Studies on Sucrose-Induced Autolysis of Clostridial Cells : Part 2. Properties of Sucrose-Induced Autolysis

Ogata, Seiya
Laboratory of Applied Microbiology, Fuculty of Agriculture, Kyushu University

Choi, Kyoung Ho
Laboratory of Applied Microbiology, Fuculty of Agriculture, Kyushu University

Yoshino, Sadazo
Laboratory of Applied Microbiology, Fuculty of Agriculture, Kyushu University

Hayashida, Shinsaku
Laboratory of Applied Microbiology, Fuculty of Agriculture, Kyushu University

https://doi.org/10.5109/23719

出版情報：九州大学大学院農学研究院紀要. 25 (1), pp.47-59, 1980-08. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 25(1), 47-59 (1980)

Studies on Sucrose-Induced Autolysis of Clostridial Cells

Part 2. Properties of Sucrose-Induced Autolysis

Seiya Ogata, Kyoung Ho Choi*, Sadazo Yoshino and Shinsaku Hayashida

Laboratory of Applied Microbiology, Fuculty of Agriculture, Kyushu University 46-02, Fukuoka 812

(Received February 28, 1980)

Sucrose-induced autolysis of *Clostridium saccharoperbutylacetonicum* (ATCC 13564) was inhibited by various chemical inhibitors, including known enzyme inhibitors (such as heavy metal ions and *p*-chloromercuric benzoate) and fixative agents (such as formalin and glutaraldehyde). Fradiomycin (neomycin) also inhibited the lysis at the concentration of above 50 μg/ml. The organisms whose growth had been inhibited by the antibiotics such as chloramphenicol, were resistant to sucrose-induced autolysis. The lysate produced by sucrose treatment had lytic activity, autolysin activity, on isolated cell wall. During the lysis, autolysin was released from the cells concomitantly with the degradation products of cell wall labelled with ^{14}C-D-alanine. In spite of these release of cell wall-degradation products and autolysin, the cell number and cellular content of macromolecules (DNA, RNA and protein) were hardly decreased. We suggest that sucrose-induced autolysis is due to the action of autolysin and progresses without destruction of protoplasmic membrane.

INTRODUCTION

Bacterial autolysis is a cellular lysis which depends upon the action of autolysin to digest the cell wall. Usually, it was accompanied with the liberation of autolysin and degradation products of cell wall from the cells into the supernatant fluid (Joseph and Shockman, 1974; Heijenoort et al., 1975). In some cases of bacterial autolysis, autolysin active on isolated cell wall (Forsberg and Ward, 1972; Heijenoort et al., 1975; Babara and Park, 1976) or whole cells (Nomura and Hosoda, 1956) were prepared from the lysate after autolysis. Heavy metal ions such as Fe^{2+} and Cu^{2+} (Ogata and Hongo, 1974) and fixatives such as osmium tetroxide and glutaraldehyde (Higgins *et al.*, 1970) were generally regarded as the potent inhibitors on bacterial autolysis. Some antibiotics, especially protein synthesis inhibitors such as tetracycline and chloramphenicol, are also suggested to inhibit autolysis. It is due to inhibition of autolysin synthesis.

Autolysins have been found in various kinds of bacteria. However, their properties are different from each other (Ogata, 1976; Ghuysen, 1976). Thus,

* Present address: Hyou-Sung Women's University, Taeku, Korea.

bacterial autolysis was also different from a bacterium to the other (Ogata, 1976). Sucrose-induced autolysis occurring on the growing cells of clostridia is a specific phenomenon as described in previous papers (Ogata *et al., 1975* a ; *1980 a).* In this paper, some properties of sucrose-induced autolysis of *Clostridium saccharoperbutylacetonicum* are investigated in detail to understand the specific autolysis.

MATERIALS AND METHODS

Bacterial strain

Clostridium saccharoperbutylacetonicum Nl-4 (ATCC 13564) was used throughout this study unless otherwise mentioned.

Medium and cultural conditions

Growth was at 30°C under a reduced atmospheric condition (5–10 mmHg) in TYA medium as described in previous paper (Ogata *et al.,* 1980 a). To obtain a young exponentially growing culture, fresh medium was inoculated with sufficient organisms to produce an initial optical density (OD_{660}) of 0.1 at 660 nm, and unless otherwise mentioned the culture was incubated until its OD_{660} became 0.3 (10^8 cells / ml).

Turbidity measurements

Optical density (OD) of the culture or bacterial suspension was measured at 660nm with a photoelectric colorimeter (model 7A, Tokyo Koden Co. or type-101, Hitachi Co.), as previously described (Ogata *et al., 1980* a).

