

Evidence for a Low Affinity but High Velocity Aspartate Transport System Needed for Rapid Growth of *Bacillus subtilis* on Aspartate as Sole Carbon Source

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Mutants (*aspT*) of *Bacillus subtilis* which lack the high affinity transport of L-aspartate and L-glutamate have been isolated by their resistance to DL-threo- β -hydroxyaspartate. They transport low concentrations (100 μM) of all three amino acids at a greatly reduced rate but they can still grow at the normal rate on high concentrations of aspartate (25 mM) as sole carbon source. The *aspT* mutation has been mapped between *argC* and *glpK*, *glpD*. In glucose/citrate medium, an *aspB* mutant, auxotrophic for aspartate, requires much lower aspartate concentrations for growth than an *aspB aspT* double mutant. These results demonstrate that a low affinity aspartate transport system is still present in *aspT* mutant strains. This was also shown by the fact that both *aspH* and *aspH aspT* mutants, which carry the gene (*aspH*) for high aspartase production, grew at the same rate in media containing high concentrations (25 mM) of L-aspartate as sole carbon source. The high affinity aspartate transport system ($K_m = 67 \mu\text{M}$) alone can satisfy the growth requirements of aspartate auxotrophs in media containing glucose or some other carbon source. However, the maximum rate (V_{max}) of this transport system is too low to allow rapid growth on aspartate as sole carbon source. For such rapid uptake the low affinity aspartate transport system is needed. Membrane vesicles, energized by glycerophosphate, exhibit only the active high affinity transport which is saturated at about 100 μM -L-aspartate ($K_m = 66 \mu\text{M}$). Apparently, the proton motive force, which energizes the high affinity aspartate (and glutamate) transport, is not (directly) required for the low affinity aspartate transport.

INTRODUCTION

Intact cells of *Bacillus subtilis* can take up high concentrations of L-malate at a faster rate (Fournier *et al.*, 1972) than can membrane vesicles (Bisschop *et al.*, 1975). Results of a study on the inhibitory effect of lipophilic acids (which destroy the proton motive force) have indicated that *B. subtilis* has two malate transport systems, a high affinity system energized by the proton motive force and a low affinity system which does not depend on this force and which is used for growth at high malate concentrations (Levin & Freese, 1977). We have similarly observed that the rate of L-aspartate uptake increases rapidly with the aspartate concentration up to about 100 μM -aspartate and then continues to increase at a lower rate up to very high aspartate concentrations. For example, when the bacteria are grown in 25 mM-sodium aspartate, the rate of continuous aspartate uptake is more than four times higher than the maximum rate of uptake observed at 100 μM -aspartate. This indicates that

Table 1. *Bacillus subtilis* strains used in genetic analysis

Strain	Genotype	Origin*
60002	<i>trpC2</i>	J. Spizizen (168)
60021	<i>amy trpC2</i>	J. Spizizen, amylase-deficient mutant of 168 strain
60348	<i>amy trpC2 aspT1</i>	Spontaneous mutant of 60021 resistant to <i>threo</i> -hydroxyaspartate (THA)
61236	<i>trpC2 argC2</i>	B. Reilly (BR85)
61459	<i>aspB66 trpC2</i>	J. Hoch (c66) (Hoch & Mathews, 1972)
61501	<i>aspH1 trpC2</i>	Spontaneous mutant of 60002 isolated for rapid growth on aspartate
61504	<i>trpC2 metA argC2</i>	Tf. of 61531 by 60136†
61505	<i>trpC2 argC2 glpD</i>	Tf. of 61531 by 61402 (Oh <i>et al.</i> , 1973)
61506	<i>trpC2 argC2 glpK</i>	Tf. of 61531 by 61154†
61507	<i>aspH1 aspT13 trpC2</i>	Spontaneous mutant of 61501 resistant to THA
61565	<i>trpC2 argC2 aspB66</i>	Tf. of 61504 by 61459
61566	<i>trpC2 aspB66 aspT1</i>	Td. of 61565 by 60348

* Tf. implies transformation (by congression) of the first strain by DNA of the second strain; Td. implies transduction of the first strain by PBS1 phage isolated from the second strain.

† The *metA* and *glpK* mutations have been isolated in this laboratory; they have not been mapped.

the high affinity active transport system is not sufficient to allow the rapid growth on aspartate as sole carbon source. This paper shows that *B. subtilis* has two transport systems: a high affinity system which is also found in membrane vesicles under the usual energization conditions, and a low affinity system (with a high V_{max}) which predominates at high aspartate concentrations and which is not observed in membrane vesicles. The high affinity system is missing in mutants (*aspT*) which can still grow on aspartate as sole carbon source.

