LYSIS OF SPHEROPLASTS OF *ESCHERICHIA COLI* BY A NON-IONIC DETERGENT

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Rod-shaped cells of *Escherichia coli* B can be converted into spheroplasts by the action of lysozyme on plasmolyzed cells (Birdsell and Cota-Robles, 1967). In such spheroplasts the L membrane component of the cell wall is intact and thus remains the outermost barrier of the cell. Upon the addition of EDTA the L membrane ruptures exposing large areas of the cytoplasmic membrane to the environment. The marked but incomplete separation of coils of the L membrane and the cytoplasmic membrane in EDTA-lysozyme spheroplasts suggested that it might be possible to separate the two membrane fractions so that they could be studied individually. A non-ionic detergent, Brij 58, was found which brings about rapid and extensive lysis of spheroplasts only if the cytoplasmic membrane is exposed as it is in EDTA-lysozyme spheroplasts. This detergent is unable to induce lysis of normal cells, washed cells, plasmolyzed cells or lysozyme spheroplasts. Since completion of this work Godson and Sinsheimer (1968) have described the lysis of *E. coli* with Brij 58 utilizing concentrations of the detergent much greater than those used in this investigation.

The purpose of this paper is to demonstrate the apparent specificity of the detergent, Brij 58, for the cytoplasmic membrane of *E. coli* and to compare the effect of Brij 58 with that of other detergents in terms of patterns of lysis and effects upon membrane-bound enzyme systems.

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Materials and Methods

The conditions for growth and spheroplast formation were the same as previously described (Birdsell and Cota-Robles, 1967). Brij 58 (Atlas Chemical Industries, Wilmington, Delaware), Triton X-100 (Rohm and Haas, Philadelphia, Penn.), sodium deoxycholate (Nutritional Biochemicals Corp., Cleveland, Ohio), and Duponal C (sodium lauryl sulfate, E. I. DuPont DeNemours and Co., Wilmington, Delaware) were dissolved in distilled water and added to cell suspensions at the concentrations noted. Lysis was scored by measuring the decrease in turbidity at 600 mμ in a Beckman DB-G recording spectrophotometer and monitored by phase microscopy. Enzymatic activity was measured either spectrophotometrically ($NADH_2$ oxidase) or polarographically ($NADH_2$ oxidase, succinoxidase, and formic oxidase). Protein concentration was estimated by the method of Lowry *et al.* (1951).

Results

The effect of the non-ionic detergent Brij 58 at a concentration of 30 μg/ml on all stages of spheroplast production is shown in Fig. 1. Regardless of the sequence of addition of detergent and EDTA neither normal cells nor washed cells were affected. Alone Brij 58 has no effect on plasmolyzed cells. The addition of EDTA to plasmolyzed cells in the presence of detergent resulted in a slow decrease in turbidity. The addition of EDTA to lysozyme spheroplast suspensions produced the rapid conversion to EDTA-lysozyme spheroplasts with concomitant lysis by Brij 58. Adding EDTA to lysozyme spheroplasts effects a rupture in the L membrane component of the cell wall. Such spheroplasts are markedly susceptible to detergent lysis. It is clear that EDTA-lysozyme spheroplasts show a unique sensitivity to Brij 58. As previously noted, in this type of spheroplast the L membrane component of the cell wall is broken and the cytoplasmic membrane exposed to the environment. These results strongly suggest that Brij 58 induces lysis only when the cytoplasmic membrane is exposed. They further suggest that the