Induction of sucrose-induced autolysis

In general, the organisms were grown anaerobically at 30°C to an OD_{660} of 0.3. Sucrose was added to this logarithmically growing culture to a final concentration of 0.35 M, incubation was continued at 30°C with or without reduction of the atmospheric pressure, and reading of OD_{660} were made at 10 min intervals.

Antibiotic treatment

Cultures grown anaerobically at 30°C to an OD_{660} of 0.3, were treated for 30 min at 30°C with antibiotics, with the exception of fradiomycin (neomycin). Excess antibiotics were removed by centrifugation (for 5min at 9000 **x g).** The harvested cells were resuspended in prewarmed TYA broth to an OD_{660} of 0.3, and the culture incubated in the usual manner with reading of OD_{660} being made at 30min intervals. A sample of antibiotic-treated culture was withdrawn at a suitable time and exposed to 0.35 M sucrose, changes in its OD_{660} being monitored for 30 min to 60min.

Antibiotics were all in the standard of medical use, and purchased from: actinomycin S, chlortetracycline and fradiomycin (Takeda Chem. Ind. Ltd.), carzinophilin A and mitomycin C (Kyowa Hakko Kogyo Ltd.), chloramphenicol (Sankyo Ltd.), mikamycin A (Kanegafuchi Chem. Ind. Ltd.), penicillin G (Meiji

Seika Ltd.) and rifampicin (Pfizer Taito Ltd.).

Count of the cell number

An aliquot of cell suspension was withdrawn at intervals (0, 30, 60min) during sucrose-induced autolysis. After an immediate fixation of the cells with 5 % (v/v) formalin, the number of intact and protoplast-like cells was repeatedly counted by using a light microscope and a hemocytometer.

Assay of macromolecules

The cells were precipitated with 5 % (v/v) of ice-cold trichloroacetic acid (TCA). After 30min of precipitation, the cells were harvested by centrifugation (3000 x *g* for 15min at 2°C) and their macromolecules were fractionated into DNA, RNA and protein according to the method of Schmidt-Thanhauser-Schneider (STS method) (Mizuno, 1974). The content of DNA was analyzed by the method of Burton, RNA by the method of Mejbaum and protein by the method of Lowry (Mizuno, 1974; Sugawara and Soejima, 1977).

Radiolabelling of cell wall and measurement of the radioactivity

The organisms were cultivated for 3 hr in the presence of 2.5 x 10^{-4} mCi/ml of ^{14}C-D-alanine. A 85% of the radioactivity incorporated into the cell was unextractable with hot TCA (the extraction was carried out for 10 min with boiling TCA). The radioisotope was purchased from Daiichi Radioisotope Lab., Ltd. The labelled cells were autolyzed in buffered sucrose solution after washing once the excess of radioisotope with buffer solution. An aliquot of the cell suspension was withdrawn at suitable intervals during autolysis. The cells were precipitated with ice-cold TCA and removed by centrifugation. Radioactivity in the supernatant fluid was monitored with a scintillation counter (model LS-250, Beckman). Scintillation fluid consisted of 0.6 % (w/v) 2,5-diphenyloxazole (P. P. O., Beckman) dissolved in toluene-methanol.

Preparation of autolysin

Autolysin used in this work was prepared from cultural fluid as follows.

The organisms were cultivated for 4 hr in TYA medium, and then, the cells were removed by centrifugation (10,000 × g for 10 min at 2°C). The enzyme in the supernatant fluid (1.0 liter) was salted twice for 15 hr at 4°C with ammonium sulfate at the concentration of 80 and 40% saturation, respectively. The salted enzyme was harvested by centrifigation (6000 × g for 30min at 2°C), dissolved again in the buffer solution (20 ml) and dialyzed for 40 hr at 4°C against buffer solution (4 liter) while stirring. After elution of the dialyzed enzyme through a column of Sephadex G-75, active fraction of enzyme (10 ml) was recovered and stocked at 0°C until use. This autolysin preparation had an lytic activity of 6 to 10 units/ml against isolated cell wall.

Assay of the autolysin activity

Lytic activity of the autolysin in the above enzyme preparation or the supernatant fluid after autolysis of the cells were also assayed as follows. A reaction mixture for the assay consisted of the enzyme preparation or the

supernatant fluid (0.2 ml) and isolated cell wall (2mg in 0.3 ml of buffer solution). After a suitable time of incubation at 30°C, the mixture was placed in boiling water for 5min to stop the further reaction of autolysin toward cell wall. The mixture was filled up to 1. Oml with buffer solution, and remaining turbidity of the mixture was measured at 660nm. Lytic activity of the autolysin was expressed as units/ml estimated by following formula: $\triangle OD_{660} \times 1000/min$