METHODS

Bacteria. All strains were derived from the transformable strain 168 (= 60002) of *B. subtilis* Marburg. The strains used are listed in Table 1.

Media and growth conditions. The synthetic salts mixture (S6) contained: 10 mM-(NH₄)₂SO₄, 5 mM-K₂HPO₄/KH₂PO₄, pH 7.0, 100 mM-2-(*N*-morpholino)propanesulphonic acid (MOPS) adjusted to pH 7.0 with KOH, 1 mM-MgCl₂, 0.7 mM-CaCl₂, 50 μM-MnCl₂, 5 μM-ZnCl₂, 5 μM-FeCl₃ and 25 μg L-tryptophan ml⁻¹. S6 + Na⁺ is S6 plus 10 mM-NaCl. Carbon sources were usually added at 25 mM and the dicarboxylic amino acids were adjusted to pH 7.0 with KOH (or NaOH where stated). Plates of synthetic media were prepared by adding the growth supplements and carbon sources to a sterile warm solution of agar (Difco) so that the final agar concentration was 1.5% (w/v).

Bacteria which had been grown overnight on plates containing tryptose blood agar base (TBAB; 33 g l⁻¹; Difco) were used to inoculate plates of synthetic medium. After incubation at 37 °C for 15 to 20 h, the growth was washed off and used to inoculate liquid medium to give an initial absorbance at 600 nm of 0.05. The cultures were incubated at 37 °C in a reciprocating water bath shaker and growth was followed by measuring A_{600} with a Gilford spectrophotometer (model 220).

The minimal glucose/citrate medium (MG) of Anagnostopoulos & Spizizen (1961) was used for transformation and the selection of transductants. PBS1 phage was multiplied in antibiotic medium 3 (AM3; Difco) using various donor bacteria.

Isolation of the *aspT* strains. Approximately 10⁷ bacteria from a culture of strain 60021 (*trpC2*) were plated on S6 + glucose plates containing 100 μM-DL-*threo*-β-hydroxyaspartate (THA). The plates were incubated at 37 °C for 72 h and colonies were purified first on fresh plates containing THA and then on TBAB plates. Ten independent mutants were obtained, each from a different single colony isolate of strain 60021. The isolates retained the THA-resistant property through five serial passages on TBAB plates. The *aspH aspT* double mutant (61507) was isolated from the *aspH* strain (61501) in a similar manner.

Transduction experiments. Transduction by PBS1 phage was performed according to Takahashi (1966) and Hoch *et al.* (1967) with the modifications described elsewhere (Iijima *et al.*, 1977).

Transformation experiments. DNA was obtained by the method of Saito & Miura (1963). A 2.5 μg portion of donor strain DNA was added per ml of recipient bacteria grown according to Anagnostopoulos &

Spizizen (1961). In order to examine the *aspT* region of the chromosome, it was necessary to construct several multiply marked strains by transformation. For example, strain 61531 (*trpC2 lys-3 argC2 gtaC*) was isolated by double transformation (congression) of strain 61530 (*trpC2 metB10 lys-3 gtaC*) obtained from F. Young (his strain RUB807) with DNA of strain 61236 (*trpC2 argC2*) obtained from B. Reilly (his strain BR85); *metB* was transformed out while *argC* was transformed in.

Isolation of an aspB aspT double mutant. DNA of strain 61459 (*trpC2 aspB66*) obtained from J. Hoch (his strain c66) was added to strain 61504 (*trpC2 metA argC2*) produced in this laboratory, to obtain, by double transformation, a *trpC2 argC2 aspB66* strain (61565); *aspB* conveyed an aspartate requirement (on MG) to this strain. Strain 61565 was then transduced by PBS1 phage grown on strain 60348 (*trpC2 aspT1*). Since the *aspT* gene is linked to the *argC* gene, many Arg⁺ transductants contained the *aspT1* marker whose presence was demonstrated by growth on MG + tryptophan + aspartate. Whereas the *aspB* mutant required only 300 μ M-aspartate for good colony growth, the *aspB aspT* double mutants required 6 mM-aspartate. One such double mutant, the *trpC2 aspB66 aspT1* strain (61566), was used in this work.

Uptake studies in whole organisms. During exponential growth in S6 or S6 + Na⁺ plus different carbon sources, the bacteria were harvested (at $A_{600} = 0.5$ or 1.0) by centrifugation (12000 g, room temp., 5 min), washed once with an equal volume of S6 and resuspended in the same medium. Portions (2 to 4 ml) were transferred to 50 or 100 ml flasks and incubated in a water bath shaker at 37 °C for 2 min. Chloramphenicol (100 μ g ml⁻¹) was included in the resuspension where indicated and, when necessary, an energy source was added just before the addition of ¹⁴C-labelled substrate as described in the text.

