JOURNAL OF BACTERIOLOGY, Mar. 1977, p. 1440-1447

Vol. 129, No. 3
Printed in U.S.A.

Sodium Effect of Growth on Aspartate and Genetic Analysis of a *Bacillus subtilis* Mutant with High Aspartase Activity

T. IIJIMA, M. D. DIESTERHAFT, AND E. FREESE*

Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland 20014

Received for publication 6 October 1976

Most strains of *Bacillus subtilis*, derived from the 168 (Marburg) strain, grow slowly on aspartate as sole carbon source. We isolated a mutant (*aspH*) that grows rapidly on aspartate because it produces aspartase constitutively. Thus, aspartase is needed for rapid growth on aspartate, whereas aspartate-α-ketoglutarate aminotransferase is not needed, as was demonstrated by a mutant lacking that enzyme activity. By two- and three-factor crosses using PBS1 transduction, the *aspH* mutation was located between the *aroD* and the *lys* markers of the genetic map. Although sodium ions do not affect growth on glucose or L-malate, they specifically stimulate growth on aspartate in both the parent and the *aspH* mutant strains. Enzyme activities of crude aspartase and fumarase and of purified aspartase do not increase in the presence of sodium. These results show that stimulation by sodium involves some reaction other than the enzymes catabolizing aspartate. The ease of purification from the *aspH* strain and the stability of aspartase suggest that the *B. subtilis* enzyme is particularly useful for aspartate determinations.

In some bacteria, such as *Bacillus megaterium* (P. H. Cooney and B. Freese, J. Gen. Microbiol., in press) or certain strains of *Escherichia coli* (2, 4), L-aspartate and/or L-glutamate can be used as rapidly metabolizable sole carbon sources for growth. Strains of *B. subtilis*, derived from the transformable 168 strain, generally grow only slowly on aspartate and almost not at all on glutamate as sole source of carbon and energy. Glutamate is frequently utilized as a slowly metabolizable carbon source for sporulation (13). In this paper, a mutant strain (gene symbol *aspH*) of *B. subtilis*, which grows rapidly on sodium aspartate but still not on glutamate is described. The *aspH* mutation produces high aspartase activity which can be easily purified. Its genetic location has been determined.

MATERIALS AND METHODS

Microorganisms. The strains used in this work are listed in Table 1. All are derivatives of the 168 strain of *B. subtilis* Marburg.

Media and growth conditions. The salts base (S5), used for growth in liquid medium, contained 30 mM $(NH_4)_2SO_4$, 5 mM K_2HPO_4/KH_2PO_4, pH 6.8, 1 mM $MgSO_4$ 30 μM $FeSO_4$, 40 μM $MnSO_4$, 50 μM $CaCl_2$ and 25 μg of L-tryptophan per ml; as carbon source, 25 or 50 mM potassium aspartate (pH 6.8) or 50 mM glucose was added. In some cases, 10 mM NaCl was added to S5 (S5 + Na) to allow rapid growth on aspartate. Petri plates contained about 25 ml of the same media and 1.5% agar (Difco Laboratories).

Cells were grown for 15 to 20 h on plates containing 33 g of tryptose blood agar base (TBAB; DIFCO) per liter and inoculated at an initial absorbancy at 600 nm (A_{600}) of 0.05. The growth reported in Fig. 1 was followed at 37°C right after inoculation. For all other growth experiments, cells were adapted to the synthetic medium by overnight incubation at room temperature; the cells were then centrifuged at room temperature and suspended at A_{600} of 0.05 in fresh synthetic medium, and growth at 37°C was followed. Without this adaptation, extremely long and variable lag times were observed.

For transformation and the selection of transductants, the minimal glucose medium (MG) of Anagnostopoulos and Spizizen (1) was utilized. The salts of that medium, without citrate and glucose, are called N. PBS1 phages were multiplied in antibiotic medium 3 (AM3; Difco) using various donor bacteria.

Selection of *aspH* mutants. Cells of strain 60002, growing exponentially in S5 plus Na plus 25 mM aspartate were plated on the same medium. After 48 h, colonies were restreaked on fresh aspartate plates. This strain selection was continued until several strains that gave good growth within 24 h were obtained. Strain 61501 (*aspH1*) was one of these mutants (Table 1). It required tryptophan for growth and retained the ability to grow rapidly on aspartate after 15 to 20 serial transfers on TBAB plates.

