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Glycolytic and Tricarboxylic Acid Cycle Enzyme Activities During Intraperiplasmic Growth of *Bdellovibrio bacteriovorus* on *Escherichia coli*

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Selected enzyme activities were measured in extracts of the total cell pellets obtained at various times during aerobic intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J on anaerobically grown *Escherichia coli* substrate cells. Initially, the glycolytic enzyme activities were associated with the input of *E. coli* and the tricarboxylic acid cycle enzyme activities with the input of bdellovibrios. During the first 90 min of *Bdellovibrio* development, the glycolytic activities declined about 25 to 60%, whereas the tricarboxylic acid cycle activities increased about 10%. Between 110 and 180 min, the glycolytic activities decreased to trace levels and tricarboxylic acid cycle activities increased about 50 to 90%. Both bdellovibrio cell extracts and the cell-free growth menstruum (obtained after bdellovibrio growth on *E. coli*) caused the inactivation of glycolytic enzymes in *E. coli* extracts.

The developmental cycle of *Bdellovibrio bacteriovorus* includes growth within the periplasmic region of a suitable gram-negative substrate bacterium. This intraperiplasmic environment can provide all nutrients necessary for bdellovibrio growth (10), and it is thought that bdellovibrio exerts a major influence on the nutritional quality of this environment by its regulated dissolution of the substrate cell (8, 9). Previous studies have indicated that the degradation of the substrate cell nucleic acids (6, 8) and lipids (7) are controlled processes leading to a high degree of reuse of the end products of bdellovibrio for synthesis of its homologous cell components. It is known that *Bdellovibrio* can produce extracellular proteases (3, 12, 13), that amino acids are used as energy sources by bdellovibrio (5), and that substrate cell activities such as permeability control (10), respiration (5, 9), and potential to synthesize ribonucleic acid and protein (15) are rendered nonfunctional shortly after bdellovibrio attack. However, little information is available with regards to bdellovibrio use of substrate cell proteins and roles of substrate cell enzyme activities during intraperiplasmic growth.

To investigate this problem, an experimental approach was devised using common cell extracts prepared from the total cell pellets obtained at intervals from an aerated synchronous culture of *B. bacteriovorus* 109J growing on *Escherichia coli* ML35 cells which had been grown under anaerobic conditions. The anaerobically grown *E. coli* have high levels of glycolytic enzymes, but low levels of tricarboxylic acid cycle (TCA) enzymes, whereas the reverse situation exists for *B. bacteriovorus* as shown by the data in Table 1. The latter organism is a strict aerobe thought to lack some glycolytic enzymes (11, 12, 14). The high levels of glucose phosphate isomerase and glyceraldehyde phosphate dehydrogenase in cell extracts of *B. bacteriovorus* 109J were unexpected, but were also observed in extracts of *B. bacteriovorus* 109D (data not shown). Since bdellovibrios apparently do not metabolize free sugars (5, 12), these two glycolytic enzymes activities could be involved in biosynthetic functions rather than energy generation.

Bdellovibrio attachment, penetration, bdelloplast (substrate cell with intracellular bdellovibrio) formation, and growth on anaerobically pregrown *E. coli* appeared very similar to bdellovibrio development on aerobically grown cells, as indicated by microscopic observations. Oxygen consumption by cell suspensions of anerobically grown *E. coli* was measured with an O_2 electrode cell as described previously (5, 10). These cell suspensions showed little ability for O_2 consumption or increased O_2 uptake when incubated with any one of a number of substrates, except formate. Shortly after the initiation of the bdellovibrio developmental cycle, the *E. coli* formate respiration declined in a manner similar to that observed previously (10) for succinate respiration of aerobically grown

TABLE 1. *Enzyme activities in cell extracts of Bdellovibrio bacteriovorus 109J and Escherichia coli ML35* [a]

