ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1982, p. 844–845
0066-4804/82/050844-02$02.00/0
Vol. 21, No. 5

Effect of Dihydrostreptomycin on Active Transport in Isolated Bacterial Membrane Vesicles

ROBERT G. EAGON,* RICHARD J. HARTLE,† JAMES B. RAKE,‡ AND SAAD ABDEL-SAYED§
Department of Microbiology, University of Georgia, Athens, Georgia 30602

Received 6 November 1981/Accepted 2 February 1982

Membrane vesicles prepared from bacterial cells grown in the absence of dihydrostreptomycin but subsequently incubated in the presence of dihydrostreptomycin transported proline normally, but vesicles prepared from cells grown in media to which dihydrostreptomycin was added 30 min before harvesting had a greatly impaired ability to accumulate proline. The latter cells extruded protons normally but were unable to maintain a proton gradient as effectively as normal cells. These data indicated that metabolism was required for dihydrostreptomycin to exert an effect on the bacterial cell membrane.

One of the early effects of streptomycin-treated bacterial cells is a loss of cell membrane permeability control resulting in the leakage of a variety of low-molecular-weight metabolites, such as cations, amino acids, and nucleotides (1–3). Whether this change in the permeability state of the cell membrane is due to the direct disruption of the cell membrane or to defective cell membrane being formed during metabolism in the presence of streptomycin has not been determined. Thus, the work presented herein was carried out to provide an answer to this question.

The organisms used in these studies were *Escherichia coli* ML 308-225 ($i^-z^-y^+a^+$) and *Pseudomonas aeruginosa* PAO1. The organisms were grown aerobically until mid-exponential phase at 37°C (*E. coli*) or 30°C (*P. aeruginosa*) on a rotary shaker in 500-ml Erlenmeyer flasks, each of which contained 50 ml of nutrient broth (Difco Laboratories, Detroit, Mich.). When the effects of streptomycin were to be determined, dihydrostreptomycin sulfate (DHS; Sigma Chemical Co., St. Louis, Mo.) was added to the cultures to a final concentration of 50 μg/ml 30 min before harvesting the cells.

Isolated membrane vesicles of *E. coli* were prepared by the Kaback procedure (5) with a slight modification. After the final washing and pelleting of the membrane fraction, the membranes were suspended in a small volume of 0.1 M potassium phosphate (pH 6.6) containing a final concentration 20 mM $MgSO_4 \cdot 7H_2O$ and 20% sucrose. This suspension was carefully layered over the same buffer solution, except that it contained 70% sucrose, in a centrifuge tube. This discontinuous sucrose-density gradient was centrifuged at 4°C for 90 min at 150,000 × *g*. The resulting interface layer was removed carefully and suspended in 0.1 M potassium phosphate (pH 6.6) containing 20 mM $MgSO_4 \cdot 7H_2O$ to a final concentration of 2 to 6 mg of protein per ml.

DHS had little or no effect on the active transport of proline in membrane vesicles prepared from *E. coli* grown in the absence of DHS (Fig. 1). Thus, the vesicles rapidly accumulated proline in the absence of DHS (Fig. 1A). When vesicles were preincubated for 15 min at 30°C in the presence of 50 μg of DHS per ml, proline was accumulated in a manner nearly identical to that observed in the absence of DHS (Fig. 1B) and, after 30 min of preincubation in the presence of DHS, the vesicles still retained the ability to transport and accumulate proline (Fig. 1C). The reduced rate and extent of proline uptake in the latter instance may be explained on the basis of the decay of transport activity in vesicles incubated for so long a period before the addition of proline to start the experiment.

Vesicles that were prepared from cells of *E. coli* grown in media to which 50 μg of DHS per ml was added 30 min before harvesting retained little or no proline transport activity (Fig. 1D).

These data clearly indicated, therefore, that the DHS-induced membrane transport defect was not mediated merely by the presence of DHS; rather, it was strongly suggested that cellular metabolism was required. These data, however, did not permit us to determine whether the failure of vesicles prepared from cells harvested from media to which DHS had been added 30 min before harvesting was due to the inability of the vesicles to transport proline or

† Present address: Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109.
‡ Present address: Smith, Kline & French Laboratories, Philadelphia, PA 19101.
§ Present address: Institut de Microbiologie, Université de Lausanne, CH-1011 Lausanne 11, Switzerland.