Preparation of cell-free extracts. Cells harvested in late exponential growth were washed twice with

TABLE 1. *Bacterial strains used in genetic analysis*

Strain	Genotype	Origin
60002	*trpC2*	Spizizen (168)
61459	*aspB66 trpC2*	J. Hoch (C66), lacks L-aspartate-α-ketoglutarate aminotransferase activity; requires aspartate for growth
61469	*lys-3 trpC2 metB10*	F. Young (BR151)
61501	*aspH1 trpC2*	Spontaneous mutant of 60002 isolated for rapid growth on aspartate.
61502	*aroD120 trpC2*	J. Hoch (SB120)
61503	*aroD120 lys-3 trpC2 gtaC*	Tf[a] of 61531 by 61502
61522	*aroB trpC2 hisB tyrA metB10*	F. Young (RUB 834)
61523	*lys-3 aroB trpC2 hisB tyrA*	Tf of 61522 by 61531
61531	*lys-3 trpC2 argC2 gtaC*	Tf of 61530 by 61236

[a] Tf implies transformation of the first strain by the DNA of the second strain.

0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), and the pellet was stored at −40°C. Cells were suspended in the same buffer at a concentration of 0.1 g/ml (wet weight) and broken by passage through a French pressure cell at 1,500 lb/in^2. Whole cells and debris were removed by centrifugation and the supernatant was retained for assay. Protein was determined by the biuret reaction (3).

Enzyme assays. Aspartate-α-ketoglutarate aminotransferase was assayed by coupling the reaction to malic dehydrogenase. The assay mixture contained 0.05 M Tris-hydrochloride (pH 7.5), 10 mM α-ketoglutarate, 0.5 mM reduced nicotinamide adeninedinucleotide, 1 IU of malic dehydrogenase, 20 μM pyridoxal phosphate, and an amount of extract containing 0.1 to 0.5 mg of protein. After 5 min of equilibration at 25°C, the rate of decrease of the A_{340} was determined in the absence and presence of 20 mM aspartate, and the difference was used to calculate the enzyme activity. This rate difference increased linearly with enzyme concentration. The specific activity of the *aspB* mutant (61487; Table 1) was less than 10% of that of strain 60002.

Aspartase was assayed by two methods. (i) Colorimetric assay: the reaction mixture contained 50 mM Tris-hydrochloride (pH 7.5), and 20 mM Tris-aspartate and cell extract; it was incubated at 30°C. Controls contained no extract or no aspartate. For the parent strain (60002), an amount of extract containing 0.5 to 2.0 mg of protein was used per ml of reaction mixture, and samples were taken at 15-min intervals for 1 h. For the *aspH* mutant (61501), 0.02 to 0.10 mg of extract protein was added, and samples were taken at 3-min intervals for 12 min. The reaction was stopped by the addition of 1 ml of Nessler reagent (Fisher Scientific Co., Pittsburgh, Pa.) per ml of reaction mixture, and the color formation was read immediately at 470 nm. The color increased linearly in the range of 0.1 to 5.0 mM NH_3. (ii) Direct assay: the reaction mixture contained 0.05 M Tris-hydrochloride (pH 7.5), 20 mM Tris-aspartate, and 0.01 to 0.05 mg of protein of extract per ml. The increase in A_{240}, which results from the formation of fumarate, was followed (14). This assay could be used with the extracts of the *aspH* mutants but not with those of the parent strain because it required high aspartase activity.

Fumarase was assayed according to Racker (10) by the increase in A_{240} owing to the fumarate produced. The reaction mixture in 0.05 M Tris-hydrochloride (pH 7.5) contained 20 mM potassium L-malate and about 0.05 mg of extract protein per ml.

The Malic Dehydrogenase assay contained 0.1 M Tris-hydrochloride (pH 8.8), 1 mM oxalacetate, and 0.15 mM reduced nicotinamide adenine dinucleotide.

