxymat apparatus (Kontron, Velizy 78, France). Tritiated water was trapped in 18 ml of solvent containing 7 g of (biphenylyl-4)-2-(*tert*-butyl-4-phenyl)-5-oxadiazole-1,3,4, 20 g of naphthalene per 700 ml of dioxane, and 300 ml of toluene. Samples were counted by liquid scintillation spectrometry. (ii) Cells were suspended in 0.5 ml of 10% trichloroacetic acid in ice for 1 h, and the suspension was then filtered on a membrane filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.) which was washed abundantly with 5% trichloroacetic acid. Tritium in the peptidoglycan deposited on the filter was converted into tritiated water as above and then counted. (iii) Cells were suspended in 0.5 ml of 8% sodium dodecyl sulfate in water at 100°C for 10 min. After cooling overnight at room temperature, 150-μl samples, in triplicate, were centrifuged at 130,000 × *g* for 30 min as before. The pellet containing the insoluble [^{3}H]DAP peptidoglycan was washed once with 100 μl of distilled water. Tritium in the pellets was then converted into tritiated water and counted as above.

RESULTS

The induction of autolysis in *E. coli* cells by different osmotic shocks was investigated. When unwashed exponential-phase cells of *E. coli* K-12 grown at 37°C in rich medium were rapidly suspended in distilled water (downshock), a 20% decrease in turbidity was observed (Fig. 1). If the shock treatment was performed by suspending cells either in growth medium diluted 10-fold or in 0.05 to 0.2 M sodium acetate, no lysis was observed. For salt concentrations between 0.3 and 0.8 M, autolysis occurred and was optimal at 0.5 M. However, only a 20% decrease in OD was obtained (Fig. 1), and no further significant change was observed even after prolonged incubation (16 h). Shock treatments with higher concentrations of sodium acetate suppressed the ability of cells to autolyze.

Unexpectedly, autolysis of unwashed exponential-phase cells of *E. coli* K-12 was efficiently promoted by first submitting them to a quick water shock (downshock) before the addition of 0.5 M sodium acetate, pH 6.5 (upshock). The association of both shock treatments had a remarkable synergistic effect on both the rate and extent of autolysis as compared with treatment with sodium acetate alone. The initial rate of lysis was increased threefold, whereas turbidity and colony-forming ability greatly decreased (Fig. 1). Concomitantly, UV-absorbing material was released. All of the experiments reported here concern autolyses promoted by the combined effect of water and buffer. Reproducibility and consistency in our results were obtained when all inductions of lysis were performed under the same experimental conditions. However, it should be stressed that the efficiency of this

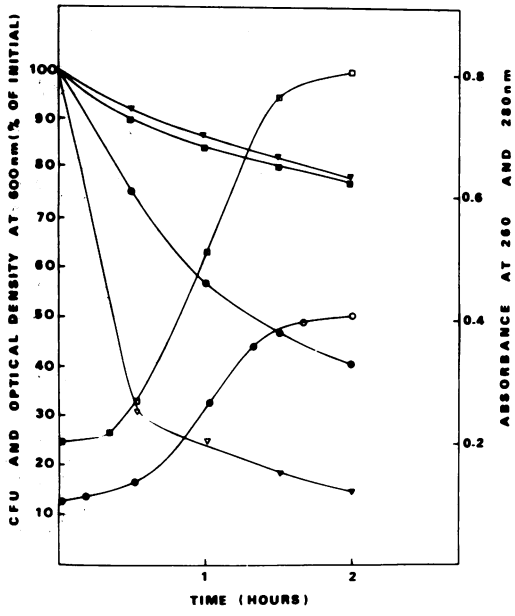

FIG. 1. *Kinetics of autolysis of E. coli K-12 HfrH. Exponential-phase cells were grown at 37°C in rich medium, and autolysis was assayed as loss of OD of the cell suspensions during incubation at 37°C. Autolysis induced by:* (▼) *0.5 M sodium acetate pH 6.5,* (■) *distilled water,* (●) *distilled water and 0.5 M sodium acetate pH 6.5.* (▽) *Loss of colony-forming units;* (□) *absorbancy of supernatants at 260 nm;* (○) *absorbancy at 280 nm. Before measuring absorbancies, samples were first centrifuged at 12,000 × g for 2 min to remove bacteria.*

procedure was to some extent dependent upon how the downshock with water was performed. Although not systematically examined, the rapidity of dispersion of cells in water, the amount of cells, the ratio of water to cell pellet, and the delay between the two shock treatments seemed to influence the intial rate constant of autolysis. Therefore, the k values given in the present paper should not be considered as absolute values.

