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Regulation of Dihydrodipicolinate Synthase During Growth and Sporulation of *Bacillus cereus*

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A four- to sixfold increase in specific activity of dihydrodipicolinic acid synthase was observed during sporulation of *Bacillus cereus*. The enzyme from cells harvested before and after the increase in specific activity appeared to be very similar as judged by pH optima, heat denaturation kinetics, apparent Michaelis constants, chromatography on diethylaminoethyl-cellulose and Sephadex G-200, and polyacrylamide gel electrophoresis. Studies with various combinations of amino acids and one of the enzyme substrates, pyruvate, failed to give evidence for control of the enzyme by activation, inhibition, repression, induction, or stabilization. Omission of calcium from the sporulation medium had no significant effect on the specific activity pattern of the enzyme as a function of age of culture.

Dipicolinic acid and diaminopimelic acid are two substances that apparently play key roles in the bacterial sporulation process. Both compounds are formed as a result of the functioning of the lysine biosynthetic pathway. Forman and Aronson (8) have shown that, in *Bacillus cereus*, aspartokinase (EC 2.7.24), aspartic β-semialdehyde dehydrogenase (EC 1.2.1.10), dihydrodipicolinic acid (DHDPA) synthase (pyruvate aspartic semialdehyde-condensing enzyme; EC 4.2.1.52), and DHDPA reductase all increase in specific activity late in sporulation. Diaminopimelate decarboxylase (EC 4.1.1.20) decreases in specific activity during sporulation of *B. cereus*, disappearing just before initiation of dipicolinic acid synthesis (11). Dipicolinic acid synthase activity is detected in sporulating cells but not in vegetative cells (3). The changes in the specific activity of the enzymes described above may be of physiological benefit to sporulating cells by making precursors available for the synthesis of dipicolinic acid and diaminopimelic acid.

DHDPA synthase is the first specific enzyme in the lysine biosynthetic pathway. It catalyzes the condensation between pyruvate and L-aspartic β-semialdehyde (L-ASA) to form DHDPA. There have been no reports of inhibition or repression of DHDPA synthase in any of the sporulating bacteria by lysine or any of the other end products or intermediates found in the aspartate family biosynthetic pathway (1, 6, 17, 21, 22). An increase in specific activity has been observed (6, 8) in both *B. cereus* and *B. subtilis* late in sporulation. It was the purpose of this investigation to determine the mechanism(s) regulating the increase in specific activity of DHDPA synthase and to determine whether the enzymes from vegetative cells and sporulating cells share the same chemical and physical properties.

MATERIALS AND METHODS

Organisms. *B. cereus* strains ATCC 10702 and T (the latter obtained from H. O. Halvorson) were the wild-type strains used. A lysine auxotroph and three thiosine-resistant mutants of *B. cereus* T were also used. The thiosine-resistant mutants that excreted lysine and the auxotroph were provided by A. I. Aronson (Purdue University).

Growth and sporulation media. G medium (18) and tris(hydroxymethyl)aminomethane (Tris)-G medium (8) were used for many of the experiments in this investigation. A modified G medium containing 0.2% glucose and 0.1% potassium phosphate was also used. Growth in G medium is limited by glucose, whereas growth in modified G medium is limited by some component in yeast extract.

In experiments in which the lysine concentration was varied, lysine assay medium (Difco Co., Detroit, Mich.) was prepared as described previously (12).

Cultivation of the organisms. Cell cultures were grown and allowed to sporulate as described previously (12).

Endotrophic sporulation (9) was used to study the effects of calcium on the synthesis of DHDPA synthase during sporulation of *B. cereus*. Cells were grown in modified G medium lacking $CaCl_2$. During the early stationary phase, 50-ml portions of the cell culture were centrifuged at room temperature at 1,000

$\times g$ for 5 min. The cells were washed twice with sterile distilled water and then suspended in 50 ml of sterile distilled water containing varying concentrations of $CaCl_2$. They were incubated at 30 C on a rotary shaker and allowed to sporulate.