Transduction. Transduction by PBS1 phage was performed according to Takahashi (13) and Hoch et al. (8) with the following modifications. To obtain phage lysates, motile donor cells were grown in AM3 to an A_{600} of about 0.5 and infected at a multiplicity of 0.1, and the mixture was shaken for 2 to 3 h at 37°C. Chloramphenicol (5 μg/ml) was then added, and the culture was left overnight (18 h) at 37°C without shaking. After addition of a few drops of chloroform and centrifugation for 10 min at 8,000 × *g*, deoxyribonuclease (10 μg/ml) was added to the clear supernatant. After 30 min at 37°C, the phage suspension was filtered through sterile membrane filters (pore size 0.65 μm; Millipore Corp.) to remove bacteria. For plaque assay, strain 61490 (*trpB* of Burkholder and Giles) was grown in AM3 medium to early stationary phase; properly diluted phages were added, and the mixture was shaken for 20 min at 37°C. A 0.2-ml portion of the bacteria-phage mixture was plated with 2.5 ml of soft AM3 agar (0.7%) on 1-day-old TBAB plates which were then incubated for 18 to 22 h at room temperature (25°C). For transduction experiments, cells grown in AM3 to an A_{600} of 1.5 were infected at a multiplicity of 0.1 to 1.0; after 20 min of slow shaking at 37°C to allow adsorption, the cells were centrifuged, resuspended in N medium and plated on selective media (usually MG plus additives). Transductants for auxotrophic markers were streaked in patches on the same selective plates and then examined for unselected markers by replica plating. The selective scoring medium for *aspH* was S5 plus sodium plus 25 mM aspartate plus additives, aspartate being the only carbon source.

Transformation. Deoxyribonucleic acid (DNA) was obtained by the method of Saito and Miura (11). A 2.5-μg portion of donor strain DNA was added per ml of recipient bacteria grown according to Anag-

nostopoulos and Spizizen (1).

Aspartase purification. Strain 60501 was grown in S5 plus sodium plus 50 mM potassium aspartate to the end of exponential growth, and cell extract was prepared from 5,000 A_{600} units of cells as described above. Protamine sulfate was added to 1% (wt/wt) of the amount of protein, and the pellet was discarded. Ammonium sulfate (salt) was added to give a final concentration of 50% saturation. The precipitate contained 50% of the protein and 80% of the aspartase activity (see Table 7). The pellet was suspended in 0.05 M Tris-PO_4, (pH 7.5), dialyzed for 4 h against several changes of this buffer, and then applied to a diethylaminoethyl (DEAE)-Sephadex (A-25) column (12 mm diameter by 10 cm long) which had been equilibrated with the same buffer. All aspartase activity remained on the column through a washing step and the first (linear) gradient of 0.05 to 0.5 M Tris-PO_4. The enzyme eluted soon after the start of the second gradient of 0.5 to 1.0 M Tris-PO_4 and was concentrated by ultrafiltration. The resulting preparation was free of fumarase activity. Addition of 10 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid, or 5 mM mercaptoethanol did not affect enzyme activity.

RESULTS

Isolation and properties of an *aspH* mutant. When cells of the 168 Marburg strain of *B. subtilis* (our isolate [called 60002] was *trpC2*; Table 1) were inoculated from a rich medium such as nutrient broth into a minimal medium (S5 plus sodium plus 25 mM aspartate; see Materials and Methods) they multiplied at the low rate shown by the lower curve in Fig. 1. Some cultures of this strain, or of mutants derived from the 168 strain, grew after different lag times at slowly increasing rates, indicating the selection of more rapidly growing mutants. Such mutants were isolated (see Materials and Methods) and one of them (61501 [*aspH1 trpC2*]) was used for further experiments (Table 1). This *aspH* mutant, which retained its high rate of growth (Fig. 1) after 20 serial transfers on TBAB plates, still grew extremely slowly on glutamate as sole carbon source. Attempts to isolate a strain that would grow well on glutamate have been unsuccessful.