Effect of pH, buffer concentration, and growth conditions. The initial rate of autolysis was dependent on the pH of the sodium acetate buffer and was optimal at pH 6.5 (Fig. 2). This effect was comparable to that observed by Mohan et al. (25) with *E. coli* B. The concentration of the sodium acetate buffer also markedly influenced the rate of cell lysis, which was optimal at 0.5 M (Fig. 3). A similar low k value was found for buffer concentrations of 0.05 and 1 M (Fig. 3) and corresponded presumably to the sole effect of the water shock, since in the absence of this treatment no detectable lysis was observed at these buffer concentrations. The initial rate of autolysis was over four times greater (k = 9 $\times 10^{-3}$ min^{-1}) with cells grown at 37°C in rich medium than with cells grown in minimal medium ($k = 2.1 \times 10^{-3}$ min^{-1}) (Table 1). Furthermore, it was observed that the rate of cell lysis was influenced by the phase of growth at which bacteria were harvested. Cells in the stationary phase autolyzed slowly, independently of the medium in which they were grown (Table 1). The effect of growth temperature was most pronounced for exponential-phase cells grown in rich medium (Table 1).

Effect of temperature. The initial rate of autolysis was clearly dependent on the temperature at which cells were incubated after induction (Fig. 4). There was no optimum between 20 and 50°C (Fig. 5). Between 20 and 30°C, cell lysis followed first-order kinetics, whereas at 37°C and above, the curves were biphasic (Fig. 4). This seemed to indicate that at the higher temperatures a competition must exist between cell lysis and a certain inactivation of the auto-

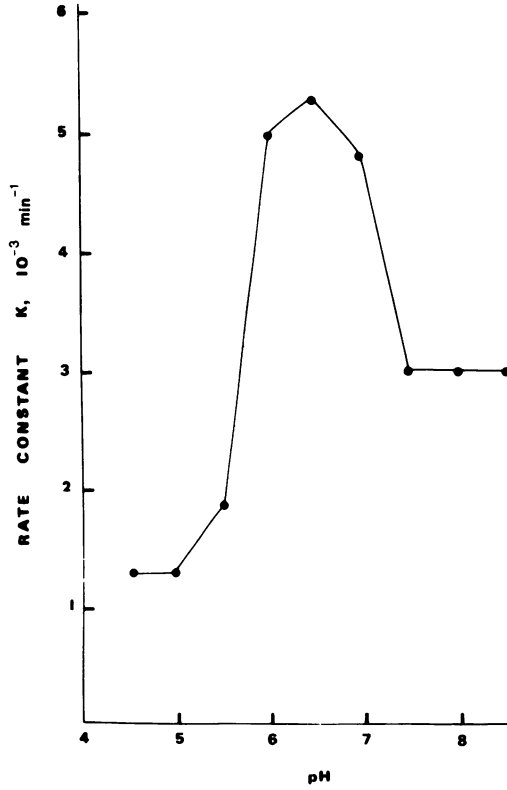

FIG. 2. *Effect of pH on the initial rate of autolysis of E. coli K-12 HfrH. Exponential-phase cells grown at 37°C in rich medium were treated in the usual way with water and 0.5 M sodium acetate of different pH values. Autolysis was assayed at 30°C as described in Material and Methods.*

