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BORIS ROTMAN

Institute for Enzyme Research and the Department of Genetics, University of Wisconsin, Madison, Wisconsin

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A common method of preparing bacterial extracts is to expose cell suspensions to sonic vibrations. The mechanism of the lysis of bacteria so treated has not been systematically investigated.

A study of bacteria treated under various conditions has shown that two steps are involved: (1) structural damage to the cells and (2) lysis. Although these steps succeed each other rapidly under common conditions, they can be resolved by treatments which inhibit lysis, namely exposure at low pH, low ionic strength or heat shock.

MATERIALS AND METHODS

Preparation of sonic treated cells. Strain K-12 of *Escherichia coli* and strain 0 of *Azotobacter vinelandii* were used. *E. coli* was grown in a chemostat (Rotman, 1955) using Davis mineral medium (Davis, 1949) with 0.1 per cent glycerol as source of carbon. *A. vinelandii* was grown in Burk's medium (Wilson and Knight, 1952).

Fifty-milliliter cultures, containing 10^9 cells per ml, were centrifuged and resuspended in distilled water to eliminate the excess of salts prior to removal of the salts by two rapid passages through a column 1 by 5 cm of Amberlite MB-3, a mixed bed ion exchange resin. The rate of flow of the column was limited only by the size of the resin. Just before passing the suspension the column was washed with cold distilled water. Desalting by resin was found more practical than washing by centrifugation because *E. coli* cells after a few washes with distilled water fail to pack well in the pellet. After passing through the resin the cells pack easily during centrifugation. This method leaves only traces of Na or K and viable cells are recovered quantitatively. The suspensions were then treated for 10 min in a refrigerated Raytheon 10-kc magnetostrictive oscillator. The cells were twice spun down at 14,000 × G for 3 min and resuspended in distilled water. The temperature throughout the whole procedure was kept below 4 C. The pH of the final suspension was about 6.6.

Suspensions which were not freed from salts by passage through the ion exchange resin underwent complete lysis during vibration. To reduce lysis to an undetectable value, the pH of the deionized suspension (50 ml) was adjusted to about 4.0 with 0.2 ml M/20 citric acid before sonic treatment. With citric acid the pH of the final sonic treated suspension after two washes was 6.1 to 6.3.

Test Method. The lysis of the cell suspensions was followed at 25 C with a Beckman DU spectrophotometer at 620 mμ in cuvettes with 1-cm light path. To 2.99 ml of cell suspension, 0.010 ml of test solution was added by means of a small glass rod. Readings were taken at 20-sec intervals and the lysis was practically completed in less than 1 min.

Leakage of intracellular material was determined by measuring the optical density at 260 and 280 mμ of the solution following centrifugation.

Viable counts were made in triplicate on spread plates of nutrient agar (Difco), with citric acid added to adjust the pH. Tetra-ethyl-pyrophosphate was kindly supplied by Dr. J. E. Casida.

EXPERIMENTAL RESULTS

Suspensions of *E. coli* and *A. vinelandii* in neutral buffer lyse completely after 5 min in the oscillator, but no significant lysis or leakage is detected after 10 min vibration if the initial pH is about 4.0.

The sonic treated cells, which look normal under the light microscope, can remain "intact"

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[2] Present address: Instituto de Química Fisiológica, Borgoño 1470, Escuela de Medicina, Santiago, Chile.

for several hours at room temperature and for days in the refrigerator.

Figures 1 and 2 are electron photomicrographs of cells before and after sonic treatment. In figure 2, severe damage to the surface of the sonic treated cells is apparent. In good agreement with the retention of intracellular material as determined photometrically, torn cells and even broken pieces of cells remain electron-dense.

When the suspension of sonic treated cells is neutralized by adding a small volume of buffer, a dramatic lysis occurs in a fraction of a second. Acetate, histidine, maleate, and phosphate buffers with either calcium, potassium or sodium