Determination of cell dry weight. The cells that were to be weighed were centrifuged from the growth medium, washed once with distilled water, and placed in tared aluminum weighing containers. The cells were dried overnight in a hot-air drying oven at 80 C and then weighed again.

Preparation of cell extracts. Cells were harvested, washed once in the breakage and dialysis buffer (see below), and then frozen. The cells were thawed, suspended in buffer, and broken routinely with a modified French pressure cell (20). The cell extracts were prepared by centrifuging the disrupted cells at 20,000 $\times g$ for 15 min at 4 C. The cells were washed, broken, and dialyzed overnight in 0.05 M sodium barbital buffer (pH 8.9) containing 2.0 M NaCl.

When it was necessary to have complete breakage of both the sporangia and spores, a Braun MSK cell homogenizer (VWR Scientific, Denver) was used. A cell suspension (15 ml) was added to each canister with 40 g of glass beads (diameter, 0.17 to 0.18 mm). Five 1-min pulses were used to break each sample. The canisters were placed in ice for at least 5 min between each pulse.

Cell extracts prepared as described above were used as the enzyme preparation in this study.

Extraction and assay of dipicolinic acid. The culture medium (50 ml) was acidified to pH 1.5 with 4 M HCl and extracted with three 100-ml portions of ethyl acetate. The ethyl acetate was evaporated to dryness, and the residue was dissolved in 5 ml of water. The resulting solution was then assayed for dipicolinic acid by the method of Janssen et al. (14). This assay was also used for determination of the dipicolinic acid content of spores.

Protein determination. Protein concentrations were determined by the biuret assay (10), with bovine serum albumin as a standard.

Pyruvate pool determination. Cells were harvested from 30 ml of culture by filtration with a membrane filter (type HA, 0.45 μm; Millipore Corp.). The filter was immediately placed in a test tube containing 6 ml of cold 3% perchloric acid. The pyruvate extracted was assayed with lactate dehydrogenase (Sigma Chemical Co.) (16).

Synthesis of L-ASA. The synthesis of L-ASA was accomplished by ozonolysis of L-allylglycine (5). The L-ASA produced was assayed by the homoserine dehydrogenase assay of Pattee and associates (15). The L-ASA was stored in 4 N HCl at −20 C.

Assay of DHDPA synthase. DHDPA synthase was assayed by a modification (17) of the *o*-aminobenzaldehyde assay (23). Unless otherwise specified, the reaction mixture contained: Tris-hydrochloride (pH 8.4), 0.1 mmol; L-ASA, 0.005 mmol; potassium pyruvate, 0.025 mmol; enzyme solution, 0.05 ml; and *o*-aminobenzaldehyde (K & K Laboratories, Inc., Hollywood, Calif.) solution (absorbancy at 440 nm, 1.6), 0.1 ml. The final volume of the reaction mixture was 0.5 ml.

One unit of enzyme is defined as that catalyzing a 1.0 increase in absorbancy at 520 nm per min at 37 C. Specific activity is defined as units of enzyme per milligram of protein.

Heat denaturation studies. DHDPA synthase from early-stationary-phase cells and sporulating cells was compared with respect to heat denaturation kinetics. Portions of the initial cell extract (0.5 ml) were placed in screwcap tubes and heated at 72 C in a water bath for designated periods of time. The heating was stopped by immersing the tubes in an ice-water bath.

Molecular weight estimation. The molecular weight of DHDPA synthase was estimated by Sephadex G-200 column chromatography as described by Andrews (1) and *Instructions for Protein Molecular Weight Determination* (Pharmacia Fine Chemicals Inc., Piscataway, N.J.).

DEAE-cellulose column chromatography of DHDPA synthase. Whatman DE 52 diethylaminoethyl (DEAE)-cellulose (20 g) was packed into a Glenco column (28 by 1.5 cm). The column was equilibrated overnight at 4 C with a buffer (pH 8.9) containing 0.1 M NaCl, 0.05 M sodium barbital, and 50 mM potassium pyruvate. Initial cell extract containing approximately 20 mg of protein was washed onto the column with 100 ml of equilibration buffer. The enzyme was eluted with a linear gradient developed with 250 ml of a buffer (pH 8.9) containing 0.1 M NaCl, 0.05 M sodium barbital, and 50 mM potassium pyruvate, and with 250 ml of a similar buffer containing 0.3 M NaCl. Fractions (3 ml) were collected at a flow rate of approximately 30 ml/h and assayed for activity.