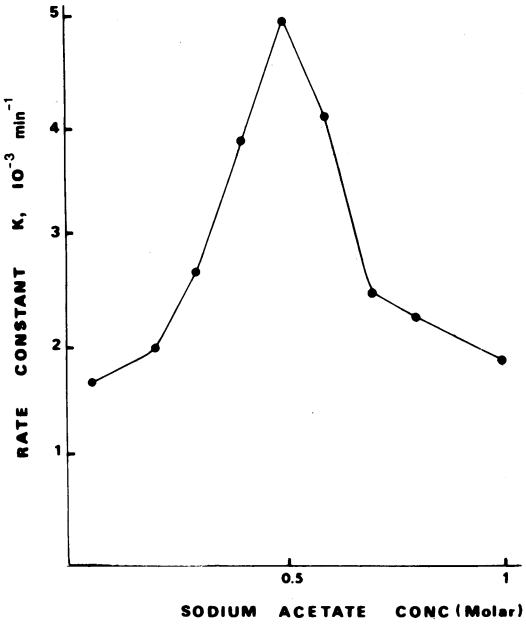

FIG. 3. *Effect of the sodium acetate concentration on the initial rate of autolysis of E. coli K-12 HfrH. Exponential-phase cells grown at 37°C in rich medium were treated in the usual way with water and sodium acetate, pH 6.5, of different concentrations. Autolysis was assayed at 30°C.*

TABLE 1. *Initial rate constants of autolysis of E. coli K-12 HfrH cells grown under different conditions*[a]

Autolysis temp (°C)	Initial rate constant (k, 10^{-3} min^{-1})					
	Exponential-phase cells grown in:				Stationary-phase cells grown in:	
	Rich medium		Minimal medium			
	30°C	37°C	30°C	37°C	Rich medium at 37°C	Minimal medium at 37°C
30	4.8	5.1	1.2	1.6		
37	5.8	9		2.1	1.6	1.1

[a] Autolysis was promoted in the usual way with water and 0.5 M sodium acetate, pH 6.5, and run at either 30 or 37°C.

lytic system. A similar situation has been described at 40°C and above with *Neisseria gonorrhoeae* (7, 13). The rate of autolysis was also influenced by the temperature at which cells were harvested. When cell cultures were cooled down rapidly to 5°C before centrifugation, the rate of autolysis was greatly decreased, even though the subsequent water and buffer treatments were carried out at 25°C. No differences were observed when cell harvesting and the shock treatments promoting autolysis were all performed at room temperature (25°C) as compared with 37°C.

Effect of different ions and chelating agents. The efficiency of potassium, calcium, and magnesium acetates in stimulating the autolysis of water-shocked cells was compared with that of sodium acetate (Fig. 6). The same kinetics were observed with K^+ as with Na^+. However, with Mg^{2+} and Ca^{2+} the rate and extent of autolysis were much lower (Fig. 6). Moreover, autolysis was immediately and totally inhibited when magnesium acetate (final concentration, 50 to 100 mM) was added to autolyzing cells 30 min after induction by the double-shock treatment with water and sodium acetate (final pH of 7.2). Concomitantly, the viability counts remained constant (3×10^8 cells/ml) for at least 2 h after the addition of magnesium acetate, whereas with the noninhibited autolyzing cells they decreased to 10^8 cells/ml during the same period of time. Addition of calcium acetate at the same concentration (50 to 100 mM) gave an