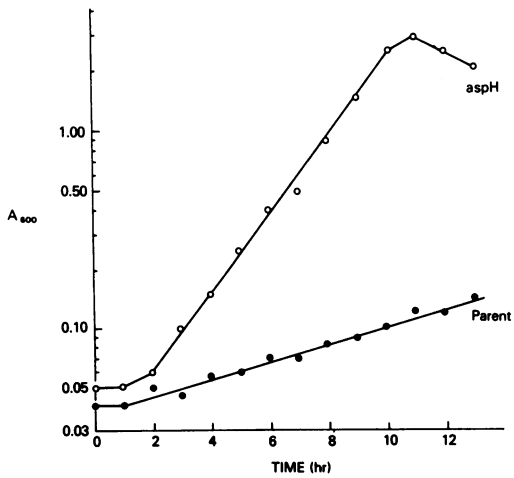

FIG. 1. *Growth of B. subtilis on L-aspartate as sole carbon source. Cells grown overnight on TBAB plates were inoculated at an A_{600} of about 0.05 into medium S5 plus sodium plus 25 mM potassium-L-aspartate. Parent strain (60002 [trpC2])* (●); *aspH1 trpC2 mutant (61501)* (○).

Table 2 shows that the parent strain (60002) had a very low aspartase activity after growth on glucose and a higher activity after growth on aspartate. Apparently, the aspartase of normal strains of *B. subtilis* is repressed by catabolites such as glucose, similar to the control described in *E. coli* by Halpern and Umbarger (5). In contrast, the *aspH* mutant (61501) contained a high aspartase activity, irrespective of whether it was grown on glucose or aspartate as sole carbon source; thus, the *aspH* strain may have a constitutive aspartase. The specific activities of aspartate-α-ketoglutarate aminotransferase, fumarase, and malic dehydrogenase were not affected by the *aspH* mutation. The aminotransferase, in particular, had negligible significance for rapid growth on aspartate as sole carbon source. This was conclusively shown by the fact that an aminotransferase mutant (*aspB*[61459]) obtained from Hoch (7) (Table 1) could grow on aspartate as sole carbon source as well as could the parent strain (60002). However, the aminotransferase activity is required for the de novo synthesis of aspartate, since Hoch has shown and we confirm that the *aspB* mutant cannot grow (in synthetic medium) without L-aspartate (8).

Genetic mapping of the *aspH* mutation. Transducing phages PBS1, grown in the *aspH* strain (61501), were added to strains containing different auxotrophic markers that were spread all over the genetic map. Prototrophic colonies were selected and tested for their rate of growth on aspartate as sole carbon source. The *aspH1* mutation was found to map closely to the *lys*, *hisB*, and *tyrA* markers (Table 3). The results of three-factor crosses in Table 4 established the order of markers as *aroD aspH lys tyrA*, and they showed that the frequency of two recombinations within one long transducing DNA piece (or of transduction by two different pieces) was extremely low. A map is given in Fig. 2.

The correlation between high aspartase activity and *aspH* (identified by good growth on aspartate as sole carbon source) bred true when *aspH* was transduced to another strain, as was

TABLE 2. *Specific activities of several enzymes involved in aspartate metabolism* [a]

Enzyme	Sp act in parent strain (60002)		Sp act in *aspH* strain (61501)	
	Glucose	Aspartate	Glucose	Aspartate
Aspartase	0.025	0.17	1.50	1.67
Fumarase	0.3	0.5	0.5	0.7
Malic dehydrogenase	0.5	1.0	0.4	1.2
Aspartate-α-ketoglutarate aminotransferase	0.02	0.03	0.03	0.04

[a] All strains were grown on S5 plus sodium plus 20 mM of the indicated carbon source and were harvested, prepared, and assayed according to Materials and Methods. Aspartase was assayed in both strains using the colorimetric assay; the direct assay for fumarate production gave similar results in the *aspH* strain but could not be used for the parent strain. Specific Activity, micromoles per minute per milligram of protein.

TABLE 3. *Linkage of aspH to lys, hisB, and tyrA* [a]

PBS1 recipient[b]	Selected marker	Growth on aspartate		Total no. tested	Recombination frequency (%)
		Slow	Rapid (*aspH*)		
61469 (*lys trpC metB*)	*lys*	135	305	440	31
61522 (*aroB trpC hisB tyrA metB*)	*his*	275	140	415	66
61522 (*aroB trpC hisB tyrA metB*)	*tyr*	215	162	377	57

[a] Selection for the following markers in other crosses showed no cotransduction with *aspH* (rapid growth in aspartate) in 60 to 130 transductants: *hisA, thr, leu, pheA, citB, pyrA, argC, cysA*. Growth on aspartate was tested as described in Materials and Methods.