Polyacrylamide gel electrophoresis. Analytical polyacrylamide gel electrophoresis was conducted by the pH 8.9 discontinuous system described by Davis (7). After electrophoresis, the gels were incubated for 2 h at 37 C in the DHDPA synthase assay mixture. The gels were then rinsed with water and placed in 0.22 M citrate-0.55 M Na_2PO_4 buffer (pH 5.0) for 30 min to allow color development of the bands. The distance traveled by the enzyme divided by the distance traveled by the tracking dye (bromophenol blue) is referred to as the R_f of the enzyme.

RESULTS

Specific activity of DHDPA synthase correlated with stages of development in B. cereus. When cells of *B. cereus* were allowed to grow and sporulate in modified G medium, sporulation was 95 to 100% complete. The synchrony of sporulation was good; refractile spores increased in number over a period of approximately 2 h. The specific activity of DHDPA synthase increased four- to sixfold during sporulation (Fig. 1). The increase in specific activity occurred approximately 1 h before the beginning of synthesis of dipicolinic acid and roughly coincided with the increase in refractility of the spores.

The increase in specific activity could have

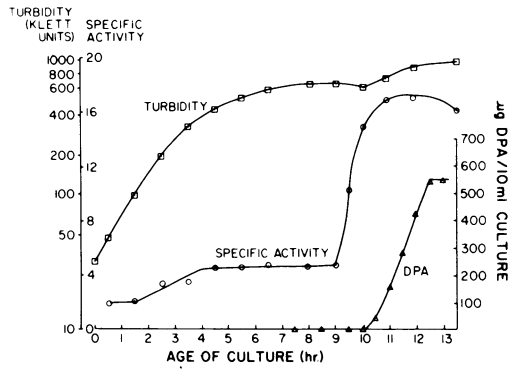

FIG. 1. *Specific activity of DHDPA synthase during growth and sporulation and accumulation of dipicolinic acid in B. cereus 10702.*

been due to a decrease in soluble proteins other than DHDPA synthase during sporulation or to an increase in total enzyme activity, or both. The cells from 100 ml of culture were harvested at the times indicated in Table 1. Complete disruption of the cells was accomplished with a Braun MSK cell homogenizer. The total enzyme activity increased approximately threefold during sporulation, whereas the specific activity increased sixfold. Thus, both phenomena are responsible for the observed increase in specific activity.

Experiments were performed to determine the effect of inhibitors of protein and ribonucleic acid biosynthesis on the increase in specific activity of the enzymes. Chloramphenicol and rifampin were added to separate cultures before and several times during the increase in specific activity. Both inhibitors prevented further increase in specific activity (Fig. 2). Thus, both ribonucleic acid synthesis and protein synthesis are required for the increase in specific activity.

Comparison of DHDPA synthase from early-stationary-phase and late-sporulating cells of B. cereus. Experiments were designed to determine whether the DHDPA synthase from late-sporulating cells could be distinguished from the enzyme from early-stationary-phase cells of *B. cereus* 10702. The term early-stationary-phase cells refers to cells that have finished growth but that do not contain refractile endospores, whereas the term late-sporulating cells refers to cells that contain refractile spores. These terms, used here and subsequently, represent stages of development before and after the increase in specific activity of DHDPA synthase. The data presented in Table 2 indicate that DHDPA synthase from cells in both stages of development is similar or identical.