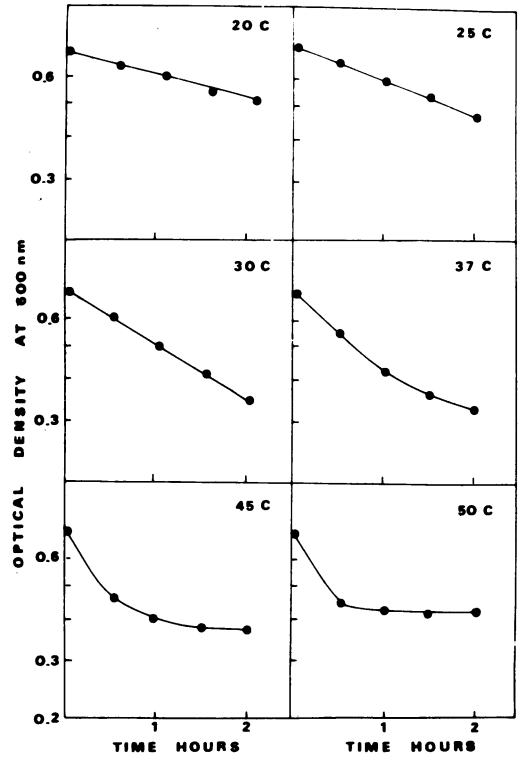

FIG. 4. *Effect of temperature on the kinetics of autolysis of E. coli K-12 HfrH. Exponential-phase cells grown in rich medium at 37°C were treated in the usual way with water and 0.5 M sodium acetate, pH 6.5. Autolysis was monitored at the different temperatures and was expressed as a logarithmic plot of OD versus time.*

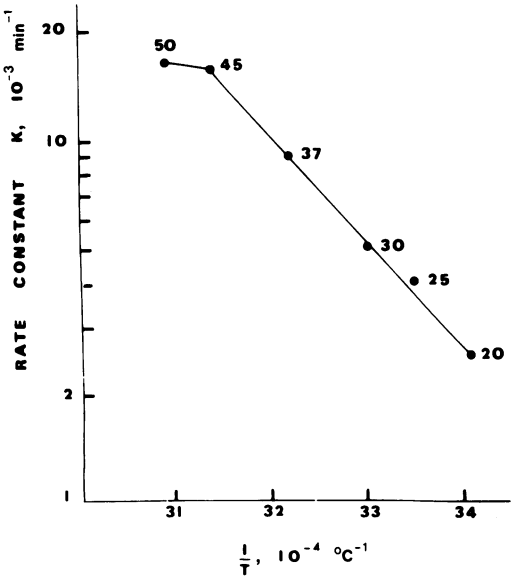

FIG. 5. *Arrhenius plot of the initial rate of autolysis. The conditions are those of Fig. 4. The numbers within the graph represent temperature in degrees centigrade.*

effect similar to that of magnesium acetate, but a lag period of 10 to 15 min was observed before autolysis stopped completely. When *E. coli* K-12 cells were submitted to a downshock with an aqueous solution of 10^{-3} M EDTA (pH 5.8) or to a shock with water followed by the addition of 10^{-3} M (final concentration) EDTA, autolysis was fully induced with the same kinetics as with the combined shock treatments with water and sodium acetate buffer (see Fig. 1). Ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid (EGTA) (10^{-3} M) had the same effect as EDTA. The effect of EDTA could be completely stopped by the addition of 10^{-2} M (final concentration) Mg^{2+} or Ca^{2+} acetate.

Relationship between autolysis and degradation of the peptidoglycan. If there is a close correlation between the degradation of peptidoglycan by specific endogenous hydrolases and cell lysis, it could be expected that peptidoglycan fragments would be released in the course of autolysis. To follow the extent of peptidoglycan degradation, the amounts of soluble and insoluble peptidoglycan material were determined at different times of autolysis. *E. coli* strain W7 was chosen since its peptidoglycan can be readily and specifically labeled with [^{3}H]DAP (42). Moreover, *E. coli* W7 cells autolyzed well (Table 2) when submitted to the double-shock treatment. At different time intervals, samples of autolyzing cells were suspended in cold 5% trichloroacetic acid and were either filtered or centrifuged for 30 min at 130,000 × *g*. Another series of samples was treated with 4% sodium dodecyl sulfate at 100°C and centrifuged for 30 min at 130,000 × *g*. The results (Fig. 7) showed that, at the different time intervals of autolysis considered, the amounts of radioactivity remaining on the filters, in the trichloroacetic acid pellets, or in the sodium dodecyl sulfate pellets were the same. Thus, the labeled peptidoglycan found in the trichloroacetic acid filtrates was not sedimentable at 130,000 × *g* nor precipitable in 4% sodium dodecyl sulfate. The loss of insoluble peptidoglycan material closely paralleled the appearance of soluble material in the supernatant (Fig. 7). Furthermore, the rate ($k = 9 \times 10^{-3}$ min^{-1}) and extent of peptidoglycan