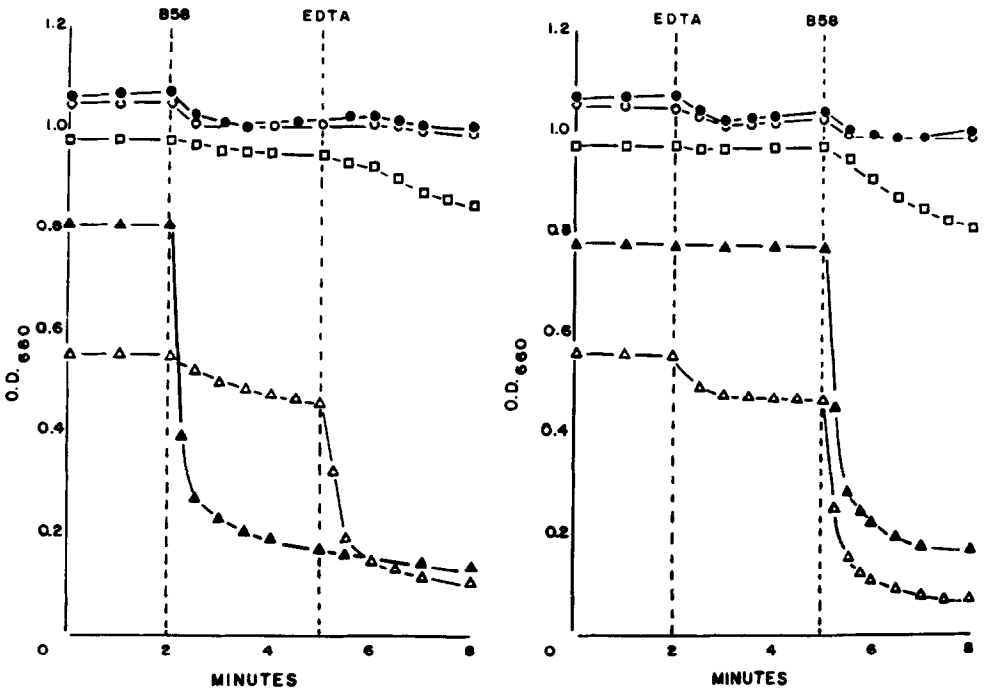

Fig. 1. Effect of Brij 58 and EDTA on all stages of spheroplast formation. Cell suspensions were prepared as described in the Materials and Methods and held in an ice bath until used. At the times indicated Brij 58 was added to a final concentration of 30 µg/ml and EDTA to a final concentration of 10^{-3} M. Symbols: ● , normal cells; ○ , washed 1X; □ , plasmolyzed; △ , lysozyme spheroplasts; ▲ , EDTA-lysozyme spheroplasts.

intact cell wall or the L membrane component of the cell wall protects the cell and/or spheroplast from Brij 58 induced lysis. Phase and electron microscopic examination of detergent lysates revealed the absence of spherical ghosts characteristically found in lysates produced by osmotic shock (Birdsell and Cota-Robles, 1967). Fig. 2a and 2b again emphasize that the susceptibility of *E. coli* to detergent lysis requires treatment with EDTA.

Four detergents, Brij 58, sodium deoxycholate (DOC), Triton X-100, and Duponal, were examined for their ability to induce lysis of cell suspensions taken from all stages of spheroplast formation. Normal cells continued to grow in the presence of any of the four detergents. Similarly, washed cells were unaffected by the four detergents. Plasmolysis rendered the cells sus-