Organism	Sp act[b]		
	E. coli		*B. bacteriovorus*
	Aerobic	Anaerobic	Aerobic
Glucokinase	48	50	2.4
Glucose phosphate isomerase	42	204	109
Phosphofructokinase	77	212	13
Fructosediphosphate aldolase	142	198	9.5
Triosephosphate isomerase	141	177	33
Glyceraldehydephosphate dehydrogenase	944	1,193	690
Pyruvate kinase	345	190	7.5
Citrate synthase	1,467	86	3,360
Isocitrate dehydrogenase	305	25	893
Fumarase	158	NA[c]	NA
Malate dehydrogenase	5,597	52	2,200
β-Galactosidase	3,100	2,810	NA

[a] *E. coli* was grown at 30°C in glucose salts medium (5) with aeration or anaerobically in filled roux bottles. *B. bacteriovorus* was grown on *E. coli* in DNB medium (13). Cell extracts were prepared, using methods described previously (5), by passage (2X) of cell suspensions through a French pressure cell. Enzyme activities in extracts were measured (1, 4), and the data are average of three determinations.

[b] Expressed as Δnanomoles per minute per milligram of protein of pyridine nucleotide oxidized or reduced, or of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed (β-galactosidase activity).

[c] NA, Not assayed.

E. coli. In addition, β-galactosidase activity of cryptic *E. coli* cells was expressed indicating loss of permeability control occurred as had been found with aerobically grown *E. coli* ML35 cells (10). Thus, we concluded that no major overall differences occurred in bdellovibrio growth on aerobically or anaerobically grown *E. coli* ML35.

Selected enzyme activities of cell extracts prepared from cells removed at various times during the cycle were measured (Fig. 1). Enzyme activities in the 0-min samples, i.e., samples removed immediately after mixing the *E. coli* and bdellovibrios, approximately equaled (±2%) the sum of the activities measured separately with individual extracts prepared from equivalent quantities of the two organisms. Enzyme activities in these latter extracts had specific activities comparable to those shown in Table 1. Of the four glycolytic enzymes assayed with the 0-min extract, over 90% of the total initial activity was associated with the input *E. coli*. Likewise, more than 90% of TCA enzyme activities were associated with the input *B. bacteriovorus* as determined by enzyme activity measurements in the individual cell extracts in the extract removed at 0 min from the combined cell suspensions.

In the first 90 min, the glycolytic activities declined some 25 to 60%, with each activity showing a different kinetic pattern. However, β-galactosidase activity had decreased only 10%. By 180 min, at which time the lysis of bdelloplasts had begun, the β-galactosidase and glycolytic enzyme activities had declined to trace levels. In contrast, the TCA enzyme activities decreased slightly during the first 20 min,