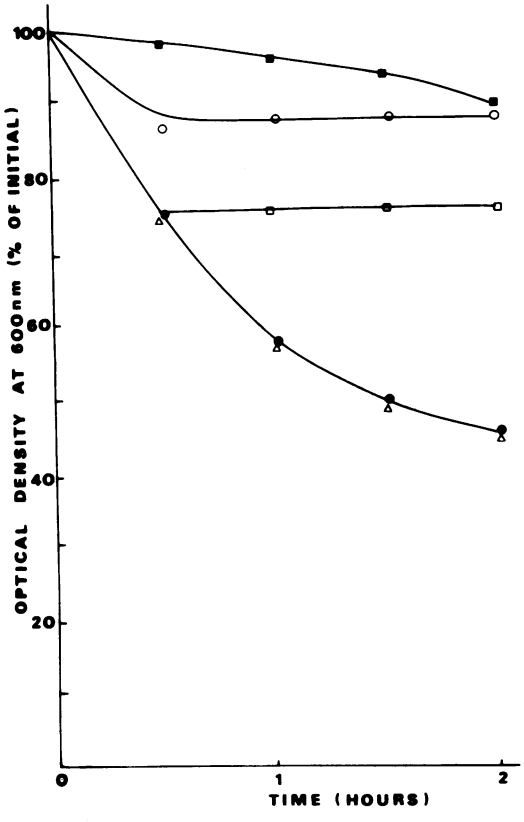

FIG. 6. *Effect of cations on the initial rate of autolysis of E. coli K-12 HfrH. Exponential-phase cells grown in rich medium at 37°C were treated in the usual way with water and the appropriate 0.5 M acetate buffer, pH 6.5. Autolysis was allowed to occur at 37°C. Symbols: (●) Sodium acetate; (△) potassium acetate; (○) calcium acetate; (■) magnesium acetate; (□) addition of solid magnesium acetate or solid calcium acetate (final concentration of 0.1 M) to the cell suspension 30 min after induction of autolysis by treatment with water and 0.5 M sodium acetate, pH 6.5.*

TABLE 2. *Initial rate constants of autolysis of E. coli strains*[a]

E. coli strain	Initial rate constant (k, 10^{-3} min^{-1})
PA3092	3.2
337	5
4655	12.6
4789	8.3
D-09-M-10-20	3.4
CE1042	7.6
K335	2.3
W7	6
K235	2.5
K-12 HfrH	4.8
K-12 HfrH	5.1
B	12.4

[a] Strains were grown in rich medium at 30°C except the last two which were grown at 37°C. After induction in the usual way with water and 0.5 M sodium acetate, pH 6.5, autolyses were all run at 30°C.

degradation were similar to those of the decrease in OD_{600} ($k = 8 \times 10^{-3}$ min^{-1}). The close relationship between autolysis and peptidoglycan degradation was further substantiated by examining the effect of magnesium ions on autolyzing [^{3}H]DAP-labeled cells of *E. coli* W7 (Fig. 7). When magnesium acetate was added at a final concentration of 0.5 M 30 min after induction of autolysis, both autolysis and the degradation of peptidoglycan were immediately blocked.

Comparison of different strains of *E. coli*. The initial rate constants of autolysis of different strains of *E. coli* were determined under the same conditions (Table 2). A fairly large range of k values was encountered. This variability was observed among wild-type strains (K-12 HfrH, B, and K235) and also with isogenetic strains such as PA3092 and 4655, which differed by a 1:4 ratio in their k values. Moreover, no direct correlation was found between the rates of autolysis and the rates of growth of the different strains.