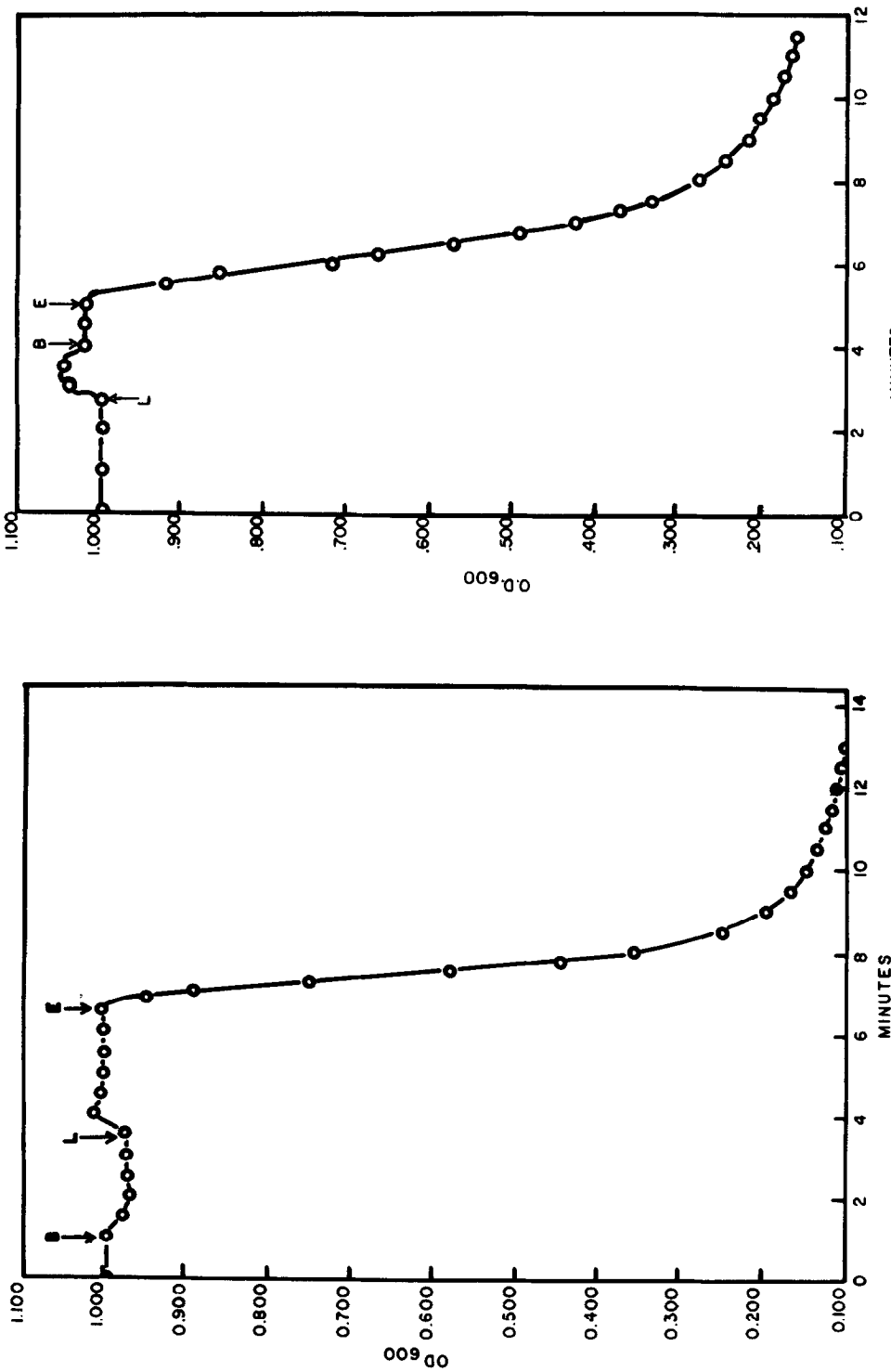

Fig. 2. Effect of sequential additions of Brij 58, lysozyme, and EDTA to plasmolyzed *E. coli*. Final concentrations: Brij 58, 30 µg/ml; lysozyme, 60 µg/ml; EDTA, 5×10^{-4} M.