[b] Donor strain: 61501 (*aspH trpC*).

TABLE 4. *Three-factor crosses for aspH*

PBS1 recipient[a]	Combinations of genotypes[b]			No. of recombinants	Frequency (%)
	aspH	*lys*	*tyrA*		
61523 (*lys aroB trpC hisB tyrA*)	1	1^s	1	186	42.2
	1	1^s	0	120	27.2
	0	1^s	1	61	13.8
	0	1^s	0	74	16.8
	1	1	1^s	160	42.4
	0	1	1^s	67	17.8
	1	0	1^s	2	0.5
	0	0	1^s	148	39.3
	aroD	*aspH*	*lys*		
61503 (*aroD lys trpC*)	1	1	1^s	11	5.0
	0	1	1^s	134	60.6
	1	0	1^s	0	0
	0	0	1^s	76	34.4
	1^s	1	1	14	3.6
	1^s	1	0	10	2.5
	1^s	0	1	0	0
	1^s	0	0	370	93.9

[a] Donor strain: 61501.

[b] 0, Recipient type for the particular gene. 1, donor type for the particular gene. 1^s, selected donor type.

demonstrated for transductants of the first cross described in Table 3. In contrast to the recipient strain (61469), the *aspH* transductants exhibited the high growth rate (on sodium-aspartate) and the high aspartase activity of the *aspH* donor (61501).

Sodium-dependent growth on aspartate. Both the parent strain (60002) (Fig. 3a) and the *aspH* mutant (61501) (Fig. 3b) grew on aspartate at a distinctly higher rate in the presence of sodium than in its absence. This effect was specific for sodium since the chlorides of Li, Cs,

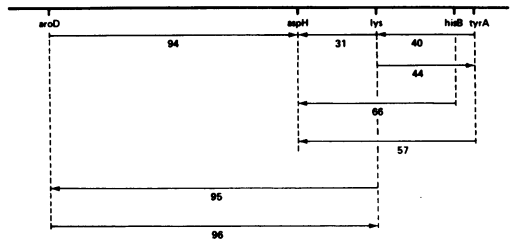

FIG. 2. *Genetic map of the area around the aspH1 marker. The arrows are directed from the selected to the unselected marker and the numbers represent the corresponding recombination frequencies in percent.*

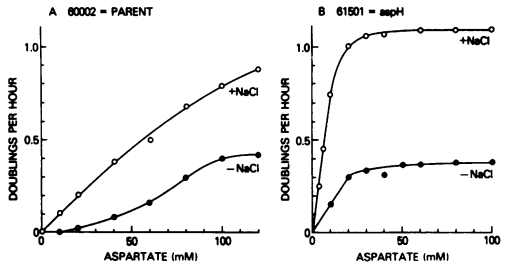

FIG. 3. *Concentration dependence of growth on aspartate in the presence (○) and absence (●) of sodium. The parent strain (60002) and the aspH strain (61501) were grown in S5 plus different concentrations of potassium L-aspartate ± 10 mM NaCl. (A) Parent strain. (B) aspH mutant.*

and Rb had no effect (Table 5). The growth stimulation increased rapidly with the sodium concentration to about 8 mM Na^+ and then continued to increase very slowly (Fig. 4). Growth in S5 plus glucose or L-malate was not stimulated by sodium; high sodium concentrations actually slightly reduced the growth rate in the glucose medium (Fig. 4).

The medium (S5) used here contained about 8 mM potassium ions as phosphate salt and, where used, 50 mM potassium as aspartate salt. If potassium was in all salts replaced by ammonium (or Tris), almost no growth was observed in either glucose or aspartate even in the presence of 10 mM NaCl (Fig. 5). The growth rate on either carbon source rapidly increased with the concentration of KCl addition, about 2 mM KCl giving maximal growth (Fig. 5). The growth requirement for K^+ is therefore not specific to aspartate. In all other experiments of this paper the growth media contained at least 8 mM K^+.