High affinity uptake was initiated by the addition of potassium L-[U-¹⁴C]aspartate (1 μ Ci μ mol⁻¹), or another amino acid, to give a final concentration of 0.1 to 0.2 mM and a radioactivity of 100 to 200 nCi ml⁻¹. Low affinity uptake was initiated by the addition of potassium L-[U-¹⁴C]aspartate or potassium L-[U-¹⁴C]glutamate (0.1 μ Ci μ mol⁻¹) to give a final concentration of 5 to 25 mM and a radioactivity of 1.0 μ Ci ml⁻¹ or 2.5 μ Ci ml⁻¹.

DL-threo- β -Hydroxy[3,4-¹⁴C]aspartic acid was prepared from [U-¹⁴C]glyoxylic acid (7.5 mCi mmol⁻¹; Amersham/Searle) as described by Kornguth & Sallach (1960). The product (7.5 mCi mmol⁻¹) was neutralized with KOH to give a solution containing 28.6 μ Ci ml⁻¹. [¹⁴C]THA, of activity 0.143 μ Ci μ mol⁻¹, was added to cells in uptake medium to give a final concentration of 0.2 mM and 28.6 nCi ml⁻¹, or of activity 0.038 μ Ci μ mol⁻¹, to give 25 mM and 0.95 μ Ci ml⁻¹.

At different times, samples (0.2 ml) were withdrawn, filtered rapidly on a membrane filter (type GN-6, pore size 0.45 μ m, Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.), washed three times with 5 ml S6 prewarmed to 37 °C, and the radioactivity on the filter was counted in a liquid scintillation spectrometer. The scintillation fluid contained 758 ml toluene, 200 ml Triton X-100 and 42 ml Spectrafluor (Amersham/Searle) per litre.

The initial rates of uptake (nmol A_{600}^{-1} min⁻¹) were usually determined from the linear portion of the uptake curves consisting of four points taken within 2 min.

Preparation of membrane vesicles and transport measurements. Membrane vesicles were prepared from cultures growing exponentially in medium containing 25 mM-glycerol and 5 mg casein hydrolysate ml⁻¹ using the procedure of Konings *et al.* (1973) with the following modifications: at the time of cell harvest, 100 μ g chloramphenicol ml⁻¹ was added to the culture to stop all protein (including protease) synthesis. After the nuclease treatment, the lysate was centrifuged at 15000 g for 45 min at room temperature. The pellets were combined, suspended in 0.05 M-potassium phosphate buffer, pH 6.6, and centrifuged at 50000 g for 15 min in the cold. The upper yellowish lipid-containing layer of the pellet was gently shaken away from the more compact darker layer of debris. The lipid layer was suspended and the process of separation repeated two to three times until only a single layer remained in the pellet. The final pellet was suspended in 0.05 M-potassium phosphate buffer, pH 6.6, at a protein concentration of 8 to 10 mg ml⁻¹ and stored in several vials at -70 °C. For the energization of transport, 20 mM-dipotassium DL- α -glycerophosphate or Tris/glycerophosphate buffer was used. These were prepared from the disodium salt by exchange on a Dowex 50 column. The concentration of the glycerophosphate was determined by glycerol-3-phosphate dehydrogenase (NAD⁺) (Michal & Lang, 1974) and the sodium content was measured by flame photometry. Transport was measured according to Kaback (1971).

RESULTS AND DISCUSSION

Isolation of aspT mutants deficient in high affinity aspartate and glutamate transport

L-Aspartate and L-glutamate are actively transported into membrane vesicles of normal strains of *B. subtilis* 168 (Konings & Freese, 1972). In order to examine the effect of this high affinity system on growth with aspartate as sole carbon source, we isolated mutants (*aspT*) resistant to DL-threo- β -hydroxyaspartate (THA) as described in Methods. Figure 1