Preparation of cell wall

Cell wall was prepared from C. *saccharoperbutylacetonicum* according to the method of Ogata *et al.* (1975 b) as follows. The cells were harvested in middle stage of logarithmic growth culture, and washed once with cold distilled water. After disruption of the cells by sonication for 15min using an insonater (model 200 M, Kubota Ltd.), the disrupted cell wall was harvested by centrifugation (10,000 × g for 30min at 2°C). The harvested cell wall immediately suspended in a buffer solution containing 1 % (w/v) sodium dodecylsulfate (SDS), and incubated for 15 hr at 37°C while stirring. After washing out remaining SDS by repeated centrifugation for 5 times, the cell wall was treated with trypsin (0.5 mg/ml) for 4 hr at 37°C, washed with distilled water for 3 to 5 times, and freeze-dried.

Expression of lysis rate

The lysis rate was expressed as the turbidity (OD_{660}) of lytic system at given time against that of zero time, as previously described (Ogata *et al., 1980* a). Besides this lysis rate, a relative lysis rate was also estimated as following formula :

$$[OD_{660}\ (0\ \text{time}) - OD_{660}\ (\text{given time})]/[OD_{660}\ (0\ \text{time})\ \text{of tested system}]$$
$$[OD_{660}\ (0\ \text{time}) - OD_{660}\ (\text{given time})]/[OD_{660}\ (0\ \text{time})\ \text{of control system}]$$

RESULTS

Inhibition of sucrose-induced autolysis

In preliminary experiment, it was found that sucrose-induced autolysis was inhibited by small concentration (10^{-4} M) of Cu^{2+} and formalin (0.1 %). Various known inhibitors of lysis (Ralston *et al.,* 1957; 1961; Doughty and Mann, 1967; Goepfert and Naylor, 1967; Higgins *et al., 1970)* were therefore tested as follows.

1) *Effect of bivalent cations*

Organisms were incubated in buffered sucrose solution containing various bivalent cations. As shown in Table 1, all tested cations showed inhibition of lysis at the tested concentrations. Especially heavy metal ions such as Fe^{*+}, Cu^{2+} and Ag^{2+} were potent inhibitors of sucrose-induced autolysis. Ni^{2+} and Co^{2+} also showed a stronger inhibition than Mg^{2+}, Ca^{2+}, Ba^{2+} and Sr^{2+}. The inhibitory action of bivalent cations on sucrose-induced autolysis may be

Table 1. Inhibition of sucrose-induced autolysis by various bivalent cations. Cells were incubated for 2 hr at 30°C in buffered sucrose solution containing bivalent cations. Lytic activity of the cells was represented as the relative lysis rate against control (none).

Compound	Concentration (M)	Lysis (%)	Compound	Concentration (M)	Lysis (%)
None		100			
$BaCl_2$	1×10^{-2}	45	$CoCl_2$	1×10^{-3}	32
$CaCl_2$	1×10^{-2}	45	$NiSO_4$	1 x10-3	14
$SrCl_2$	1×10^{-2}	52	$FeSO_4$	1×10^{-4}	8
$MgCl_2$	1×10^{-2}	45	$AgCl_2$	1×10^{-4}	0
$MnSO_4$	1×10^{-2}	50			0

Table 2. Inhibition of sucrose-induced autolysis by various enzymatic inhibitors. Cells were subjected to sucrose-induced autolysis in the presence of inhibitors. The concentration of each inhibitor represents the minimum concentration of it for showing a complete inhibition of lysis.

Inhibitors	Concentration
Formalin	0. 1 % (v/v)
Glutaraldehyde	0. 1 % (v/v)
Osmium tetroxide	0. 01 % (w/v)
Uranyl acetate	0. 2 mM
PCMB[1)]	0. 1 mM

1) PCMB: p-chloromercuric benzoate.

based on at least two different actions of them. One may inhibit the action of autolysin as frequently demonstrated by heavy metal ions. The other ac-

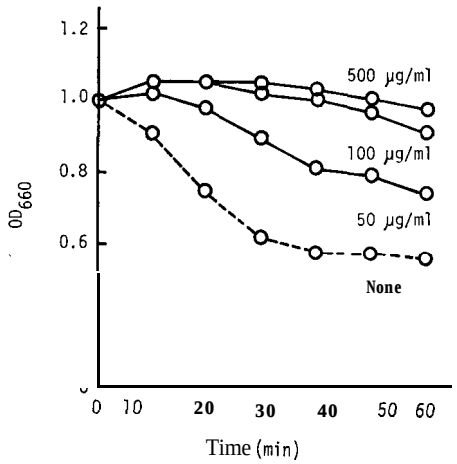

Fig. 1. Inhibitory effect of fradiomycin (neomycin) on sucrose-induced autolysis. Cells were incubated in buffered sucrose solution (0.35 M) containing fradiomycin.

tion may be more closely related to the stabilization of the developed protoplast-like cells than direct inhibition of autolysin activity. The weak inhibition of lysis exhibited by Mg^{2+} and others was probably due to the latter action of them because those ions showed little inhibition on the lysis (digestion) of isolated cell wall by autolysin at the same concentration of them as above experiment. Moreover, it was clear that Mg^{2+} had an effect on stabilization of the protoplast-like cells (Ogata *et al.*, 1980 a; *1980* b).