When aspartate was not used as sole carbon source but only as growth supplement of the aspartate-requiring *aspB* mutant (61487) (grown in S5 plus glucose), 1 mM potassium aspartate sufficed to allow growth at the maximal rate; thus, the addition of 10 mM of NaCl did not affect the growth rate at any aspartate concentration.

Purification and properties of aspartase. The sodium effect could result from an increase of aspartate transport or aspartate metabolism. To examine the latter possibility, the effect of sodium on the activities of the first three enzymes needed to metabolize aspartate via the citrate cycle and of the aspartate-α-ketoglutarate aminotransferase were measured (Table 6). In the presence of excess substrate, no significant change in the activity of any of these enzymes was observed when 10 mM NaCl was added to the reaction mixture. In particular, aspartase activity showed no increase when 10 mM NaCl or KCl was added to crude cell extracts of the *aspH* mutant grown in S5 + potassium aspartate or glucose or both carbon sources with or without NaCl (not shown).

Since aspartase is the first enzyme of aspartate catabolism, it was purified from extracts of the *aspH* strain as described in Table 7. The enzyme remained stable throughout purification and was free from fumarase activity after

TABLE 5. *Effect of alkali ions on the growth of the aspH strain*[a]

Addition	Doublings/h
None	0.33
NaCl	0.80
LiCl	0.32
CsCl	0.34
RbCl	0.31

[a] Strain 61501 was grown in S5 plus 50 mM potassium L-aspartate in the presence of 10 mM of the stated salts. The medium contained 58 mM total potassium.

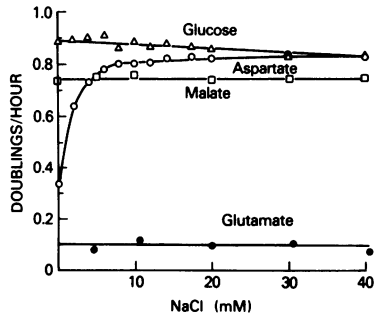

FIG. 4. *Sodium concentration dependence of growth. The aspH strain was grown from an initial A_{600} of 0.05 in S5 plus different concentrations of NaCl with 50 mM potassium L-aspartate (○), L-glutamate (●) or L-malate (□), or with 50 mM D-glucose (△) as the sole carbon source.*

the DEAE-Sephadex-column step. Since the specific activity of aspartase was already six to nine times higher in the *aspH* strain than in its parent, the purified enzyme had more than 150 times the specific activity of the standard strain. In kinetic analysis, the enzyme displayed saturation at about 6 mM potassium or Tris-L-aspartate, the potassium salt giving a higher maximal activity (Fig. 6). The saturation curves obtained with Tris-aspartate in the presence of 10 mM NaCl or KCl were similar to the one obtained without these salts. Thus, NaCl did not increase the aspartase activity at any (even nonsaturating) aspartase concentration. Aspartase reacted specifically with L-aspartate, 20 mM D-aspartate and DL-threo-β-hydroxyaspartate giving 10% of the deamination rate of L-aspartate. Figure 7 shows the effect of NaCl and KCl on the aspartase activity, measured with 20 mM Tris-aspartate. The activity was not significantly affected by concentrations of these salts employed for cell growth. At higher concentrations, both salts inhibited aspartase, the effect of NaCl being more pronounced.

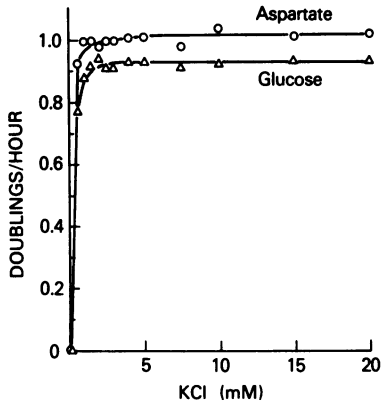

FIG. 5. *Potassium concentration dependence of growth. The aspH strain (61501) was grown in S5, in which all potassium was replaced by NH_4^+, plus 50 mM ammonium-aspartate* (○) *or 50 mM glucose* (△). *KCl was added to these media of different concentrations.*