Effect on activity of DHDPA synthase of mixing initial cell extracts obtained from early-stationary-phase and late-sporulating cells. Mixed-extract experiments were performed to determine whether something in the initial cell extract of late-sporulating cells would cause an increase in activity of DHDPA synthase from early-stationary-phase cells, or whether something in the initial cell extract of early-stationary-phase cells would cause a decrease in activity of the enzyme from late-sporulating cells. Cells at both stages of development were broken, and the enzyme preparations (without centrifugation to remove cell debris and without dialysis) were incubated together for 2 h at 30 C in buffer (pH 7.0 and

TABLE 1. *Comparison of the specific activity of DHDPA synthase with total activity from B. cereus cells harvested at different times and broken in a Braun cell homogenizer*[a]

Age of culture (h)	Sp act[b]	Total activity[c]
5.00	2.92	10.4
6.25	4.70	12.4
7.25	19.10	33.3
8.25	20.40	29.6
9.50	9.85	16.6

[a] There were no unbroken cells or spores remaining after breakage as determined by observation with a phase-contrast microscope.

[b] Units of enzyme per milligram of protein.

[c] Total activity is expressed as the change in optical density (520 nm) per 15 min per ml of culture.

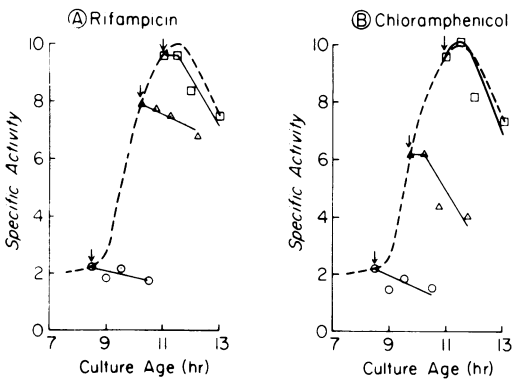

FIG. 2. *Effect of rifampin and chloramphenicol on the increase in specific activity of DHDPA synthase in sporulating cells of B. cereus 10702. The dashed line indicates the increase in specific activity in cells grown in the absence of inhibitor. The arrows indicate time of addition of the inhibitor. The concentrations of rifampin and chloramphenicol used were 10 and 100 μg/ml, respectively.*

TABLE 2. *Properties of dihydrodipicolinate synthase from early-stationary-phase cells and late-sporulating cells of B. cereus 10702*

Determination	Enzyme from early-stationary-phase cells	Enzyme from late-sporulating cells
pH optimum[a]	8.4	8.4
Rate constant (k) for heat inactivation at 72 C	5.1×10^{-4}/s	5.1×10^{-4}/s
K_m (pyruvate)	3.3 mM	4.0 mM
K_m (L-ASA)	0.33 mM	0.45 mM
Apparent mol wt[b]	107,000	107,000
NaCl concn for elution from DEAE-cellulose[b]	0.15 M	0.15 M
R_f on polyacrylamide gels[b]	0.43	0.43

[a] The activity profiles at pH values above and below the optimum were identical for the two enzyme preparations.

[b] Only one peak or band of activity was observed after cochromatography or coelectrophoresis of the two enzyme preparations.

8.9) with and without NaCl (2 M) and pyruvate (0.05 M). Enzyme assays after removal of the cell debris revealed no evidence of any activating or deactivating factor in either extract. The specific activities were equal to the average of the specific activities of the enzyme preparations assayed separately.

Effect on specific activity of DHDPA synthase of the addition of culture medium removed from late-sporulating cells to early-stationary-phase cells. It is possible that some factor present in the medium of late-sporulating cells stimulated synthesis of DHDPA synthase. Alternatively, the absence of some component at this stage of development might allow derepression of enzyme synthesis. To test these possibilities, cells harvested after 6.5 h were suspended in a medium obtained by the removal of cells after development for 10 h. The cells were incubated, and portions of the culture were removed at different times for assay of DHDPA synthase. The specific activity increased at the same time as it did in a control culture; i.e., no premature increase in specific activity occurred.

Effect of medium additives on synthesis of DHDPA synthase in B. cereus 10702 and B. cereus T. The compounds shown in Table 3 were added to cultures of *B. cereus* 10702 to determine whether any of them were involved in control of synthesis of DHDPA synthase. Inducers were expected to cause a premature increase in the specific activity, whereas repressors were expected to abolish the normal increase in specific activity.