2) *Lysis inhibition by fixatives and enzymatic inhibitors*

Fixatives such as formalin, glutaraldehyde, osmium tetroxide and uranyl acetate and enzymatic inhibitor such as *p*-chloromercuric benzoate (PCMB) were potent inhibitors of sucrose-induced autolysis, as shown in Table 2. These inhibitors protect the bacteria from autolysis (Higgins *et al., 1970).* The concentrations of fixatives used in this experiments were much lower than their conventional concentrations used for fixation of the bacterial cells in electron microscopic experiments (1% of them is used).

Fradiomycin (neomycin) was demonstrated to inhibit the activities of phage lysins and clostocin 0 lysin (Ogata *et al.*, 1974 b; *1974 c).* As shown in Fig. 1, sucrose-induced autolysis was also inhibited by fradiomycin at the concentration of above 50 μg/ml. This results suggests that fradiomycin inhibits sucrose-induced autolysis without preincubation of bacterial cells. The mode of action of fradiomycin on phage endolysin and others has been described elsewhere (Ogata and Hongo, 1979).

Resistance to sucrose-induced autolysis of antibiotic-treated organisms

Treatment with antibiotics such as chloramphenicol results in rapid decrease in the rate of autolysis (Shockman, 1965; Higgins *et al.*, 1970; Pooley and Shockman, 1970; Stewart and Marmur, 1970). Ogranisms were therefore treated with various kinds of antibiotics (inhibitors on the biosynthesis of DNA, RNA, protein or peptidoglycan). Lysis was performed on the cells pretreated with each antibiotic as described in Materials and Methods.

As shown in Table 3, the cells treated with antibiotics which had their first target on the biosynthesis of DNA, RNA or protein resisted sucrose-induced autolysis, even though resistibility of the cells was different from drug to drug tested. The highest resistibility was evident on the cells treated with protein synthesis inhibitors such as chlortetracycline. On the other hand, peptidoglycan synthesis inhibitors made the cells slightly more sensitive to lysis. The high resistibility of rifampicin-treated cells, distinguishable from the cells treated with other nucleic acid synthesis inhibitors, was possibly linked more closely with the secondary action of it to inhibit the protein synthesis than with the primary action of it to inhibit the RNA synthesis (Sayare *et al., 1972).* Each antibiotic used in this experiment was an enough concentration for showing a heavy inhibition on the growth of C. *saccharoperbutylacetonicum* (Ogata *et al.*, 1974 a).

Further experiment was performed to understand the resistant action of protein synthesis inhibitors on sucrose-induced autolysis in detail. Cells har-

Table 3. Lytic activity of the cells treated with antibiotics. Cells were cultivated for 30min in the presence of antibiotics from when the cultural turbidity reached 0.3. Antibiotic-treated cells were autolyzed for 2 hr in fresh medium containing sucrose. The lysis rate of each antibiotic-treated cells represents the relative lysis rate of them against control (none).

Primary target of antibiotic	Antibiotics	Concentration (μg/ml)	Lysis rate (%)
On the biosynthesis of DNA	None	—	100
	Mitomycin C	1	85
	Carzinophiiin A	5[1]	53
RNA	Rifampicin	1	25
	Actinomycin S	1	82
Protein	Chloramphenicol	10	a
	[illegible]ycline	0.5	16
	Mikamycin A	10[1]	0
			13
Peptidoglycan	Bacitracin	1	103
	Penicillin G	25	107

[1] unit.