DISCUSSION

In the present paper, different osmotic shocks promoting the autolysis of *E. coli* cells were studied. Conditions leading to cell lysis have been investigated recently in a few gram-negative bacteria (4, 7, 21, 25). Among these, the use of EDTA was reported (6, 7, 27, 34), but as far as we are aware, the association of a downshock with water and an upshock with sodium acetate has not yet been described. The effects of certain factors influencing the rate of lysis of *E. coli* K-12, such as pH, temperature, and buffer concentration, closely resemble those described previously for *E. coli* B (25) and *N. gonorrhoeae* (13). However, it is difficult to make quantitative comparisons between our results and those of Mohan et al. (25), since they washed their cells with 0.2 M sodium bicarbonate before treating them with sodium acetate.

Although little is known about the effects of osmotic shocks on *E. coli*, it has been shown (2, 8, 17) that they can lead to the release of various cations (K^+, Ca^{2+}, Mg^{2+}, etc.) and small components (amino acids, sugars, etc.). Our results suggest that the release of Mg^{2+} or Ca^{2+} may play a key role in the triggering of autolysis. The

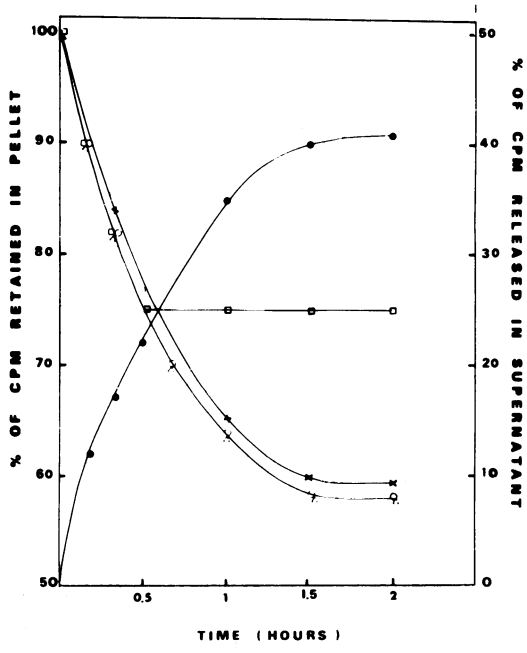

FIG. 7. *Degradation of the peptidoglycan of E. coli during cell lysis. Exponential-phase cells of E. coli W7 were grown at 37°C in rich medium containing 10^{-4} M [^{3}H]DAP (60 mCi/mmol) and washed once with culture medium containing unlabeled DAP (10^{-4} M). Cells were submitted to the double-shock treatment with water and sodium acetate. At different times, samples of autolyzing cells were suspended either in 5% trichloroacetic acid or 4% sodium dodecyl sulfate and centrifuged. The radioactivity present in the trichloroacetic acid pellets (○), in the sodium dodecyl sulfate pellets (△), in the trichloroacetic acid supernatants (●), on the membrane filters (×), and in the trichloroacetic acid pellets after addition of magnesium acetate to autolyzing cells (□) was determined. Results were expressed as percentages of the radioactivity found in the pellets at zero time (11,700 cpm for 0.5-ml samples of cell culture). Owing to the endogenous DAP and peptidoglycan precursors, the amount of radioactivity present in the trichloroacetic acid supernatant at zero time was far from negligible (9,000 cpm for 0.5-ml samples of cell culture). This background value was substracted before calculating the percentage of cpm released in the supernatant at the different times of autolysis considered.*

importance of these cations was stressed by the fact that they could prevent or immediately terminate cell lysis. The commitment to autolysis is thus not irreversible. It should be noted that when Mg^{2+} or Ca^{2+} acetate was added to autolyzing cells, the viable counts remained constant for at least 2 h. Furthermore, autolysis could be efficiently brought about by a rapid downshock with 10^{-3} M EDTA or EGTA and also subsequently blocked by the addition of Mg^{2+} or Ca^{2+}. The stimulation of autolysis by such chelating agents and the inhibiting effect of divalent cations have been observed with other gram-negative bacteria (6, 7, 27, 34). It can therefore be speculated that one of the effects of the different osmotic shocks described here is to remove Mg^{2+} and Ca^{2+} from the cell and that this removal is an essential initial step in the induction of the autolytic process. Preliminary results showed that sodium chloride was far less efficient than sodium or potassium acetate in the upshock. Presumably the acetate anion acts as a weak chelating agent. The synergistic effect of the two osmotic shocks could perhaps be explained by the fact that the initial downshock with water not only removes some divalent cations from the cell, but in some way facilitates the accessibility of acetate ions to critical divalent ion binding sites. An alternative explanation could be that acetate ions prevent divalent cations released by the downshock from being recaptured by the cell.