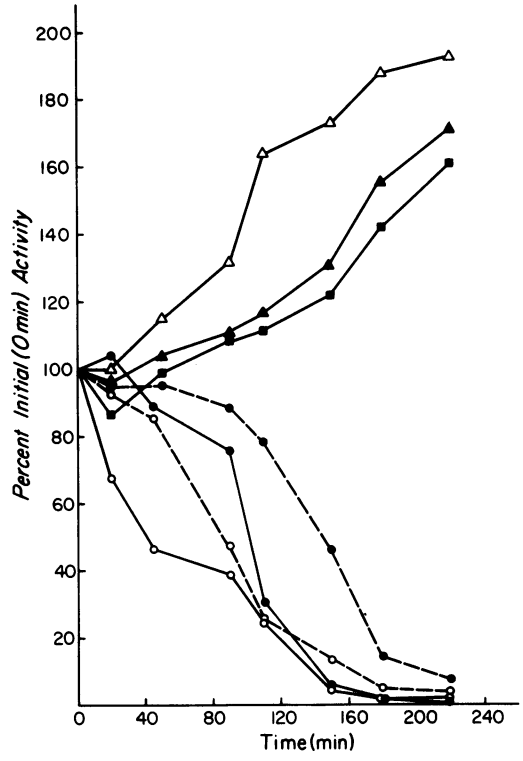

FIG. 1. *Changes in enzyme activity levels during intraperiplasmic growth of B. bacteriovorus 109J on E. coli ML35. Parallel developmental cultures (300 ml) were prepared (9) to initially contain 7 × 10⁹ B. bacteriovorus and 4 × 10⁷ anaerobically grown E. coli cells/ml. At the indicated time, a culture was harvested by centrifugation, an extract was prepared from the resultant total cell pellet, and enzyme activities in the extract were measured (methods cited in Table 1 were used). The enzyme activities are expressed as the percentage of the activity measured with the 0-min extract. The data are the average of three separate experiments. Phosphofructokinase* (●——●), *fructose diphosphate aldolase* (○——○), *pyruvate kinase* (○- - -○), *β-galactosidase* (●- - -●), *citrate synthase* (▲), *isocitrate dehydrogenase* (△), *and malate dehydrogenase* (■).

but then continued to increase throughout the rest of the developmental cycle. Maximal increases in TCA activities occurred after 100 min. The initial, slight decreases in TCA enzyme activities were similar in magnitude to the TCA activities associated with the input of *E. coli*. The initial decrease in TCA activities may have been due to loss of much of these *E. coli* enzyme activities.

The observed decreases in the *E. coli* enzyme activities during bdellovibrio development can result from any combination of at least three distinct processes: (i) inhibition of enzyme activity without alteration of the enzyme; (ii) inactivation of enzyme activity by minor alteration of the enzyme, e.g., removal of a cofactor or activator; (iii) degradation of the enzyme by proteases to provide energy substrates and biosynthetic monomers for bdellovibrio growth. To evaluate the first two of these hypotheses, the following experiments were done. When a cell extract prepared from bdellovibrios or from 90-min bdelloplasts was mixed with an *E. coli* cell extract, the glycolytic and TCA enzyme activities in the resultant extract were essentially equal to the sum (±2%) of the activities measured in the individual extracts. Similar results were obtained with extracts prepared from cell suspensions made by mixing *E. coli* cells and bdellovibrio cells or bdelloplasts. If the bdellovibrio or bdelloplast extract contained an inhibitor or if intraperiplasmically located bdellovibrios produce inhibitor(s) not made by free bdellovibrios, then this would result in lower than expected enzyme activities in the resultant mixed cell extract. If bdelloplast enzyme activities were decreased via loss of cofactors, the added *E. coli* cell extract would most probably have ample amounts of these cofactors and the resultant mixed cell extract would have higher levels than expected of glycolytic activities. Since neither of these results was found and the decreases in glycolytic activities during bdellovibrio development were time dependent (Fig. 1), the data suggest that the first two hypotheses are unlikely and that degradation by proteases is more probable.

To gain some information as to whether degradation by enzymes of *E. coli* or *Bdellovibrio* or both could be responsible for the loss of glycolytic enzyme activities, *E. coli* extracts were incubated in various suspending fluids (Fig. 2). Little or no loss of fructose diphosphate aldolase activity was observed with extracts incubated in buffer or buffer plus bovine serum albumin. This suggests that endogenous *E. coli* proteases of cells not attacked by bdellovibrios are not the major causes of inactivation. However, cell-free suspending fluid (obtained after bdellovibrio