DISCUSSION

Our results demonstrate that *B. subtilis* grows on aspartate as sole source of carbon and energy at a distinctly higher rate in the presence of sodium ions (optimum 8 to 10 mM); the effect is specific to Na^+. Growth on glucose or malate as sole carbon source is not affected by Na^+. The growth rate on aspartate is further enhanced by a mutation (*aspH*) which causes the production of a high (apparently constitutive) aspartase activity. An additional (*aspB*) mutation removing aspartate-α-ketoglutarate aminotransferase activity does not affect growth on aspartate. This shows that aspartase is necessary for rapid growth on aspartate as sole carbon source. At 25 mM aspartate, the effects of the *aspH* mutation and of sodium are additive. Thus, both aspartase activity and the

TABLE 6. *Absence of a sodium effect on the specific activities of several enzymes involved in aspartate catabolism*[a]

Enzyme	Sp act in parent strain (60002)		Sp act in *aspH* strain (61501)	
	−NaCl	+NaCl	−NaCl	+NaCl
Aspartase	0.025	0.022	1.6	1.3
Fumarase	0.4	0.3	0.5	0.45
Malic dehydrogenase	0.6	0.5	0.4	0.3
Aspartate-α-ketoglutarate aminotransferase	0.03	0.02	0.04	0.05

[a] Cells were grown in S5 plus glucose, and extracts were assayed for enzyme activity as in the legend to Table 1, except that 10 mM NaCl was included in the reaction mixture where indicated. Specific activity, micromoles per minute per milligram of protein.

TABLE 7. *Purification of aspartase from strain 61501*[a]

Step	Volume (ml)	Protein (mg/ml)	Total protein (mg)	Sp act (U of protein per mg)	Total activity (U)
1. Extract	80	8.0	640	1.0	655
2. Protamine sulfate	84	7.1	590	1.1	630
3. Ammonium sulfate					
0 to 50%	20	11	220	2.5	550
50 to 75%	20	12	240	0.08	20
4. DEAE-Sephadex	1.6	9	15	30.0	450

[a] For details, see Materials and Methods. Aspartase activity was measured by the direct assay.

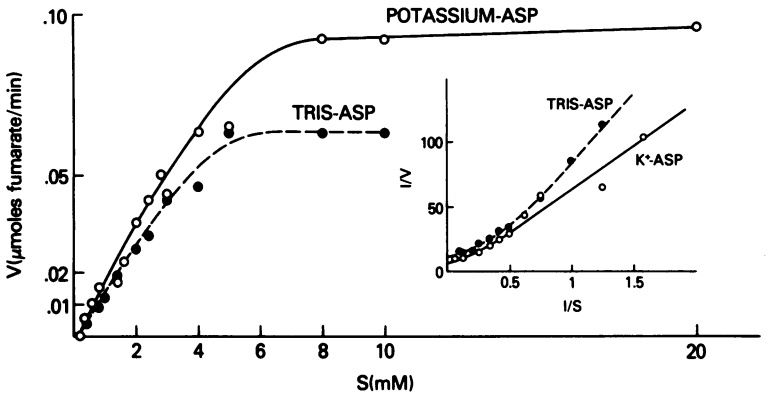

FIG. 6. *Concentration dependence of aspartase activity. Partially purified aspartase was assayed by the increase in A_{240} due to fumarate production. Substrates were K-aspartate (○) or Tris-aspartate (●). Addition of 50 mM NaCl to the reaction mixture reduced the aspartase activity by 50 to 60% at all concentrations of Tris-aspartate, whereas addition of 10 mM NaCl reduced the rates by about 10%. Insert: double reciprocal plot.*

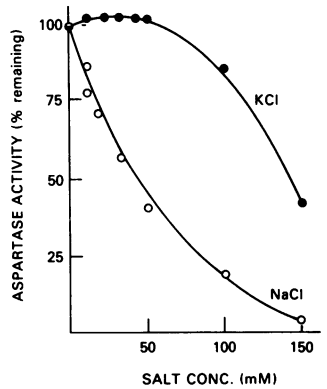

FIG. 7. *Inhibition of aspartase by high concentrations of salt. Aspartase, purified as described in Table 7, was assayed in the presence of different concentrations of NaCl (○) and KCl (●) by the spectrophotometric method using 20 mM Tris-L-aspartate as substrate.*

activity controlled by sodium limit the rate of growth on aspartate. While aspartase is only needed for aspartate catabolism, as was already suggested by Halpern and Umbarger for *E. coli* (5), aspartate-α-ketoglutarate aminotransferase is only needed for aspartate synthesis in the absence of extracellular aspartate.