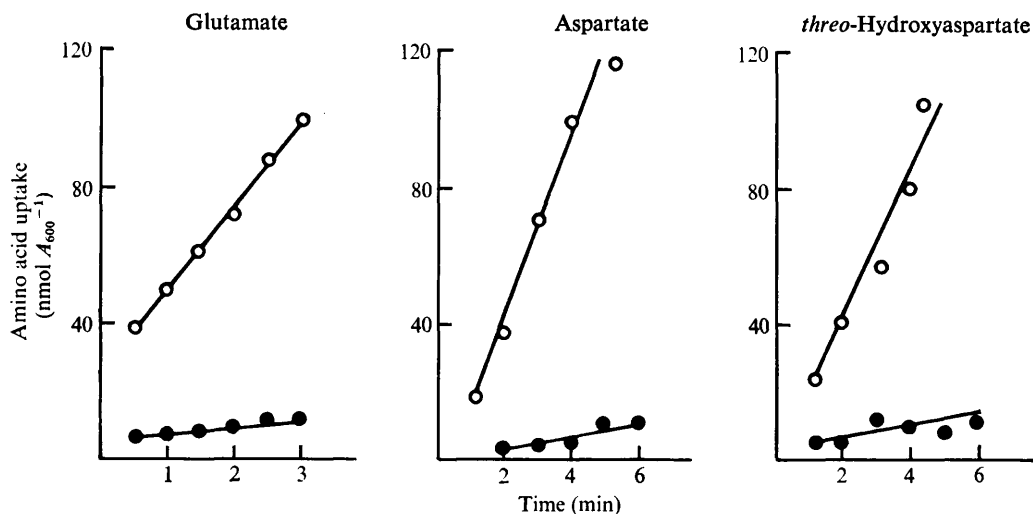


Fig. 1. Amino acid uptake by whole organisms. The standard strain 60002 (○) and the *aspT* mutant 60348 (●) were grown in S6+glucose to $A_{600} = 1.0$, and uptake was measured with 15 mM-glucose as energy source as described in Methods.

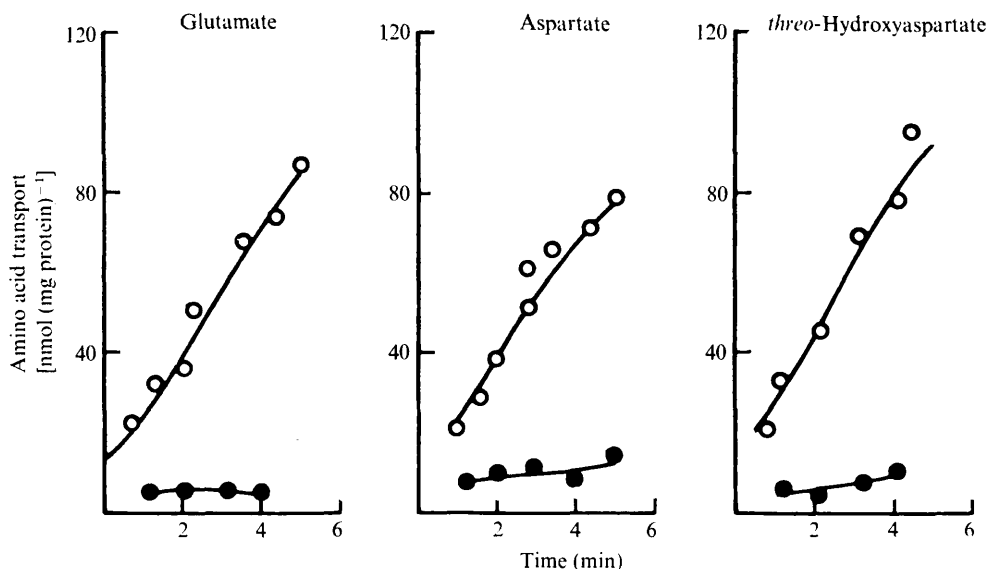


Fig. 2. Amino acid uptake by membrane vesicles. The standard strain 60002 (○) and the *aspT* mutant 60348 (●) were grown to $A_{600} = 1.0$, vesicles were prepared and uptake was measured in the presence of 20 mM-Tris/glycerophosphate as energy source as described in Methods.

demonstrates that intact bacteria of one of these mutants (*aspT1*, 60348) were deficient in the high affinity transport of aspartate (at 100 μM), as well as that of glutamate (at 500 μM) and THA (at 200 μM). The same transport deficiency was observed in membrane vesicles energized by glycerophosphate (Fig. 2). The results demonstrate that L-aspartate, L-glutamate and THA are transported in *B. subtilis* by the same 'high affinity' transport system which is apparently energized by the proton motive force (generated in this case by glycerol-

Table 2. Linkage of *aspT* to *argC* and *metA*The donor strain was 60348 (*trpC2 aspT1*).

PBS1 recipient		Selected marker	Non-selected <i>aspT</i> marker		Total number tested	Recombination frequency (%)
Strain	Genotype		Sensitive to THA	Resistant to THA		
61236	<i>trpC2 argC2</i>	<i>arg</i>	214	120	334	64
61504	<i>trpC2 metA argC2</i>	<i>met</i>	184	46	230	80

Other recipient strains, in which 110 to 140 transductants of each of the following markers were selected, showed no cotransduction with *aspT* (resistance to THA): *hisA*, *thr*, *leu*, *lys*, *citB*, *pyrA*, *purB*.

