CONTROL OF PERMEATION TO GLYCEROL IN CELLS OF ESCHERICHIA COLI[1]

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Two types of membrane transport systems have been distinguished for small molecules: "active transport" in which a substance is moved across a biological membrane against an electrochemical gradient at the expense of metabolic energy and "facilitated diffusion" in which the specific membrane carriers catalyze the equilibration of intracellular and extracellular substrate concentrations without energy coupling. The term "facilitated diffusion" was coined by Danielli (1954) to describe the mechanism of entry for glycerol into human erythrocytes. Knowledge of this process stems from observations of Jacobs that erythrocytes of certain species possess a special process for glycerol permeability (Jacobs, 1931) which is non-

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competitively inhibited by traces of copper (Jacobs and Corson, 1934; Jacobs and Stewart, 1946; and Jacobs, 1950) and competitively inhibited by ethylene glycol (Jacobs, 1954).

Similar investigations were extended to sugar permeability in the human erythrocytes by LeFevre (1954), Widdas (1954), and Wilbrandt (1954). A facilitated-diffusion mechanism for sugar uptake was discovered in yeast by Burger et al (1959) and was studied further by Okada and Halvorson (1963, 1964). Kaback and Stadtman (1968) have recently suggested that glycine may enter E. coli by a similar mechanism.

Rapid entry of glycerol into bacteria was noted as early as 1903. The high permeability to glycerol was inferred by the failure of this compound to sustain osmotic pressure across the cell membrane (Fischer, 1903; Mitchell and Moyle, 1956; Mager et al, 1956; Avi-Dor et al, 1956; and Bovell et al, 1963). More recently, cells of E. coli K12 lacking glycerol kinase were found unable to accumulate labeled material when incubated with ^{14}C-glycerol. The lack of active transport of glycerol and the additional finding that the "K_m" for growth (0.9 μM) closely reflected the K_m of glycerol kinase (1.3 μM) led to the belief that the compound readily equilibrates across the membrane by free diffusion and that trapping of the substrate depended upon phosphorylation (Hayashi and Lin, 1965).

To test whether glycerol indeed enters primarily by an uncatalyzed process, wild-type E. coli K12 and two mutants were examined for the rate of change in cell volume after being exposed to a hypertonic solution of glycerol. Cells of strain 1 (wild type, see Koch et al, 1964) grown in 40 ml of a mineral medium (Tanaka et al, 1967) containing 1% casein hydrolysate as carbon source were harvested at 1.3 O.D. (550 mμ), washed once

with cold Tris (0.02 M, pH 7.5), and suspended in a solution of low osmotic pressure (4 mM Tris, pH 7.5) at an O.D. of 1.5.

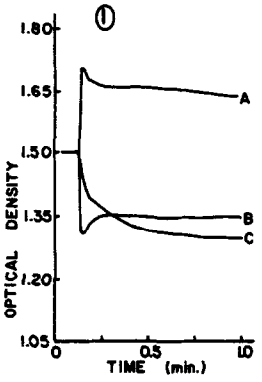

Fig. 1. The change of O.D. of cell suspensions of strain 1 upon being mixed with various diluents: hypertonic NaCl (A), water (B), and hypertonic glycerol (C). The cells were grown on 1% casein hydrolysate. The first 0.1 min of each curve is believed to be inaccurate due to instrumental artifacts. See text for details.

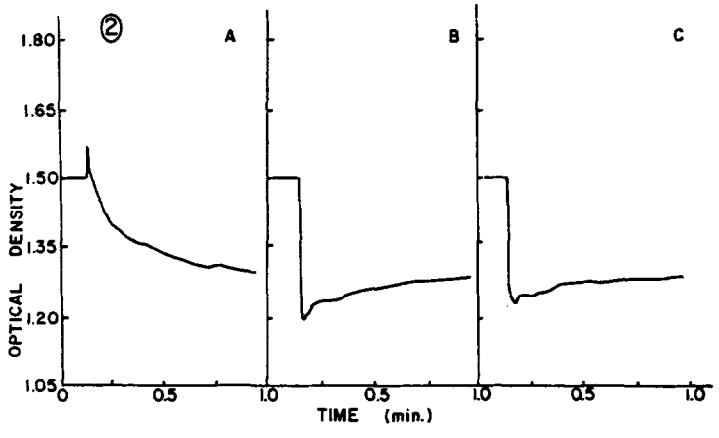

Fig. 2. Glycerol permeability of strain 1 cells grown on 1% casein hydrolysate with 0.01 M glucose (A), with 0.02 M glycerol (B), and with 0.04 M DL-α-GP (C).

To 2.5 ml of such a suspension in a cuvette (1 cm light path) placed in the cell compartment of a Model 2000 Gilford recording spectrophotometer, 0.5 ml of 2.4 M NaCl was rapidly injected through a hypodermic needle. The change in O.D. of the suspen-

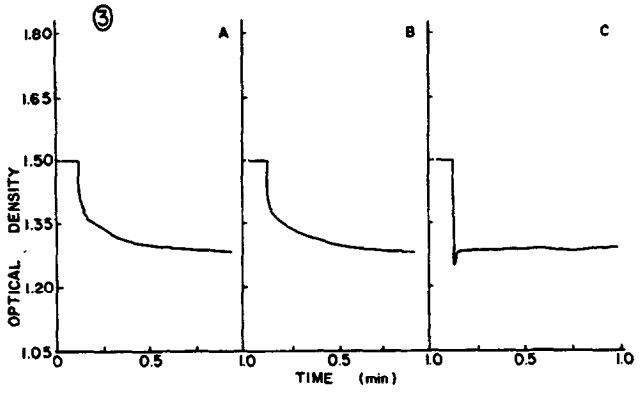

Fig. 3. Failure of glycerol to act as an inducer for the permeability factor in cells lacking glycerol kinase. Cells of strain 75 (a glycerol kinase-negative mutant isolated by Dr. J. P. Koch) were grown on 1% casein hydrolysate with no supplementation (A), with 0.02 M glycerol (B) and with 0.04 M DL-α-GP (C).