Little evidence was found to suggest that any of the combinations tested are of significant importance in control of the enzyme. None caused a premature increase in specific activity (7-h samples). Cultures treated with lysine, lysine plus diaminopimelate, and lysine plus diaminopimelate plus dipicolinic acid had specific activities about 25% lower than the control in the 11.75-h samples. Lysine auxotrophs of *B. cereus* T were grown in lysine assay medium in the presence of limiting amounts of lysine to determine whether synthesis of DHDPA synthase became derepressed as the lysine concentration became limiting. No derepression oc-

TABLE 3. *Effects of addition of various compounds on the synthesis of DHDPA synthase from B. cereus*[a]

Compound[b]	Percentage of control[c]	
	7-h samples	11.75-h samples
Lys	98	72
DPM	99	89
DPA	90	119
Lys + DPM	96	80
Lys + DPM + DPA	91	77
Lys + Met + Thr + Ileu + homoserine (added at 0 and 6 h)	107	92
Lys + Met + Val + Leu + Ileu	135	99
Asp	138	123
Asp + Met + Thr + Ileu + homoserine	155	110
Asp + Leu + Val	95	113
Val + Leu	92	134
Pyruvate	98	88
Lys + Met + Thr + Ileu + 1% Casamino Acids (added at 0 and 6 h)	125	125
Lys + Met + Thr + Ileu + 1% Casamino Acids (added at 0 and 6 h)[d]	93	72

[a] All cells were grown in modified G medium unless specified otherwise. In this medium, growth is limited by some component of yeast extract.

[b] All concentrations of the compounds listed above were 10 mM unless otherwise specified. All compounds were added at 6 h unless otherwise specified. Abbreviations: Lys, lysine; Met, methionine; Thr, threonine; Leu, leucine; Ileu, isoleucine; Val, valine; DPM, diaminopimelic acid; DPA, dipicolinic acid; Asp, aspartic acid.

[c] Specific activity of the enzyme is expressed as a percentage of the specific activity of the enzyme in a control culture.

[d] Cells were grown in G medium, in which growth is limited by glucose.

curred when lysine became limiting (Fig. 3). Three lysine-excreting, thiosine-resistant mutants of *B. cereus* T also exhibited normal patterns of specific activity as a function of stage of development (data not shown).

Effect of calcium concentration in the medium on the increase in specific activity of DHDPA synthase during sporulation in B. cereus. Black et al. (4) demonstrated that sporulating cells deprived of calcium produce spores that are deficient in dipicolinic acid. The observation of Forman and Aronson (8) that calcium is needed for the normal increase in specific activity of DHDPA synthase during sporulation provided a reasonable explanation for this deficiency in dipicolinic acid. Table 4 shows the results of an experiment that was performed with varying concentrations of $CaCl_2$ in the distilled water used for endotrophic sporulation. The dipicolinic acid content of the spores was dependent upon the amount of $CaCl_2$ present during endotrophic sporulation. The specific activity of DHDPA synthase was independent of the $CaCl_2$ concentration, however. We obtained essentially identical data with *B. cereus* T, the strain used by Forman and Aronson (8).

The possibility existed that dipicolinic acid was being formed normally in the absence of $CaCl_2$ but was being released into the medium. To test this hypothesis, the culture medium (50 ml) from cells harvested at 24 h was extracted with ethyl acetate and the dipicolinic acid assay was performed using the extract. No dipicolinic acid was detected. The calcium-dependent reaction required for normal synthesis of dipicolinic acid remains unknown.

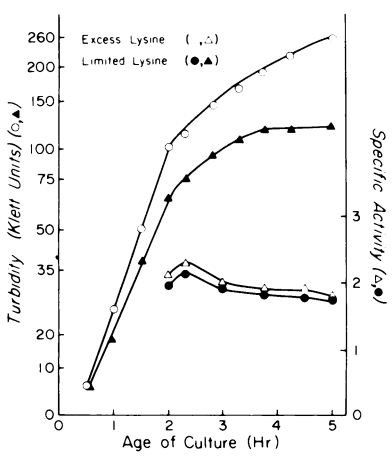

FIG. 3. *Effect of lysine-limited growth on the specific activity of DHDPA synthase in a lysine auxotroph of B. cereus T. Lysine assay medium was the basal medium used for growth. "Excess lysine" refers to the culture supplemented with 1.0 mM lysine. "Limiting lysine" refers to the culture supplemented only with the lysine carried over from a starter culture containing 0.05 mM lysine.*