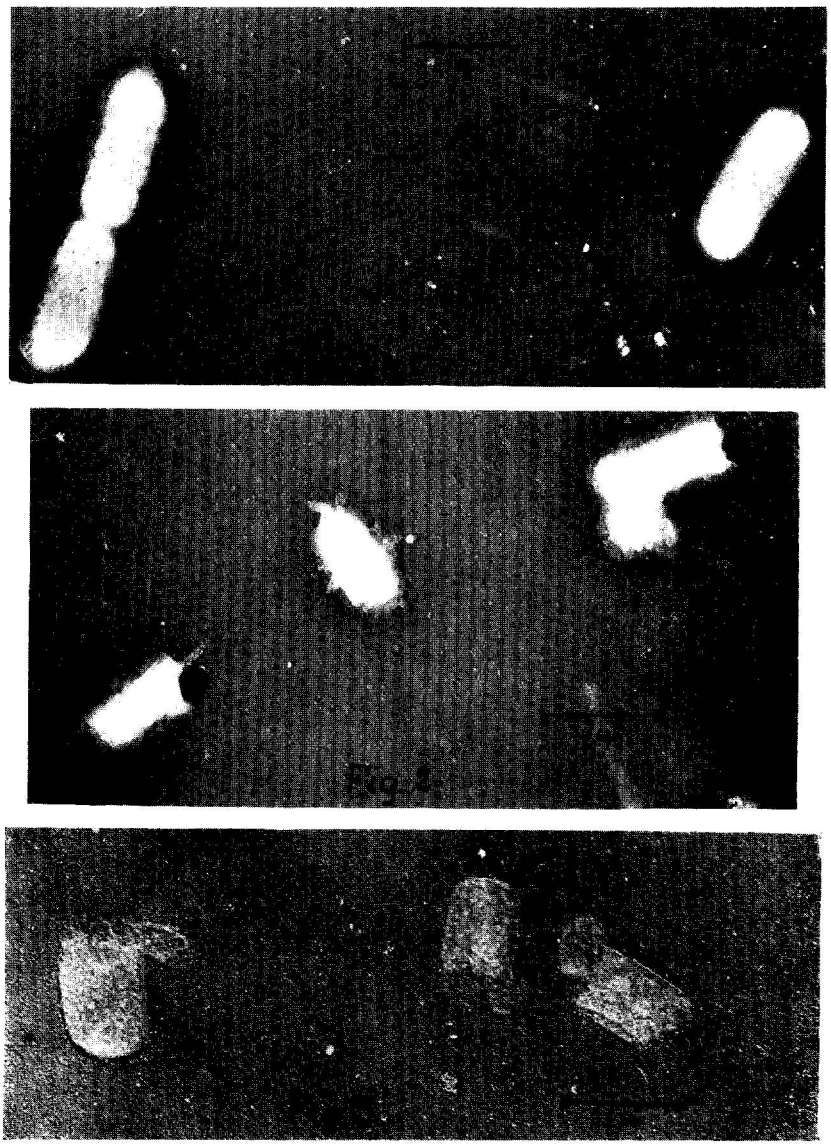

Figure 1. Normal cells of *Escherichia coli* prior to sonic oscillation.*
Figure 2. Cells after 10 min sonic oscillation.*
Figure 3. Same as figure 2 but with addition of phosphate buffer pH 7.2 to M/3,000 final concentration.*

* Uranium shadowing. Electron photomicrographs courtesy of Dr. Paul J. Kaesberg.

TABLE 1

Viability of sonic-treated cells

Optical density before lysis = 2.10
Optical density after lysis = 0.286

	pH of Agar	Viable Cells/ml
Before sonic vibration.....	7.0	1.0×10^{10}
Before sonic vibration.....	5.6	7.8×10^{9}
After sonic vibration.....	7.0	4.3×10^{4}
After sonic vibration.....	5.6	2.7×10^{5}

TABLE 2

pH effect: 0.01 ml of M/2 *Na-PO$_4$ buffer was added to 2.99 ml of sonic-treated cells of optical density (O.D.)*
O.D. = 0.635

pH of Buffer	O.D. after 1 min	Final pH
No buffer added	0.635	6.25
4.75	0.640	5.8
5.5	0.585	6.2
6.0	0.480	6.6
6.5	0.308	7.1
7.25	0.275*	7.65
7.7	0.277*	7.95
8.2	0.271*	8.15
N/2 NaCl	0.540	—
20% glucose	0.635	—

* No intact cells were found in the lysates by microscopic examination.

have been used with similar effects. Only ghosts (as depicted in figure 3) were found by microscopic examination after lysis of the suspension. No intact cells were seen. The viability of sonic treated cells, if plated without addition of buffer, i. e., prior to lysis, was about 5-fold greater on nutrient agar at pH 5.6 than at pH 7.0, as shown in table 1.

The results obtained with deionized cells with or without addition of citric acid were similar. The only detectable difference was that extensive lysis occurred during vibration in the absence of citric acid and therefore the yield of "intact" cells was greatly reduced.