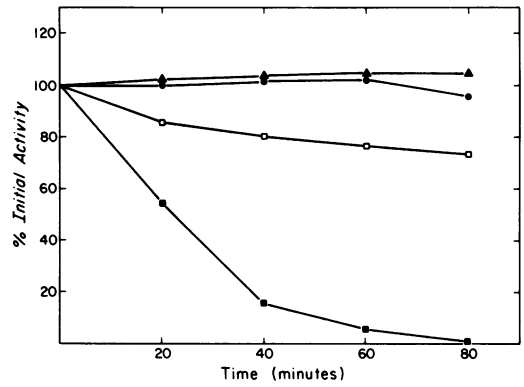

FIG. 2. *Inactivation of fructose diphosphate aldolase activity. An E. coli extract was diluted (final concentration, 5 mg of protein/ml) in 10^{-3} M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, pH 7.6 (●), in HEPES buffer + 5 mg of bovine serum albumin per ml (▲), in HEPES buffer + 5 mg of B. bacteriovorus cell extract per ml (□), or the cell-free HEPES suspending buffer obtained after growth of 8 × 10^9 cells of B. bacteriovorus/ml on 6 × 10^9 E. coli cells/ml (■). During incubation at 30°C with gentle aeration, samples were removed, cooled to 4°C, and assayed immediately for enzyme activity.*

growth on *E. coli*) catalyzed a loss of activity as did bdellovibrio cell extracts. Prior heat treatment (100°C, 10 min) of bdellovibrio extracts and the suspending fluids prevented this loss of activity. In similar experiments, the same pattern of results was observed for the inactivation of *E. coli* phosphofructokinase activity. These data, in conjunction with the published observations (2, 3) showing bdellovibrio extracellular proteases can degrade *E. coli* protein, suggest that bdellovibrio enzymes are responsible for the observed loss of substrate cell glycolytic enzyme activities during intraperiplasmic growth. However, definitive evidence to verify this hypothesis is not yet available. Such evidence would have to clearly demonstrate that during intraperiplasmic growth, specific bdellovibrio porteases are active on substrate cell proteins within the bdelloplast environment.

In regards to the various enzyme activity levels present during *Bdellovibrio* development (Fig. 1), if these levels correlate well with the actual levels of enzyme proteins, then several observations can be made. First, the kinetic patterns of β-galactosidase and glycolytic enzyme activities during growth of bdellovibrios on *E. coli* indicate that *E. coli* proteins are differentially degraded. For some proteins, degradation begins shortly after initiation of the *Bdellovibrio* developmental cycle. Since the glycolytic enzymes in fact constitute only a

small fraction of the total substrate cell protein, the average of these enzyme activity levels at any one time (e.g., ca. 50% decrease by 90 min) may or may not reflect the overall degree of degradation of total substrate cell protein. The former possibility is more likely as previous studies have shown that substantial degradation (more than 90%) of other substrate cell macromolecules, such as deoxyribonucleic acid (8) and ribonucleic acid (6), occurs during the first 90 min of the developmental cycle. Secondly, the kinetic patterns of the TCA enzyme activities show increases starting at 20 min into the cycle, whereas increased oxygen consumption by bdellovibrios does not begin to occur until 50 to 60 min (5, 10). After 60 min, the kinetic patterns of TCA enzyme activities (Fig. 1) and bdellovibrio oxygen consumption rates (5, 10), more or less, parallel one another. This suggests that initially substrate cell-derived amino acids are used more by bdellovibrios for protein synthesis rather than for catabolsim for energy. Later, amino acids are used extensively by *Bdellovibrio* for both purposes as a *Bdellovibrio* respiratory quotient characteristic of amino acid catabolism is evident at 60 min (5). The former point is also consistent with the fact that actual initiation of bdellovibrio protein synthesis must begin very early in the cycle since both penetration into the substrate cell and degradation of substrate cell nucleic acids are inhibited by chloramphenicol (5, 6, 8). Thirdly, even though the TCA enzymes are only a small part of the total *Bdellovibrio* protein, the observed kinetic patterns of TCA enzyme activities may indeed reflect the general synthesis pattern for total bdellovibrio protein. If so, then the bulk of bdellovibrio protein is made after the first 100 min of the cycle at which time concomitant synthesis of bdellovibrio nucleic acids also occurs (6, 8). Our data showing changes in enzyme activity levels during *Bdellovibrio* development are of a limited nature, and further evidence is necessary to clearly establish the validity of the previous three points. However, the data presented are compatible with the concept that bdellovibrio carries out a regulated degradation and utilization of substrate cell components (9).

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