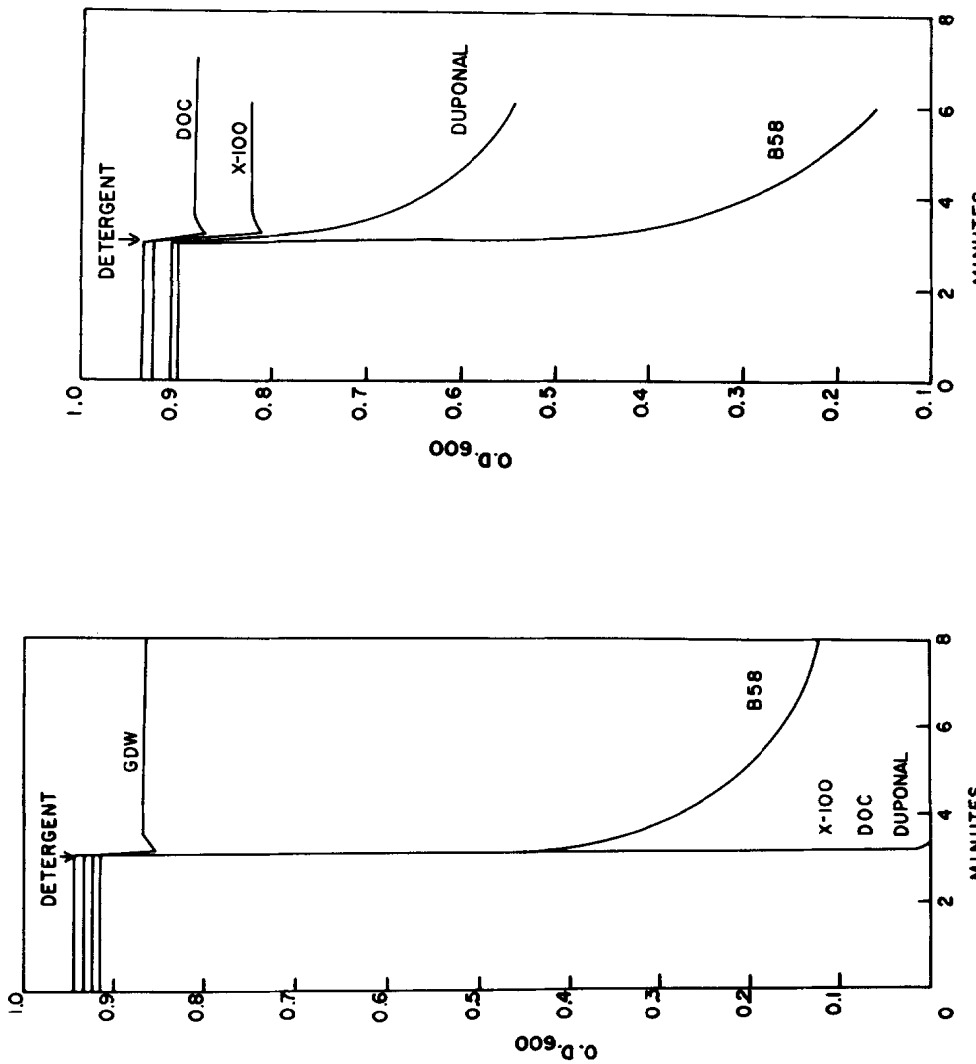

Fig. 3. Effect of Brij 58, Triton X-100, Duponal, and DOC on EDTA-lysozyme spheroplasts. Cell suspensions were prepared and held as previously described. Detergent concentrations were as follows: 3a; Brij 58, 50 µg/ml; Triton X-100, 2.5 mg/ml; Duponal, 0.5 mg/ml; and DOC, 5 mg/ml. 3b: All at 50 µg/ml.

ceptible to extensive lysis by Triton X-100, Duponal, and DOC. However, Brij 58 was without effect on plasmolyzed cells. The same patterns of lysis were evident when lysozyme spheroplasts were treated with the four detergents. As seen in Fig. 3a, lysis of EDTA-lysozyme spheroplasts was instantaneous and complete with the high concentrations of Triton X-100, Duponal, and DOC used. The low concentration of Brij 58 used also induced rapid lysis; however, lysis was not as extensive as with the other detergents. Fig. 3b shows the extent of lysis of EDTA-lysozyme spheroplasts when the detergents were used at the same concentration (50 µg/ml). The turbidity decrease associated with DOC and Triton X-100 treatment barely exceeded that of the distilled water control. Duponal produced a 50% decrease in turbidity. At this low detergent concentration Brij 58 far exceeded the other detergents in effecting lysis of EDTA-lysozyme spheroplasts.

The effects of the above four detergents on the stability of cyanide-sensitive $NADH_2$ oxidase (spectrophotometric) are shown in Table 1. Duponal

Table 1. Effect of Brij 58, Triton X-100, Duponal, and DOC on the stability of $NADH_2$ oxidase

Detergent	Concentration	Units of $NADH_2$ oxidase after treatment	Percent of control
None		141,700	100
Brij 58	1 mg/ml	42,650	30.5
Triton X-100	1 mg/ml	31,100	21.9
Duponal	1 mg/ml	0	0
Deoxycholate	10 mg/ml	0	0

Spheroplasts were prepared in the usual manner, divided into 5 aliquots and centrifuged at 10,000 x g for 5 minutes. The pellet was resuspended in distilled water plus the detergents at the concentrations given, incubated for 10 minutes at room temperature, and assayed spectrophotometrically for $NADH_2$ oxidase activity. The numbers given represent cyanide sensitive $NADH_2$ oxidase.