Table 3. Three-factor crosses for *aspT*The donor strain was 60348 (*trpC2 aspT1*).

PBS1 recipient strain	Combinations of genotypes*					Number of recombinants	Frequency (%)
	<i>metA</i>	<i>argC</i>	<i>aspT</i>	<i>glpD</i>	<i>glpK</i>		
<i>trpC2 argC2 metA</i> (61504)	1	1 ^s	1			36	16.1
	1	1 ^s	0			69	30.9
	0	1 ^s	1			76	34.1
	0	1 ^s	0			42	18.8
	1 ^s	1	1			46	20.0
	1 ^s	1	0			38	16.5
	1 ^s	0	1			0	0
	1 ^s	0	0			146	63.5
<i>trpC2 argC2 glpD</i> (61505)		1 ^s	1	1		20	6.6
		1 ^s	1	0		138	45.7
		1 ^s	0	1		1	0.3
		1 ^s	0	0		143	47.5
		1	1	1 ^s		26	8.9
		0	1	1 ^s		78	26.7
		1	0	1 ^s		2	0.7
		0	0	1 ^s		186	63.7
<i>trpC2 argC2 glpK</i> (61506)		1 ^s	1		1	26	8.1
		1 ^s	1		0	161	50.2
		1 ^s	0		1	1	0.3
		1 ^s	0		0	133	41.4
		1	1		1 ^s	30	10.0
		0	1		1 ^s	82	27.4
		1	0		1 ^s	2	0.7
		0	0		1 ^s	185	61.9

* 0, Recipient type; 1, donor type; 1^s, selected donor type (= wild type).

phosphate oxidation). Eight other *aspT* isolates also lacked the high affinity transport of aspartate.

Mapping of the *aspT* mutation

The *aspT1* mutation (strain 60348) cotransduced with markers of the *argC* and *metA* genes (Table 2). In three-factor crosses (Table 3), the order of genes was established as *metA argC aspT (glpK glpD)*. Figure 3 shows a genetic map of the *aspT* area, in which the marker distances (recombination frequencies) of all crosses performed are summarized. Using transduction, it was also shown that THA resistance and lack of high affinity aspartate uptake were strictly correlated. The THA resistance of eight other independent *aspT* mutant isolates also cotransduced with the *argC* gene.

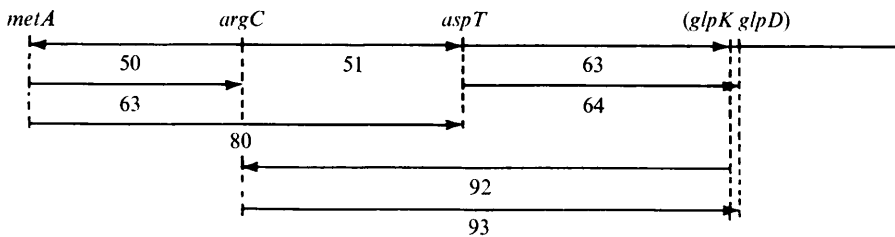


Fig. 3. Genetic map of the area surrounding *aspT*. The arrows are directed from the selected to the unselected marker. The numbers indicate the recombination frequencies (%).

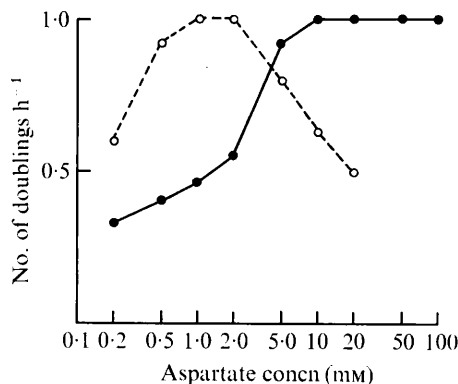


Fig. 4. Effect of the *aspT* mutation on growth of aspartate auxotrophs. The *aspB* strain 61459 (○), lacking aspartate- α -ketoglutarate aminotransferase activity, and the *aspB aspT* strain 61566 (●), lacking also the high affinity aspartate transport, were grown in S6+25 mM-glucose+10 mM-sodium citrate with different concentrations of potassium aspartate. Growth curves were plotted semilogarithmically and the linear portion was used to determine the doubling time.