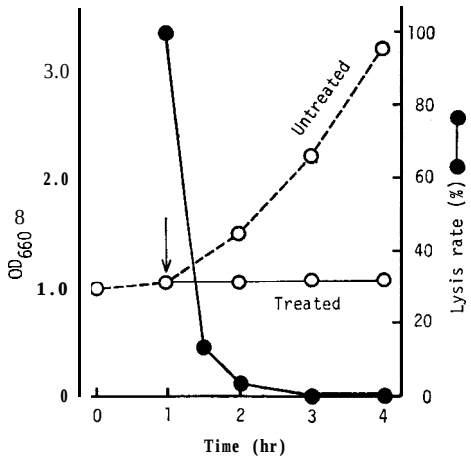

Fig. 2. Resistance to sucrose-induced autolysis of chlortetracycline-treated cells. A 0.5 μg/ml of chlortetracycline (TC) was added to the culture at this time indicated by arrow. Sucrose (0.35 M) was added at various times, changes in OD_{660} being monitored for 30 to 60min. The lysis rate represents the relative lysis rate of TC-treated cells against control (untreated).

vested at different time of chlorteracycline (0.5 μg/ml) treatment were submitted to sucrose-induced autolysis. As shown in Fig. 2, the chlortetracycline-treated cells completely stopped growing about 60min after exposure to the antibiotic, and developed their insensitivity to sucrose-induced autolysis after some 30 min. A similar result was also obtained from chloramphenicol (10 μg/ml)-treated cells, but slow lysis was demonstrable at all times tested. When

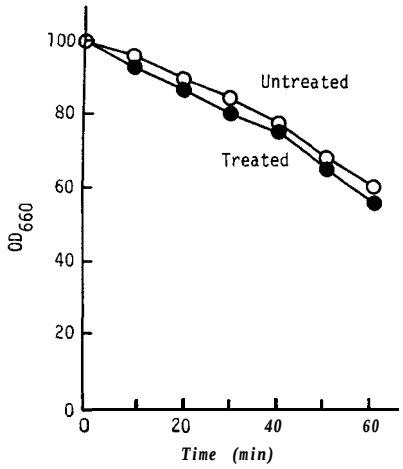

Fig. 3. Digestion of cell wall of chlortetracycline-treated clostridial cells. Cells were cultivated for 2 hr in the presence of 10 μg/ml of chlortetracycline. Their cell wall was isolated and reacted with autolysin preparation.

Table 4. Conservation of cell number and the content of cellular macromolecules. Cells were autolyzed in buffered sucrose-solution after adjustment of the initial turbidity to 0.3. The cell number was counted by using a light microscope and a hemocytometer after fixation of the cells with 5 % formalin. Each macromolecule was fractionated and then its content was determined according to the methods described in Materials and Methods.

Experiment	Time of lysis (min)		
	0	30	60
Decrease in turbidity (%)	0	42	58
Cell number (cells/ml)			
Intact cell	8.9×10^7	0.3×10^8	9.1×10^5
Protoplast-like cell	—[1]	0.9×10^8	1.4×10
Total	8.9×10^7	1.2×10^8	1.4×10^8
macromolecular content (μg/ml)			
DNA	39.6	37.4	37.0
RNA	41.4	39.4	38.9
Protein	82.1	62.3	60.3

[1] No protoplast-like cell was detected.

these antibiotics were directly (without preincubation of the organisms exposing to the drugs) used in sucrose-induced autolysis, they were not chemical inhibitors such as fradiomycin (neomycin). Moreover, they did not inhibit *in vitro-lysis* (digestion) of isolated cell wall by autolysin so far as tested concentrations of them (1-1000 μg/ml). As shown in Fig. 3, there were no significant difference in the progress of digestion of both cell walls isolated from normal cells and chlorteracycline-treated cells.

Thus, sucrose-induced autolysis is inhibited by antibiotics known to impair

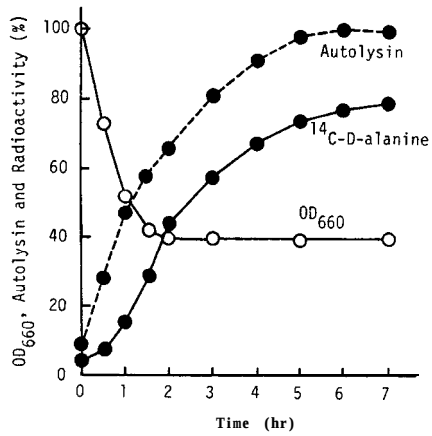

Fig. 4. Release of cellular autolysin and ^{14}C–D–alanine of cell wall during sucrose-induced autolysis. Cells were labelled with ^{14}C–D–alanine. The released radioactivity was represented as the per cent of total radioactivity incorporated into the cells. The autolysin activity was represented as the per cent of maximum activity of enzyme released during lysis.

autolysis. Autolysin system involved in sucrose-induced autolysis probably undergo turnover so rapidly that the ready synthesized autolysin are substituted with newly synthesized autolysin within 30min or so.

Conservation of cell number and cellular macromolecules during sucrose-induced autolysis

The changes of cells in their number and macromolecular content during sucrose-induced autolysis were investigated to know whether or not the lysis progressed destroying inner protoplasmic membrane. As shown in Table 4, more than 99% of the cells were converted into protoplast-like cells after 60 min. In contrast to decrease in the turbidity of lytic system, the number of formed protoplast-like cells showed an increase of 13 % than that of the initial whole cells. The content of macromolecules (DNA, RNA and protein) was almost constant. This result indicates that sucrose-induced autolysis progresses without destruction of protoplasmic membrane.