TABLE 4. *Effect of calcium on specific activity of DHDPA synthase and dipicolinic acid content of sporulating cells of B. cereus 10702*

Percent calcium ($\times 10^2$)[a]	Sp act[b]	Dipicolinic acid content[c]
0.00	9.08	8.9
0.008	7.43	17.4
0.08	10.09	23.4
0.4	8.88	60.6
0.8	8.94	72.3

[a] Percent calcium is given as the amount present in the distilled water used for endotrophic sporulation.

[b] The specific activity (units of enzyme per milligram of protein) was determined when the age of the culture was 9 h. The enzyme was assayed in extracts prepared from cells of different ages. The specific activity was maximal at 9 h.

[c] The amount of dipicolinic acid present in the spores is given as micrograms of dipicolinic acid per milligram of dry spores. The age of the culture was 24 h.

Intracellular control of degradation of DHDPA synthase in B. cereus 10702. We have observed that pyruvate is effective in stabilizing DHDPA synthase in vitro during storage at 4 C. The increase in specific activity during sporulation could be due to a decrease in the rate of enzyme degradation in vivo, perhaps caused by an increased pool size of pyruvate. This hypothesis was tested by measuring the pyruvate pool size during growth and sporulation. The pyruvate pool size remained constant during sporulation. The fact that addition of pyruvate to cells did not cause a premature increase in specific activity (Table 3) constitutes additional evidence that pyruvate does not cause the increase by preventing enzyme degradation. Of course, it is possible that other substances that change in concentration during this time do cause an increase in enzyme stability.

Effects of addition of various aspartate family amino acids and other substances on the activity of DHDPA synthase from B. cereus 10702. Experiments were performed to determine whether the activity of DHDPA synthase from *B. cereus* was affected by various substances (Table 5). There was no evidence for stimulation or inhibition by any of the substances tested.

DISCUSSION

The specific activity of DHDPA synthase was observed to increase four- to sixfold late during

TABLE 5. *Effects of addition of various aspartate family amino acids and other substances on the activity of DHDPA synthase from early-stationary-phase and late-sporulating cells of B. cereus*

Substance added[a]	Activity (% control activity)	
	Enzyme from early-stationary-phase cells[b]	Enzyme from late-sporulating cells[c]
Lys (30 mM)	87	93
DPA (30 mM)	92	98
DPM (30 mM)	96	99
Lys + DPM (10 mM each)	89	94
Lys + DPM + DPA (10 mM each)	86	89
Asp (30 mM)	93	98
Homoserine (30 mM)	86	94
Homoserine + Thr + Met (10 mM each)	94	97
Yeast extract (1.0%)	90	93
Casamino Acids (1.0%)	83	87

[a] The concentrations indicated are the final concentrations in the reaction mixture. Abbreviations are as in Table 3.

[b] The specific activity of the enzyme from early-stationary-phase cells was 3.6 enzyme units per mg of protein. Cells were harvested at 6 h. The reaction was started by addition of enzyme.

[c] The specific activity of the enzyme from late-sporulating cells was 14.3 enzyme units per mg of protein. Cells were harvested at 11.75 h.