Evidence for a low affinity aspartate transport system

When sodium aspartate was used as sole carbon source (S6+25 mM-sodium aspartate) the *aspT* mutants, which lack the high affinity aspartate transport, grew at about the same rate (2.5 to 3 h doubling time) as the strains having the normal (high affinity) aspartate transport. Therefore, the physiological effect of the *aspT* mutation on growth could be observed (in the absence of the inhibitor THA) only in an aspartate auxotroph. Such a mutant is the *aspB* strain (isolated by J. Hoch), which requires aspartate for growth in minimal glucose medium because it lacks glutamate-oxaloacetate aminotransferase activity (Hoch & Mathews, 1972). The *aspB* marker was combined with the *aspTI* marker in an *aspB aspT* double mutant (61566) (see Methods). Figure 4 shows that the double mutant could grow in S6+Na⁺+25 mM-glucose at a reasonably high rate only if the aspartate concentration was higher than 2 mM. Apparently, the strain had to use the low affinity aspartate transport system to provide enough aspartate for protein, nucleotide and cell wall synthesis even when glucose could provide energy and other cell components. In contrast, an *aspB* strain (61459) required much less aspartate for optimum growth. [The *aspT* strain (60348) naturally did not require any aspartate.] High concentrations of aspartate inhibited growth of the *aspB* strain (61459), possibly because aspartate (being actively transported into the cells) could not be metabolized rapidly enough since the strain did not carry the high aspartase activity resulting from the *aspH* mutation (see below). This inhibition was not further investigated.

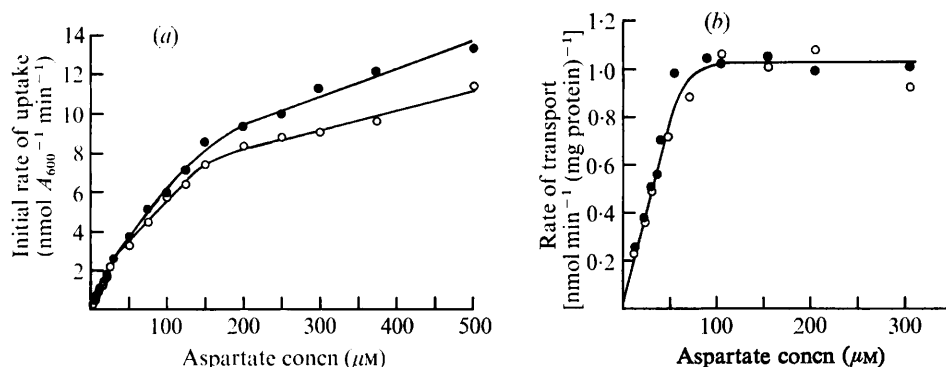


Fig. 5. Concentration dependence of aspartate uptake by (a) whole organisms and (b) membrane vesicles of strain 61501 (*aspH*) in the absence (○) or presence (●) of 10 mM-NaCl. (a) Strain 61501 was grown in S6+25 mM-sodium aspartate+25 mM-glycerol and uptake of aspartate was measured in the presence of chloramphenicol (100 $\mu\text{g ml}^{-1}$) and dipotassium glycerophosphate (20 mM) as energy source as described in Methods. (b) Membrane vesicles were isolated as described in Methods and uptake was measured in the presence of Tris/glycerophosphate (20 mM) as energy source.

Properties of the high affinity aspartate transport system

Normal *B. subtilis* strains grew on aspartate as sole carbon source at a low rate (doubling times 2 to 3 h) even if the medium contained Na^+ (10 mM) which distinctly increased the rate of growth on aspartate; Na^+ had no effect on growth in media containing glucose or malate. During other work in this laboratory (Iijima *et al.*, 1977), a mutation (*aspH*) had been isolated that produced rapid growth on sodium aspartate because the strain (61501) had a high (apparently constitutive) aspartase activity. Again, growth on aspartate was greatly stimulated by the presence of Na^+ . To measure the relative effects of the low and the high affinity transport systems on uptake of and growth on aspartate, we have used only strains that contained the *aspH* mutation.