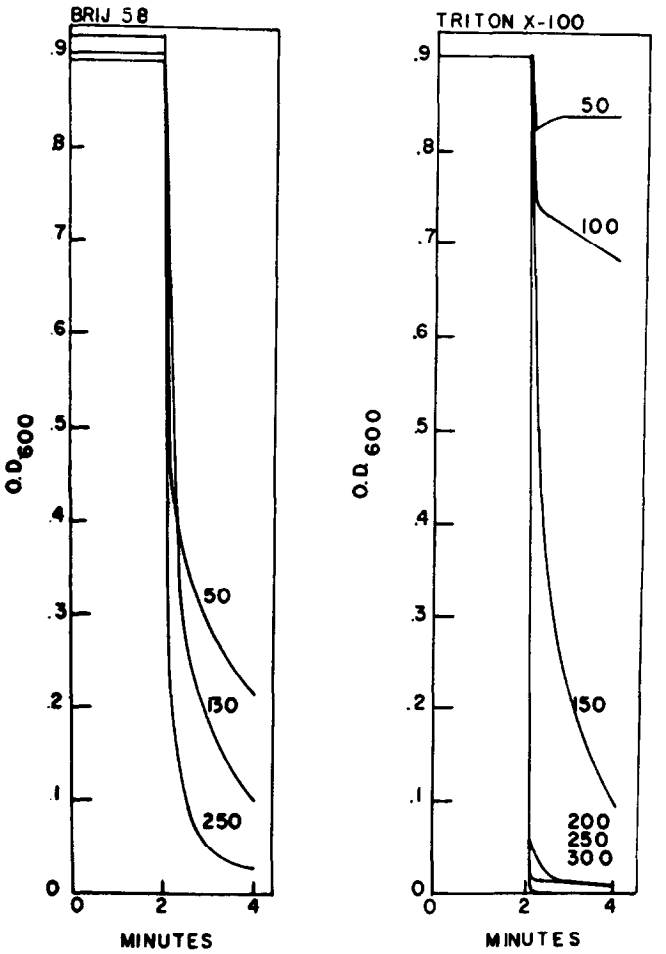

Fig. 4. Effect of various concentrations of Brij 58 and Triton X-100 on EDTA-lysozyme spheroplasts. The cell suspension was the same as in Fig. 3. The numbers above the curves refer to the final detergent concentration (μg/ml).

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and DOC completely destroyed $NADH_2$ oxidase activity (both cyanide sensitive and insensitive). Brij 58 and Triton X-100 treated suspensions retained 30 and 22% respectively of the $NADH_2$ oxidase activity. Of the $NADH_2$ oxidase activity which survived detergent treatment, 99% was sensitive to inhibition by cyanide. In these experiments the detergent treatment was carried out at room temperature. When lysis is carried out at 2-4 C, 90-95% of $NADH_2$ oxidase, succinoxidase, and formic oxidase activities survive Brij 58 treatment (Birdsell and Cota-Robles, in preparation).

The effect of different concentrations of Brij 58 and Triton X-100 on EDTA-lysozyme spheroplasts is shown in Fig. 4. Below 150 µg/ml Brij 58 was more effective than Triton X-100 in lysing EDTA-lysozyme spheroplasts. Above this concentration Triton X-100 produced more extensive lysis than did Brij 58. Furthermore, Triton X-100 treatment at a concentration of 100 µg/mg protein released 65% of the cytochrome b_1 and 83% of the flavoprotein into a form not sedimentible at 100,000 X g for 4 hours. Concomitant with this release was loss of $NADH_2$ oxidase activity and solubilization of succinic dehydrogenase activity.

Discussion

As reported by Godson and Sinsheimer (1968), *E. coli* is sensitive to lysis by the non-ionic detergent Brij 58 only after prior treatment with EDTA and lysozyme. Our results can be contrasted to those of Godson and Sinsheimer in that we found that much less detergent is required to produce extensive lysis of *E. coli*. In our hands rapid lysis of *E. coli* spheroplasts was obtained when we utilized concentrations equivalent to 1/50th of that used by Godson and Sinsheimer. Our results suggest that lysis by low concentrations of Brij 58 can be obtained if the cytoplasmic membrane is exposed and not protected by the L membrane component of the cell wall. We believe that lysis results from the disruption of the integrity of the cytoplasmic membrane rather than an expansion of the murein layer of the cell wall as proposed by Godson and Sinsheimer. It is conceivable that our observations can be reconciled with those of Godson and Sinsheimer. However, this reconciliation notwithstanding, it is clear that EDTA-lysozyme spheroplasts can be lysed very effectively by relatively low (30 µg/mg protein) concentrations of the non-ionic detergent, Brij 58.

The low concentrations of Brij 58 used in this study have enabled the isolation and characterization of a membrane fraction enriched for the electron transport system (Birdsell and Cota-Robles, in preparation). Brij 58

may prove extremely useful as a relatively innocuous general lysing agent for studies such as those of Godson and Sinsheimer (1968 a and b) and Birdsell and Cota-Robles (in preparation).

Acknowledgments

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References

Birdsell, D. C. and E. H. Cota-Robles. *J. Bacteriol.* 93, 427 (1967).
Birdsell, D. C. and E. H. Cota-Robles. (1968). In preparation.
Godson, G. N. and R. L. Sinsheimer. *Biochim. Biophys. Acta*, 149, 476 (1968).
Godson, G. N. and R. L. Sinsheimer. *Biochim. Biophys. Acta*, 149, 489 (1968).
Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* 193, 265 (1951).