sporulation. The total activity was observed to increase threefold. The increase in activity could be due to: (i) a modification of the presporulation enzyme to a more active sporulation form, (ii) synthesis of a different sporulation enzyme, or (iii) more rapid synthesis or less rapid degradation of the presporulation enzyme than occurs earlier in development. Chloramphenicol and rifampin, when added to the cells before and several times during the increase in specific activity, were both able to prevent any further increase in specific activity. The data from these experiments suggest that it is unlikely that a modifying enzyme is altering the early-stationary-phase DHDPA synthase to yield a more active late-sporulation form of the enzyme. If a modifying enzyme was present during the time of increase in specific activity, then it is probable that addition of the inhibitors after the increase began would not have completely prevented further increase in specific activity. The increase would have been expected to continue due to preexisting modifying enzyme, although at a reduced rate, the reduction being due to blockage of synthesis of the modifying enzyme by the inhibitors. The results of the mixed-extract experiment also indicate that modification of DHDPA synthase from early-stationary-phase cells does not occur. It is possible, however, that the incubation conditions were not proper for the activity of a modifying enzyme. Further evidence against enzyme modification was obtained from the studies comparing enzymatic and physical properties of DHDPA synthase obtained from early-stationary-phase and late-sporulation cells. The pH optima, heat denaturation kinetics, apparent K_m values with respect to pyruvate and L-ASA, column chromatographic properties on DEAE-cellulose and Sephadex G-200, and the electrophoretic mobility during polyacrylamide gel electrophoresis indicate that the enzymes from early-stationary-phase and late-sporulating cells are very similar or identical. The results of these experiments also suggest that a new form of DHDPA synthase is not produced during the late stages of sporulation.

Grandgenett and Stahly (11) have shown that diaminopimelate decarboxylase activity becomes nondetectable late in sporulation. The loss in activity is accompanied by a drop in the lysine pool size of the cells. It seemed reasonable to postulate that DHDPA synthase was derepressed as the lysine pool was being depleted. Evidence against this mechanism of control was obtained when lysine was added to the medium during sporulation. The specific activity of DHDPA synthase was not significantly lowered in cells receiving this treatment. Further evidence against repression of DHDPA synthase by lysine was obtained by growing a lysine auxotroph of *B. cereus* in a medium containing a growth-limiting amount of lysine. The enzyme was not derepressed as the lysine was depleted from the medium. The pattern of enzyme activity as a function of stage of development was also normal in thiosine-resistant mutants that excreted lysine. Vold and associates (19) also found no evidence for repression of DHDPA synthase in *B. subtilis* when using lysine auxotrophs and thiosine-resistant mutants. Experiments with intermediates and other end products of the aspartate biosynthetic pathway failed to give evidence for repression or induction of synthesis of DHDPA synthase.

We have been unable to substantiate the findings of Forman and Aronson (8) that calcium is needed for the increase in specific activity of DHDPA synthase. Our data (Table 4) show that calcium is needed for the normal production of dipicolinic acid but is not needed for normal synthesis of DHDPA synthase.

The fact that DHDPA synthase was stabilized in vitro by one of its substrates, pyruvate, suggested that this compound might be stabilizing the enzyme in vivo during sporulation. A decrease in the rate of degradation would result in the observed increase in specific activity of DHDPA synthase. However, the pyruvate pool size was not found to increase late in sporulation when the specific activity of DHDPA synthase increased. Also, the addition of 10 mM potassium pyruvate to cultures before the increase in specific activity of DHDPA synthase (Table 3) failed to cause a premature increase. These data do not rule out the possibility that other substances are stabilizing the enzyme and slowing its rate of degradation during sporulation. The other substrate of DHDPA synthase, L-ASA, would be a possible stabilizer of the enzyme. Enzyme stability studies with this compound are difficult due to its instability at physiological pH values.

The above studies have failed to reveal a control mechanism for DHDPA synthase. Perhaps there is an increased rate of synthesis of the enzyme owing to a change in the transcribing ability of the ribonucleic acid (RNA) polymerase during sporulation. This possibility is discussed in more detail in a subsequent publication (13).

In addition to the absence of success in finding a low-molecular-weight substance that induced or repressed synthesis of DHDPA synthase, no substance was found that activated or inhibited the enzyme (Table 5). Thus, this enzyme appears to be protected from environmental alteration. Perhaps the increase in specific activity of DHDPA synthase late in sporulation is a key step in sporulation. This is logical in that a large quantity of dipicolinic acid must be made for incorporation into spore core, as well as diaminopimelic acid for inclusion into spore cortex peptidoglycan. If continued cell differentiation depended on the increase in activity, it obviously would be of value to the cell to have a system that was not influenced by changes in the medium composition.

ACKNOWLEDGMENTS

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