During exponential growth of the *aspH* strain (61501) in S6+25 mM-sodium aspartate+25 mM-glycerol (to induce glycerophosphate dehydrogenase, NAD^+ independent), bacteria were removed (at $A_{600} = 0.5$), washed in S6, and the uptake of L-aspartate was measured in the presence of dipotassium glycerophosphate as energy source and 10 mM-NaCl. Figure 5(a) shows that the initial rate (within 2 min) of aspartate uptake by whole organisms was almost the same with and without Na^+ at low aspartate concentrations ($< 50 \mu\text{M}$) and exhibited an Na^+ dependence at higher concentrations. In membrane vesicles, the aspartate uptake, energized by Tris/glycerophosphate, exhibited no Na^+ dependence even at high aspartate concentrations (Fig. 5b). In the absence of Na^+ , the apparent K_m was about 67 μM for intact organisms and 66 μM for membrane vesicles. When the vesicles were prepared and assayed in different buffers, such as choline/ PO_4 , choline/Cl, Tris/Tes [Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid], Tris/ P_i or KP_i , different rates of aspartate transport (differences of a factor of 5) were obtained, phosphate-containing buffers giving higher rates than other buffers. Inclusion of 10 mM-NaCl in these buffers did not change the rate of aspartate transport (measured at 60 μM -L-aspartate).

Whereas aspartate uptake by intact bacteria increased slowly with concentration beyond 100 μM -aspartate, it levelled off at this concentration for membrane vesicle transport. These results indicated that the high affinity transport, observed in whole organisms and membrane vesicles, did not depend on the presence of Na^+ . The low affinity uptake of aspartate was observed only in whole organisms, and it showed a slightly higher rate in the presence of Na^+ . However, the 'initial' rate was determined from the uptake in the first 2 min and may contain a significant contribution from aspartate metabolism. No conclusions can, therefore, be drawn from these experiments about the mechanism by which sodium stimulates growth. This aspect will be analysed elsewhere.

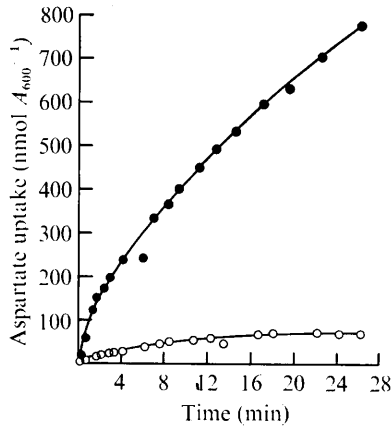


Fig. 6. Aspartate uptake at low and high concentrations. The *aspH* strain (61501) was grown to $A_{600} = 0.5$ in $S6 + Na^+ + 25$ mM-glucose (○) and in $S6 + 25$ mM-sodium aspartate (●). The organisms were then washed and resuspended in $S6$. Aspartate uptake was measured in $S6 + Na^+ + 25$ mM-glucose + $100 \mu M$ -aspartate (○) and in $S6 + 25$ mM-sodium aspartate (●).

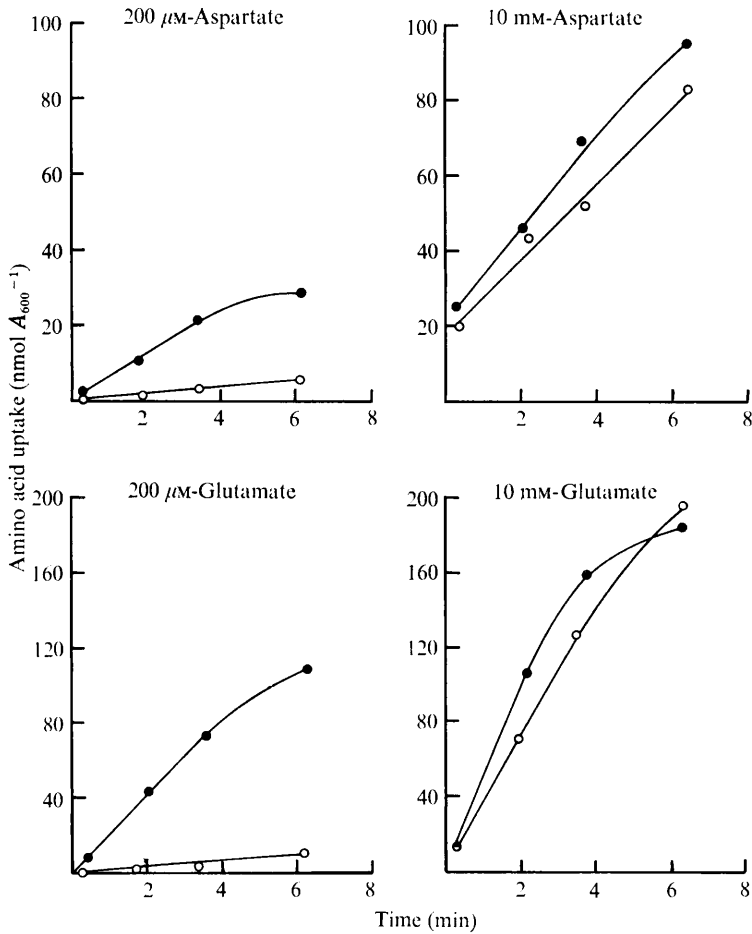


Fig. 7. Uptake of L-aspartate and L-glutamate at $200 \mu M$ and 10 mM by the *aspH* strain 61501 (●) and the *aspH aspT* strain 61507 (○). Each strain was grown in $S6 + 10$ mM-glycerol + 0.2% casein hydrolysate to $A_{600} = 0.5$ and uptake was measured in the presence of 20 mM-disodium glycerophosphate as described in Methods.