A close correlation was established between the degradation of peptidoglycan and autolysis. Both phenomena were induced by the same shock treatment, followed similar kinetics, and were efficiently blocked by addition of divalent cations. It is difficult to consider the degradation of peptidoglycan by specific hydrolases and the subsequent cell lysis as normal physiological processes. The existence of peptidoglycan hydrolases has led to the idea that they must play a role in bacterial growth which requires rapid modifications of the peptidoglycan network (10, 11). Thus, under normal growth conditions, there must exist rapid and sensitive regulatory mechanisms that enable these enzymes to carry out their physiological functions without expressing their potentially dangerous deleterious action. It is not yet clear how this discrimination is accomplished in the cell envelope.

If it is assumed that the removal of Mg^{2+} and Ca^{2+} is a key factor in promoting autolysis, it remains to be understood how these cations influence either directly or indirectly the action of peptidoglycan hydrolases. It has been found that there is an optimal Mg^{2+} concentration for the peptidoglycan hydrolases of *E. coli* functioning together in vitro (12). However, since it is unknown which hydrolases are involved in the autolytic process, it is presently impossible to say whether there is any direct in vivo effect of Mg^{2+} or Ca^{2+}. Another possibility is that the control of these hydrolases is dependent on the state of the envelope as a whole and that the removal of divalent cations from the cell induces some disorganization of the envelope, which in turn triggers the uncontrolled action of peptidoglycan hydrolases by a yet unknown mechanism. Different observations partially substantiate this point of view. For instance, the organization of the envelope of *E. coli* is dependent on divalent cations, since treatments with EDTA have been shown to increase the permeability of the outer membrane (19), dissociate lipopolysaccharide (3, 26), and promote the release of lipopolysaccharide (18). Addition of divalent cations reverses the first two effects (3, 19, 26). Another point is that a proper association between the outer membrane and peptidoglycan seems to be a critical factor in the control of the autolytic system (31). In *E. coli* these two structures are closely associated owing to linkages mediated by outer membrane proteins (5). Recently, Hirota et al. (14, 31) have isolated a mutant (*lpo*) lacking the lipoprotein of Braun. This mutant was hypersensitive to EDTA, which caused a very rapid cell lysis. The addition of Mg^{2+} neutralized the effect of EDTA. The importance of proper organization of the cell envelope for the control of peptidoglycan hydrolases has also been stressed by Hartman et al. (12), who investigated conditions promoting the degradation of peptidoglycan independently of cell lysis. It was found that in *E. coli* the peptidoglycan-hydrolyzing system does not act spontaneously, but that it must be induced by more or less drastic treatments. It was concluded from these results that in intact cells, a delicate barrier exists between peptidoglycan and its degrading hydrolases. Its maintenance was said to depend on the presence of divalent cations and on preservation of the integrity of the cell envelope (12).

Recently, important results have been obtained in the study of the control of autolysis in gram-positive bacteria. Specific cell envelope components such as lipoteichoic acids have been found to play a critical role in the regulation of autolysins, and a general scheme for their control has been proposed (32). In *E. coli,* it is still unknown whether any particular envelope component has a similar function or whether the control of autolysins depends more on general properties of the envelope (membrane potential, ionic state of the periplasm, etc.). Undoubtly, further studies of *E. coli* mutants having deficiencies in the cell envelope as well as determi-

nation of the main peptidoglycan hydrolases involved in the autolytic process should provide more information on the mechanism of autolysis. Mutants of *E. coli* with altered peptidoglycan hydrolases have been described (16, 22, 35), but the autolytic process has not yet been investigated in these strains.

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