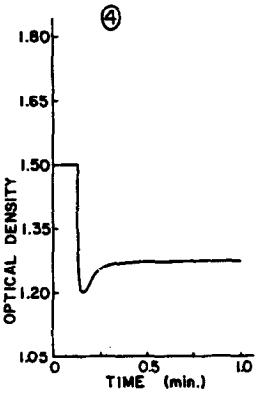

Fig. 4. Constitutivity of the glycerol permeability factor in a mutant derepressed in the L-α-GP regulon. Cells of strain 72 (Cozzarelli et al, 1968) were grown on casein hydrolysate alone.

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sion was recorded within 2 sec after mixing. Fig. 1, curve A, shows that this addition caused the O.D. of the suspension to rise. The decrease of transmitted light was due to the increase in scattering resulting from plasmolysis. This cytoplasmic shrinkage was maintained because of the relative impermeability of the cell membrane to NaCl. Curve B of Fig. 1 shows the effect

of simple dilution of a cell suspension with 0.5 ml of water. When a similar suspension was exposed to a hypertonic solution of glycerol (0.5 ml of 4.8 M) osmotically equivalent to the NaCl solution, the cells shrank briefly and then regained their original volume as glycerol equilibrated across the plasma membrane, as shown in curve C. The rate of approach to a steady O.D. value is directly related to the rate of entry of glycerol. The final O.D. of the cell suspension treated with glycerol was lower than that of the suspension diluted with an equal volume of water, which is probably due to differences in refractive indices of the two media.

The presence of glucose in a casein-hydrolysate medium repressed the cellular permeability to glycerol as indicated by the slower approach to equilibrium (Fig. 2A) in comparison with cells grown on amino acids alone (Fig. 1, curve C). On the other hand, growth in the presence of either glycerol or L-α-glycerophosphate (L-α-GP) greatly increased the permeability to glycerol so that its rate of equilibration was too rapid to be recorded (Fig. 2 B and C).

The increase in permeability to glycerol, however, could not be induced by this compound in cells lacking glycerol kinase activity. Thus in a kinase-negative mutant, L-α-GP but not glycerol acted as an inducer (Fig. 3). Such an induction pattern suggests that this system for facilitated diffusion belongs to the L-α-GP regulon (Cozzarelli *et al*, 1968). The hypothesis is confirmed by the fact that this system is constitutive in cells which are genetically derepressed in glycerol kinase, the L-α-GP transport system and L-α-GP dehydrogenase (Fig. 4).

These preliminary results indicate that the entry of glycerol into *E. coli* is not by simple diffusion but is controlled by a

specific gene product. Also, the technique based upon measurement of volume changes can be usefully applied to the study of selective permeability of bacterial cell membranes.

REFERENCES

Avi-Dor, Y., Kuczynski, M., Schatzberg, G., and Mager, J., J. Gen. Microbiol. 14, 76 (1956).

Bovell, C. R., Packer, L., and Helgerson, R., Biochim. Biophys. Acta 75, 257 (1963).

Burger, M., Hejmová, L., and Kleinzeller, A., Biochem. J. 71, 233 (1959).

Cozzarelli, N. R., Freedberg, W. B., and Lin, E. C. C., J. Mol. Biol. 31, 371 (1968).

Danielli, J. F., In Recent Developments in Cell Physiology (ed. Kitching, J. A.), New York, Academic Press, 1954, pp. 1-14.

Fischer, A., In Vorlesungen ueber Bakterien, Gustav Fischer, Jena, 1903.

Hayashi, S., and Lin, E. C. C., Biochim. Biophys. Acta 94, 479 (1965).

Jacobs, M. H., Ann. New York Acad. Sci. 50, 824 (1950).

Jacobs, M. H., Biol. Bull. 107, 314 (1954).

Jacobs, M. H., Ergebn. Biol. 7, 1 (1931).

Jacobs, M. H., and Corson, S. A., Biol. Bull. 67, 325 (1934).

Jacobs, M. H., and Stewart, D. R., Amer. J. Med. Sci. 211, 246 (1946).

Kaback, H. R., and Stadtman, E. R., J. Biol. Chem. 243, 1390 (1968).

Koch, J. P., Hayashi, S., and Lin, E. C. C., J. Biol. Chem. 239, 3106 (1964).

LeFevre, P. G., Symp. Soc. Exper. Biol. 8, 118 (1954).

Mager, J., Kuczynski, M., Schatzberg, G., and Avi-Dor, Y., J. Gen. Microbiol. 14, 69 (1956).

Mitchell, P. and Moyle, J., in Bacterial Anatomy, Sixth Symp. Soc. Gen. Microbiol., University Press, Cambridge, 1956, p. 150.

Okada, H., and Halvorson, H. O., J. Bacteriol. 86, 966 (1963).

Okada, H., and Halvorson, H. O., Biochim. Biophys. Acta 82, 538 (1964).

Tanaka, S., Lerner, S. A., and Lin, E. C. C., J. Bacteriol. 93, 642 (1967).

Widdas, W. F., Symp. Soc. Exper. Biol. 8, 163 (1954).

Wilbrandt, W., Symp. Soc. Exper. Biol. 8, 136 (1954).