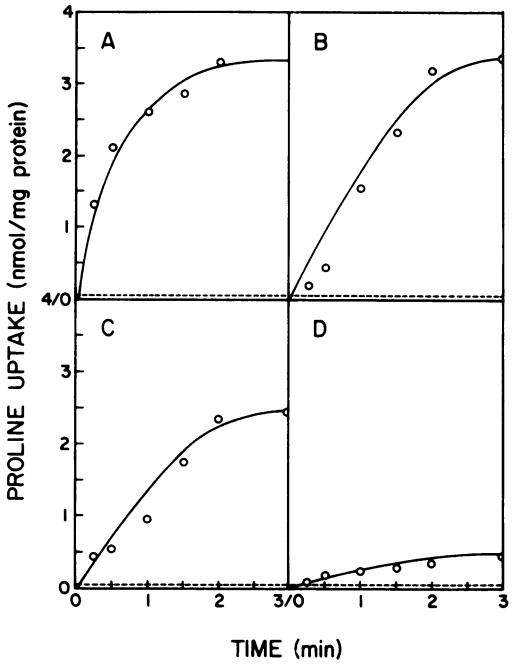

FIG. 1. Uptake of [U-^{14}C]proline (specific activity, 5 μCi/μmol; New England Nuclear Corp., Boston, Mass.) in isolated membrane vesicles of *E. coli* energized by ascorbate-reduced phenazine methosulfate as determined by the method described by Kaback (5). The broken horizontal lines represent intravesicular equilibration of proline with the external environment. Uptake of proline in vesicles prepared from cells grown in DHS-free media: (A) no treatment; (B) vesicles preincubated for 15 min in 50 μg of DHS per ml; (C) vesicles preincubated for 30 min in 50 μg of DHS per ml. (D) Uptake of proline in vesicles prepared from cells grown in media to which 50 μg of DHS per ml was added 30 min before harvesting.

whether, instead, the vesicles were unable to retain and thereby accumulate proline after its uptake.

In an effort to answer this question, we measured proton extrusion by the procedure described by Rake and Eagon (6) in intact normal cells and in cells from culture media to which 50 μg of DHS per ml were added 30 min before harvesting. These experimental results showed that the latter cells extruded protons normally. For example, both normal cells of *P. aeruginosa* and cells harvested from media containing DHS gave an H^+/O ratio of 6.8 ± 0.4 (data not shown). However, proton resorption times were different. Normal cells showed proton resorption half-times of 51 ± 5 s, whereas cells from media containing added DHS showed proton resorption half-times of 26 ± 5 s. Thus, these data suggested that membranes of cells cultured in media with added DHS were more permeable than normal cell membranes, but their primary energy transduction was not impaired, only their ability to maintain a proton gradient as efficiently as normal cell membranes. This in turn would be expected to impair both active transport and the ability of such cells to retain intracellular solutes. This implies, therefore, that the membrane supramolecular structure was not so disrupted as to derange electron transport, but it was sufficiently disordered to enhance permeability.

Finally, it is interesting that, in contrast to our findings on the effect of DHS on the cell membrane, streptomycin has been reported to permeabilize the outer membrane of *P. aeruginosa* by physical interaction (4).

This investigation was sponsored by the U.S. Army Medical Research and Development Command, Fort Detrick, MD 21701, under contract no. DAMD-17-79-C-9022.

LITERATURE CITED

1. **Anand, N., and B. D. Davis.** 1960. Effect of streptomycin on *Escherichia coli*. Nature (London) **185**:22–23.
2. **Bryan, L. E., and H. M. Van Den Elzen.** 1976. Streptomycin accumulation in susceptible and resistant strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **9**:928–938.
3. **Dubin, D. T., R. Hancock, and B. D. Davis.** 1963. The sequence of some effects of streptomycin in *Escherichia coli*. Biochim. Biophys. Acta **74**:476–489.
4. **Hancock, R. E. W., V. J. Raffle, and T. I. Nicas.** 1981. Involvement of the outer membrane in gentamicin and streptomycin uptake and in killing in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **19**:777–785.
5. **Kaback, H. R.** 1973. Transport in isolated bacterial membrane vesicles. Methods Enzymol. **31**:698–709.
6. **Rake, J. B., and R. G. Eagon.** 1980. Inhibition, but not uncoupling, of respiratory energy coupling of three bacterial species by nitrite. J. Bacteriol. **144**:975–982.