FORMATE-CONTROLLED ACCUMULATION OF INORGANIC AND ORGANIC SUBSTANCES IN ESCHERICHIA COLI

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The penetration and accumulation of certain inorganic and organic compounds in bacteria occurs at the expense of energy derived from the metabolism of either cellular reserves (1), or externally provided substrates (2). In cells depleted of reserves, the accumulation of an oxidizable organic substrate is autocatalytic since the oxidation of the substrate is the sole source of energy for its own accumulation. This imposes considerable restrictions on studies of the initial phases of uptake of compounds as the energy available for accumulation is limited. Clearly, it would be desirable to provide an energy source that is independent of the accumulation process. In Escherichia coli hexoses, amino acids and carboxylic acids are compounds which are actively accumulated (3); formate and glycerol, on the other hand, enter the cell by diffusion (4). This paper reports experiments showing that Na-formate can serve as a source of energy for the uptake of organic and inorganic compounds by resting cells. Formate is particularly suitable as a ready source of energy for E. coli for several reasons: the cell is freely permeable to the compound, it is converted stoichiometrically to H_2O and CO_2 by a single oxidative reaction, it is respired more rapidly than most other compounds (5) and its K_m for respiration is less than 100 μM which permits the study of the accumulative process as a function of the energy provided to the cell.

E. coli strain B was grown in Penassay broth at 37°C with forced aeration. The cells were harvested by centrifugation after 12 - 14 hours growth, washed twice with an equal volume of distilled water and resuspended in 20 mM tris-(hydroxy-methyl)-aminomethane-HCl (Tris) - 300 mM KCl pH 7.5 to a final concentration of 0.5 - 1.0 mg cellular protein/ml (6). The suspension was aerated for 1 - 2 hours prior to the experiments to deplete cellular reserves. The uptake of the test compounds was followed at 25°C by measuring the decrease in light (546 mμ) scattered at 90° due to deplasmolysis following the addition of the compound (4). Simultaneous

measurements of respiration were made using a vibrating platinum electrode immersed in the cuvette.

Figure 1 shows the results of a typical experiment. Here formate serves as the source of energy for the uptake of K-malate. The cells were delivered to the cuvette of a Brice-Phoenix light scattering photometer and the test compounds were added after 75 seconds. It can be seen that the addition of formate alone causes no change in the amount of light scattered by the cell suspension, whereas the addition of K-malate or K-malate plus Na-formate leads to a 40 per cent decrease in light scattering. The accumulation of malate in the absence of formate is described by a sigmoid curve: the rate of uptake increases, becomes maximal and then decreases as saturation of the cell is approached. Addition of formate and malate simultaneously leads to a rapid decrease in light scattering that is complete in 2 minutes. The same type of kinetics describes the accumulation of hexoses, amino acids and carboxylic acids. The rate of accumulation and the total decrease in light scattered is characteristic of the compound taken up. However, succinate, alpha-ketoglutarate and malate are accumulated at similar rates, as are glucose and mannose. Table 1 records in seconds, the reciprocal of the time required for 1/2 maximal saturation of the cells by the compounds brought about by 400 μM formate.

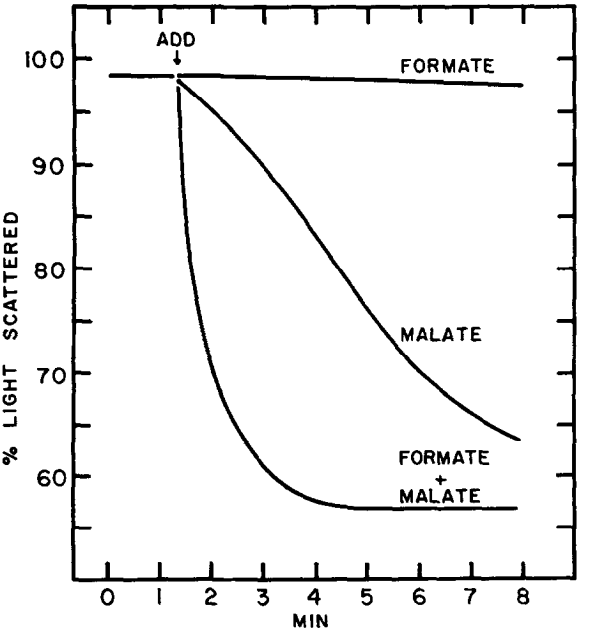

Figure 1. Effect of formate on the uptake of K-malate by E. coli. The cuvette contained 5.0 ml cell suspension (0.8 mg protein/ml) in Tris-KCl medium. The substrates were added after 75 seconds to give a final concentration of formate of 200 μM and malate of 5 mM.

TABLE I

Uptake of Organic Compounds by E. coli in the Presence of Na-formate

The reaction mixture contained 5.0 ml cells suspended in 20 mM Tris - 300 mM KCl pH 7.5. Formate and the test compounds were added simultaneously to give concentrations of 200 μM and 5 mM respectively. Data are reported as the reciprocal of the time in seconds required for 1/2 maximal saturation of the cells.

Compound	$1/T_{1/2}$ (sec^{-1})
K-malate	0.042
K-alpha ketoglutarate	0.050
Na_2succinate	0.050
K-glutamate	0.027
K-aspartate	0.016
Glucose	0.057
Mannose	0.057
Fructose	0.050

These results suggested that it might be interesting to study the effects of formate oxidation on the accumulation of inorganic salts. It was found that the addition of salt to E. coli suspended in 20 mM Tris pH 7.5 causes an instantaneous increase in light scattering response as the cells plasmolyse. Subsequent addition of formate (200 μM) caused a rapid decrease in light scattering as the salt is accumulated and the cells deplasmolyse. Table 2 shows the reciprocal time for 1/2 maximal saturation of E. coli with the chloride salts of potassium, sodium, lithium, magnesium and strontium. Manganese, calcium and ammonium do not show light scattering changes in the presence of formate and thus appear not to be accumulated under the test conditions employed.

It was found that preincubation of the cells in the presence of 4-hydroxybenzalmalononitrile, an uncoupler of oxidative phosphorylation in mitochondria (7), at 6 x 10^{-5}M prevents the uptake of both inorganic and organic compounds, but stimulates the respiration of formate 1.5 fold. This result suggests that the test compounds cannot be accumulated when respiration is uncoupled.

TABLE II

Uptake of Inorganic Salts by E. coli in the Presence of Na-formate

The cuvette contained 5.0 ml of cells suspended in 20 mM Tris pH 7.5. The chloride salts of the cations were added to a final concentration of 100 mM. Two minutes later Na-formate was added to a concentration of 200 μM. Data are given as the reciprocal of the time in seconds required for 1/2 maximal saturation of the cells.

Compounds	$(1/T_{1/2})sec^{-1}$
$MgCl_2$	0.100
KCl	0.133
NaCl	0.057
$SrCl_2$	0.133
LiCl	0.017

Owing to the unique features of formate oxidation in E. coli, its expenditure affords a rapid and quantitative approach for distinguishing energy requirements associated with the accumulation of substances into bacterial cells. The stimulation of formate oxidation by compounds such as malononitrile derivatives, which uncouple mitochondrial respiration, further suggest an approach to evaluating the nature of respiratory control mechanisms in bacteria.

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