Table 2 shows that lysis is inhibited at pH values below 6. Nevertheless, pH alone does not determine lysis but a certain ionic concentration seems to be required. This is clear from comparison of lines 1 and 3 of table 2. In contrast to cells without buffer, cells with buffer

TABLE 3

Lysis inhibition

Assay by addition of 0.01 ml of M/2 K-PO$_4$, pH 72 to 0.99 ml of sonic-treated cells

Experiment	Treatment	O. D.* after 1 Min
A	Initial O.D. = 0.549	
	No treatment	0.270
	4 min at 70 C	0.460
	8 min at 70 C	0.477
	12 min at 70 C	0.520
B	Initial O.D. = 0.850	
	No treatment	0.430
	7 min at 30 C in 7×10^{-3} M TEPP (pH 5.5)	0.370
	240 min at 30 C in 7×10^{-3} M TEPP (pH 5.5)	0.875
	240 min at 30 C	0.475
	240 min at 30 C in M/50 HCl	0.360
	30 min in M/1000 NaN_3	0.430
	5 min in 0.1% TCA at 25 C	0.425
C	When buffer was added to a mixture 1:1 of cells without heat treatment and cells held 8 min at 70 C, the O.D. after lysis was 0.375. The calculated value assuming no lysis of the latter ones is 0.365.	

* Optical density.

pH 5.5 show a degree of lysis in spite of the similar pH attained by both suspensions at the end of the experiment. From line 9 of table 2 it can be inferred that neutral salts alone do not cause lysis.

Lysis inhibition by heat treatment and other agents is described in table 3. Even storage in the refrigerator for several days caused some inhibition. Among the chemicals only tetraethyl-pyrophosphate (TEPP) was found to be inhibitory at relatively high concentrations compared with those used for proteolytic enzymes (Jansen *et al.*, 1951). Metabolic inhibitors like azide, cyanide, arsenic or dinitrophenol and protein denaturants such as trichloroacetic acid, mercuric chloride or hydrochloric acid did not inhibit lysis. Glucose, sucrose and lactose did not inhibit nor cause lysis even at high concentrations (10 per cent).

Experiments were conducted to determine whether heat shock inhibits an enzymatic lytic reaction. Sonic-treated cells were mixed with an

equal volume of the same suspension which had been submitted to heat shock after vibration. As indicated in table 3, no lysis of the heat-treated cells was observed when buffer was added to the mixture. Analogous experiments with cells treated with benzene, lysozyme, or heat gave identical results.

Experiments with *A. vinelandii* did not differ from those with *E. coli*.

DISCUSSION

The primary action of sonic vibrations appears to be damage of the cell structure most clearly demonstrated by the appearance of the cell wall under the electron microscope.

The question left open by the experiments described here concerns the nature of the lysis following sonic treatment. Two alternatives were considered but none could be excluded:

(1) The lysis is enzymatic in nature. The sonic vibrations would put the digestive enzymes in contact with their substrates by breakage of physical barriers or would permit the normally inactive enzymes to be reached by activators or to release bound inhibitors.

(2) The lysis occurs by dissolution of the cytoplasm. The sonic vibrations would destroy the physical barriers between the cytoplasm and the medium. The cytoplasm would undergo a gel-sol transition after contact with salts at neutral pH.

The enzymatic hypothesis is supported by the following: (a) Lysis does not occur at pH values below 6.0; (b) Heat treatment inhibits lysis; (c) Incubation with 7×10^{-3} M TEPP inhibits lysis; (d) It is known that ribonuclease and autolytic enzymes become active upon destruction of the cell (Manson, 1953); (e) The lysis described here resembles the Nakamura effect with lysozyme as modified by Grula and Hartsell (1954). In their experiments, cells previously treated with lysozyme were lysed when the pH was raised to about 10 with NaOH.

On the other hand, the fact that under no conditions could bacteriolytic activity of the lysates be demonstrated does not sustain the enzymatic hypothesis unless one postulates that the bacteriolytic enzyme(s) has to act from within or that it becomes extremely labile under extracellular conditions.

To accept that dissolution of the coagulated cytoplasm is the cause of lysis requires prior explanation of how broken cells retain their cytoplasm even at pH values of 6.6 or in the presence of M/300 NaCl. The possibility that the cytoplasm is gelated under these conditions cannot be excluded.

A certain proportion of the sonic treated cells can give rise to clones if plated under conditions which inhibit lysis, i. e., nutrient agar at pH 5.6. This could be interpreted as recovery from sonic injury, although the type of injury could be entirely different from that suffered by the cells shown in figure 2.

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SUMMARY

Bacterial suspensions (*Escherichia coli*, *Azotobacter vinelandii*) do not lyse under sonic vibrations when suspended in medium of low ionic strength or at low pH. After sonic treatment, lysis can be brought about by adding neutral buffer. Sonic vibrations are shown to cause structural damage to the cell but no leakage of intracellular material occurs under the conditions described. The nature of the lytic process following vibration is discussed in terms of enzymatic lysis or of solation of the cytoplasm.

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