Release of digestion product of cell wall and cell wall-bound autolysin during sucrose-induced autolysis

As many cases of bacterial autolysis, lytic enzyme (autolysin) active on isolated cell wall was found in the supernatant fluid after sucrose-induced autolysis. Experiment was therefore carried out to investigate the relationship between the digestion of cell wall and release of autolysin bound to it. Cell wall of the organisms was labelled with ^{14}C–D–alanine before exposure to sucrose. Most of ^{14}C–D–alanine incorporated into the cells must be associated with the cell wall: in the bacteria, D-alanine is a specific amino acid of cell wall and a 85% of the activity was found in the unextractable fraction with hot TCA (in bacteria, high polymer component, especially peptidoglycan, is

responsible to the unextractable fraction). As shown in Fig. 4, radioisotope and autolysin were concomitantly released from the cells to the supernatant fluid as soon as lysis occurred. Approximately 77 % of the radioactivity incorporated into the cells was released after 7 hr of lysis. This release of radioactivity indicates that at least 90% of the cell wall was dissolved. However, the per cent of autolysin released was unclear because absolute activity of autolysin in the cells before cellular lysis was unclear.

Another important observation in this experiment was that the concomitant release of radioisotope and autolysin continued for several hours after lysis had stopped. Approximately one half of both the activities were estimated to be released after lysis had stopped. This delay in release of the radioisotope and autolysin may suggest that cell wall is separated from the protoplasmic bodies as some large fragments during sucrose-induced autolysis. Details of this phenomenon will be described in future paper of this series.

The same experiment as above was attempted using N-acetyl-1-^{14}C-D-glucosamine which was also a major component of the cell wall peptidoglycan. This attempt was, however, unsuccessful because the organisms did not incorporate the compound intensively into the cell wall, but they were uniformly labelled.

Effect of autolysin addition on sucrose-induced autolysis

Experiment was carried out to know the effect of added autolysin on the progress of sucrose-induced autolysis. Cells were autolyzed in buffered sucrose solution containing autolysin preparation. As shown in Fig. 5, the lysis was slightly accelerated during the early stage of lysis, while the final lysis rate was hardly affected.

An interesting observation in this experiment was that the cells were

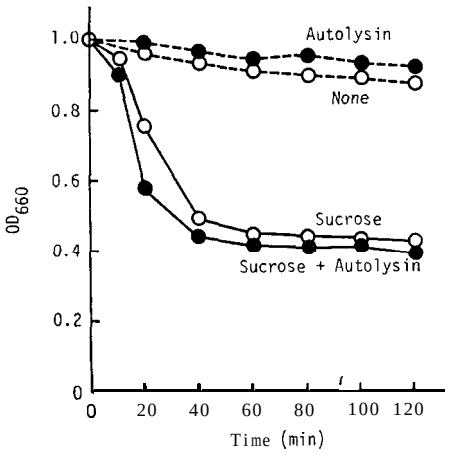

Fig. 5. Acceleration of sucrose-induced *autolysis* by addition of autolysin preparation. Autolysin preparation (1.0 ml) was added to lytic system (100 ml) and then incubation was continued. Heat-treated autolysin preparation *was* used for the control system (none and sucrose).

resistant against the added autolysin when the lytic system did not contained sucrose, but they became sensitive to autolysin in the presence of sucrose. The same result was also observed when an autolysin preparation isolated from the supernatant fluid after sucrose-induced autolysis was used instead of extracellular autolysin of exponentially growing culture. This result suggests that there occur some changes on the cell surface by sucrose treatment which makes the cells to be converted into sensitive phase to the autolysin added. Details of this phenomenon also will be described in future paper of this series.

DISCUSSION

There were indications that sucrose-induced autolysis was enzymatically caused, e. g., it was inhibited by chemicals known to be inhibitors of enzymes and of autolysis. The cultural conditions which favoured lysis were those in which there was marked synthesis of autolysin (Ogata *et al.*, 1980 a). Furthermore, the lysate produced by sucrose treatment had lytic activity against isolated cell wall. Since no lytic enzymes other than autolysin are known to exist in normal growing organisms, our findings suggest that the lysis by sucrose treatment is catalysed by autolysin. Autolysin may play some part in the normal growth of bacteria (Ogata, 1976); action of autolysin on peptidoglycan can cause local weakening of the rigid cell wall and so facilitate of newly synthesized wall material.