Aspartate is utilized as carbon and energy source by its metabolism to fumarate, malate, and then via the rest of the citrate cycle; it can also be used for gluconeogenesis. Since Na^+ does not enhance the growth on L-malate, it could stimulate aspartate catabolism only by increasing the activity of the first two enzymes, aspartase or fumarase. We did not find such an effect. In particular, the activity of the first metabolic enzyme, aspartase, is not stimulated by Na^+ at any aspartate concentration (high Na^+ concentrations actually inhibit the enzyme). Therefore, we conclude that sodium does not affect the rate of intracellular aspartate catabolism.

In other microorganisms, growth on L-glutamate requires sodium ions (2), apparently to activate glutamate transport, as was reported for *E. coli* (4, 9) and *Mycobacterium phlei* (6). In *B. subtilis*, the maximal rate of growth of the *aspH* mutant is obtained at about 30 mM aspartate, whereas the maximal rate of (isolated) aspartase is obtained at about 6 mM aspartate. Therefore, at up to 30 mM aspartate the rate of growth of the *aspH* mutant is apparently limited by the rate of aspartate transport rather than by that of catabolism. Since the growth rates obtained at different aspartate concentrations in the presence of Na^+ reach a higher plateau than those obtained without Na^+, but both saturate at about 30 mM, Na might increase the maximal velocity of aspartate transport. However, it is also possible that Na^+ prevents the production or function of a growth inhibitor that is derived from aspartate. These alternatives are under investigation.

ACKNOWLEDGMENTS

We wish to thank Cheryl Marks for her excellent assistance.

LITERATURE CITED

1. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. 81:741–746.
2. **Frank, L., and I. Hopkins.** 1969. Sodium-stimulated transport of glutamate in *Escherichia coli*. J. Bacteriol. 100:329–336.
3. **Gornall, A. G., C. J. Bardowill, and M. M. David.** 1949. Determination of serum protein by means of the biu-

ret. J. Biol. Chem. 177:751-766.
4. **Halpern, Y. S., H. Barash, S. Dover, and K. Druck.** 1973. Sodium and potassium requirements for active transport of glutamate in *Escherichia coli* K-12. J. Bacteriol. 114:53-58.
5. **Halpern, Y. S., and H. E. Umbarger.** 1960. Conversion of ammonia to amino groups in *Escherichia coli*. J. Bacteriol. 80:285-288.
6. **Hirata, H., F. C. Kosmukos, and A. F. Brodie.** 1974. Active transport of proline in membrane preparations from *Mycobacterium phlei*. J. Biol. Chem. 249:6965-6970.
7. **Hoch, J.** 1972. Genetic studies in *Bacillus subtilis*, p. 113-116. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
8. **Hoch, J., M. Barat, and C. Anagnostopoulos.** 1967. Transformation and transduction in recombination-defective mutants of *Bacillus subtilis*. J. Bacteriol. 93:1925-1937.
9. **Miner, K. M., and L. Frank.** 1974. Sodium-stimulated glutamate transport in osmotically shocked cells and membrane vesicles of *Escherichia coli*. J. Bacteriol. 117:1093-1098.
10. **Racker, E.** 1950. Spectrophotometric measurements of the enzymatic formation of fumaric and cis-aconitic acids. Biochim. Biophys. Acta 4:211-214.
11. **Saito, H., and K. Miura.** 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. Biophys. Acta 72:619-624.
12. **Sterlini, J. M., and J. Mandelstam.** 1969. Commitment to sporulation in *Bacillus subtilis* and its relationship to development of actinomycin resistance. Biochem. J. 113:20-37.
13. **Takahashi, I.** 1966. Joint transfer of genetic markers in *Bacillus subtilis*. J. Bacteriol. 91:101-105.
14. **Williams, V. R., and D. J. Lartigue.** 1967. Quarternary structure and certain allosteric properties of aspartase. J. Biol. Chem. 242:2973-2980.