Treatment of rapidly growing organisms with antibiotics results in thickening of their cell wall or cell surface (Shockman, 1965; Higgins et *al.*, 1970), rapid loss of their ability to autolyse, and a gradual decrease in their content of the active form of autolysin (Shockman, 1965; Higgins *et al.*, 1970; Pooley and Shockman, 1970; Stewart and Marmur, 1970). At present, we have no precise knowledge of the condition of antibiotic-treated organisms, but it is clear that chlortetracycline or chloramphenicol treatment results in a rapid decrease in the rate of sucrose-induced autolysis. Antibiotic-treated organisms developed their insensitivity to sucrose-induced autolysis before growth completely stopped. The continuing (but weak) senstitivity of chloramphenicol-treated organisms could be due to the continuing synthesis of autolysin and cell wall, since chloramphenicol still permitted growth (Ogata and Hongo, 1974). These results indicate that sucrose-induced autolysis is inhibited by those antibiotics known to inhibit autolysis. It may also be said that the resistance to sucrose-induced autolysis of antibiotic-treated organisms would depend on the decrease in their content of autolysin and the increase in thickness of their cell wall or cell surface. Fradiomycin (neomycin) were specific inhibitors of lytic enzymes of phages of C. *saccharoperbutylacetonicum*, whose properties had reported in greater detail elsewhere (Ogata, 1977; Ogata and Hongo, 1979).

Joseph and Shockman (1974) reported that cellular autolysin began to be released from the cells after a 60 % of the marker (isotope-labelled amino acid) of cell wall of *Streptococcus faecalis* had been released. On the contrary, Coyette and Shockman (1973) reported a concomitant release of autolysin and

digestion of cell wall during autolysis of ***Lactobacillus*** *acidophilus*. The manner of release of digestion products of cell wall and cell wall-bound autolysin may be different with the bacterial strain and their lytic conditions.

From these observations, it may be concluded that the rapid lysis of clostridial cells by the adition of sucrose is due to the action of autolysin. The role of sucrose and autolysin on sucrose-induced autolysis will be reported in detail in future papers. However, it is now said that sucrose treatment may result in the artificial conversion of clostridial cells from nonsensitive to sensitive phase to autolysin, because the resistance of clostridial cells against supplemented autolysin preparation were changed to be sensitive in the presence of sucrose (Fig. 5).

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in Aid of Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Babara. B. and J. T. Park 1976 Activity of three murein hydrolases during the cell division cycle of *Esherichia coli* K-12 as measured in Toluene-treated cells. *J. Bacteriol.*, *126*: *1250-1260*

Coyette. Y. and G. D. Shockman 1973 Some properties of the autolytic N-acetylmuramidase of *Lactobacillus acidophilus*. *J. Bacteriol.*, 114: *34-41*

Doughty, C. C. and J. A. Mann 1967 Purification and properties of a bacteriophage-induced cell wall peptidase from *Staphylococcus aureus*. *J. Bacteriol.*, *93: 1089-1095*

Forsberg, C. W. and J. B. Ward 1972 N-acetylmuramyl-L-alanine amidase of *Bacillus licheniformis* and its L-form. *J. Bacterial.*, 110: 878-888

Ghyusen, J. M. 1976 Use of bacteriolytic enzymes in determination of cell wall structures and their role in cell metabolism. *Bacteriol. Rev., 32: 507-518*

Goepfert, J. M. and H. B. Naylor 1967 Characteristics of a lytic enzyme induced by bacteriophage infection of *Micrococcus lysodeikticus*. *J. Virol.*, 1 : 701-710

Heijenoort, J. V., C. Parquet, H. Huluouret and Y. V. Heijenoort 1975 Envelope-boud N-acetylmuramyl-L-alanine amidase of *Esherichia coli K-12* : Purification and properties of the enzyme. *Eur. J. Biochem., 58:* 611-619

Higgins, M. L., H. M. Pooley and G. D. Shockman 1970 Site of initiation of cellular autolysis in *Streptococcus faecalis* as seen by electron microscopy. *J. Bacteriol., 103: 504-514*

Joseph, R. and G. D. Shockman 1974 Autolytic formation of protoplasts (autoplasts) of *Streptococcus faecalis 9790:* Release of cell wall, autolysin and formation of stable autoplasts. *J. Bacteriol., 118: 735-746*

Mizuno, S. 1974 *Kakusan no Ippan-teki Bunri-teiryo Ho (General Methods for Isolation and Determination of Nucleic Acids).* Tokyo Univ. Press, Tokyo, pp. 19-56 (Japanese)

Nomura, N. and J. Hosoda 1956 Nature of the primary action of the autolysin of *Bacillus subtilis*. *J. Bacteriol.*, **72**: 573-581

Ogata, S. 1976 Autolysis, autolysin, autoplast. *J. Agric. Chem. Soc.* Japan, 50: R69-R77 (Japanese)

Ogata, S. 1977 Bacteriophage no yokin koso (Lytic enzymes of bacteriophages). *In* "Yokin Koso (Lytic Enzyme)," ed. by M. Funatsu and D. Tsuru, Kodansha Scientific, To-

kyo. pp. 129-151 (Japanese)

Ogata, S., K. H. Choi and M. Hongo 1975a Sucrose-induced autolysin and development of protoplast-like cells of ***Clostridium saccharoperbutylacetonicum. Agric. Biol. Chem., 39: 1247-1254***

Ogata, S., K. H. Choi, M. Hongo and S. Hayashida 1980a Studies on sucrose-induced autolysis of clostridial cell Part 1. Induction of rapid bacterial autolysis by sucrose treatment. *J. Fac. Agr.*, ***Kyushu Univ., 25: 33-45***

Ogata, S., K. H. Choi, M. Hongo and S. Hayashida 1980b Studies on sucrose-induced autolysis of clostridial cell Part 4. Properties of protoplasts produced by sucrose-induced autolysis. *J. Fac.* ***Agr., Kyushu Univ.,*** in preparation

Ogata, S., K. H. Choi, Y. Ikeda and M. Hongo 1974a Bacterial lysis of ***Clostridium*** species III. Properties of premature lysis provoked by antibiotics in mitomycin C-induced lysis of ***Clostridium saccharoperbutylaceionicum. J. Gen. Appl.*** *Microbicl.*, ***20: 153-168***

Ogata, S. and M. Hongo 1974 Lysis induced by sodium ion and its relation to lytic enzyme systems in ***Clostridium saccharoperbutylacetonicum. J. Gen.*** *Microbiol.*, ***81: 315-323***

Ogata, S. and M. Hongo 1979 Bacteriophages of the genus ***Clostridium. In*** "Advance in Applied Microbiology," Vol. 25, ed. by D. Perlman, Academic Press, New York, pp. 241-273

Ogata, S., Y. Tahara and M. Hongo 1975b Chemical composition of cell wall peptidoglycan from ***Clostridium saccharoperbutylacetonicum*** studied with phage endolysins and gas chromatography. ***J. Gen. Appl.*** *Microbiol.*, ***21: 65-74***

Ogata, S., A. Umeda and M. Hongo 1974b Bacterial lysis of ***Clostridium*** species IV. Fradiomycin and some other antibiotics are specific inhibitors against endolysin of an induced bacteriocin. ***J. Gen.*** *Appl. Microbiol.*, ***20:*** 169-177

Ogata. S., A. Umeda and M. Hongo 1974c Bacterial lysis of ***Clostridium*** species Part 5. Effect of fradiomycin on the activities of various lytic enzymes active toward *Clostridium* ***saccharoperbutylacetonicum. J.*** *Fac.* ***Agr., Kyushu Univ., 19: 49-59***

Pooley. H. M. and G. D. Shockman 1970 Relationship between the location of autolysin, cell wall synthesis, and the development of resistance to cellular autolysis in ***Streptococcus faecalis*** after inhibition of protein synthesis. ***J.*** *Bacteriol.*, ***103: 457-466***

Ralston, D. J.. B. Baer, M. Lieberman and A. P. Krueger 1961 Virolysin, a virus-induced lysin: its appearance and function in phage-infected staphylococci. ***J. Gen.*** *Microbiol.*, ***24: 313-325***

Ralston, D. J., M. Lieberman, B. Baer and A. P. Krueger 1957 Staphylococcal virolysin, a phage-induced lysin: its differentiation from autolysin of normal cells. ***J. Gen.*** *Physioz.*, ***40:*** 791-807

Sayare, M., L. Daneo-Moore and G. D. Shockman 1972 Influence of macromolecular biosynthesis on cellular autolysis in ***Streptococcus faecalis. J.*** *Bacteriol.*, ***112: 337-344***

Shockman, G. D. 1965 Symposium on the fine structure and replication of bacterial and their parts IV. Unbalanced cell-wall synthesis: autolysis and cell-wall thickening. *Bacteriol.* ***Rev., 29: 345-358***

Stewart, C. R. and J. Marmur 1970 Increase in lytic activity in competent cells of ***Bacillus subtilis*** after uptake of deoxyribonucleic acid. ***J.*** *Bacteriol.*, 101: 449-455

Sugawara, K. and M. Soejima 1977 ***Tanpakushitsu no Teiryo Ho (Methods for Protein*** *Analysis*). Tokyo Univ. Press, Tokyo, pp. 14-15, pp. 95-131 (Japanese)