Table 4. Initial rates of uptake of amino acids and keto acids by strains 60002 (*trpC2*) and 61507 (*aspH1 aspT13 trpC2*)

The bacteria were grown in S6 + 10 mM-glycerol + 0.2% casein hydrolysate to $A_{600} = 0.5$, centrifuged and then uptake was measured in the presence of chloramphenicol ($50 \mu\text{g ml}^{-1}$) and 20 mM-disodium glycerophosphate as energy source. The uptake was initiated by the addition of ^{14}C -labelled substrate to a final concentration of 0.2 mM.

Substrate	Initial rate of uptake (nmol $A_{600}^{-1} \text{ min}^{-1}$)		Substrate	Initial rate of uptake (nmol $A_{600}^{-1} \text{ min}^{-1}$)	
	60002	61507		60002	61507
Glycine	6.5	5.6	L-Histidine	1.1	1.2
L-Alanine	6.6	8.5	L-Arginine	1.0	1.0
L-Valine	6.1	5.1	L-Methionine	1.0	0.9
L-Leucine	6.0	5.6	L-Proline	1.2	8.3
L-Isoleucine	3.6	4.1	L- α -Aminoisobutyrate	4.3	4.6
L-Serine	10.8	10.8	DL- <i>threo</i> - β -Hydroxy- aspartate	7.9	0.08
L-Threonine	7.2	3.8	L-Malate	2.7	3.0
L-Aspartate	13.5	0.9	L-Succinate	3.2	4.0
L-Asparagine	17.6	15.6	L-Fumarate	3.5	3.8
L-Glutamate	25.1	1.4	L-Pyruvate	0.4	0.3
L-Lysine	0.9	1.2			

Properties of the low affinity transport system

An *aspH* culture growing in S6 + 25 mM-sodium L-aspartate consumed aspartate at a rate of $33 \text{ nmol } A_{600}^{-1} \text{ min}^{-1}$ (measured after the initial high rate of aspartate uptake) (Fig. 6). This rate was about four times higher than the maximum rate ($8 \text{ nmol } A_{600}^{-1} \text{ min}^{-1}$) of aspartate uptake in a culture growing in S6 + Na^+ + 25 mM-glucose, measured at $100 \mu\text{M}$ -aspartate, the concentration at which the high affinity aspartate transport system was about saturated. Thus, aspartate can be taken up at a higher rate than would be possible by the high affinity transport system, but this uptake is effective only at very high aspartate concentrations, i.e. it has a low affinity (K_m) but a high velocity (V_{max}) for aspartate. The high rate may indicate the presence of a large number of transport molecules on the cell surface. The saturation of the aspartate transport in membrane vesicles above $100 \mu\text{M}$ and the absence of transport in vesicles of *aspT* mutants indicates that the low affinity transport system is not energized by the proton motive force (except if these transport molecules are lost upon membrane vesicle isolation).

To measure the properties of the low affinity aspartate uptake system without any background of high affinity transport, a spontaneous double mutant *aspH aspT13* (61507) was isolated on S6 + glucose plates containing $100 \mu\text{M}$ -THA. This double mutant was defective in the high affinity aspartate and glutamate transport (Fig. 7). With the ^{14}C -labelled compounds at $200 \mu\text{M}$, the uptake rates of aspartate and glutamate were distinctly lower than in the *aspH* strain. THA was also no longer taken up whereas the uptake of other amino and keto acids was not impaired (Table 4); compared to the standard strain (60002), the uptake of threonine was reduced by a factor of 2 and that of proline increased by a factor of 7. This may be due to derepression resulting from the reduced amount of aspartate and glutamate taken up by the mutant bacteria or it may result from a secondary mutation. However, the uptake rates of 10 mM-aspartate and glutamate were about the same in both strains (Fig. 7), i.e. the low affinity aspartate transport system was not impaired.

Conceivably, the aspartate uptake observed in the *aspH aspT* strain could result from an exchange with intracellular aspartate or some other compound (glutamate) that had accumulated in the organisms during prior growth. To exclude this possibility, the effect of inhibitors of the electron transport system and of uncouplers, i.e. compounds destroying the proton motive force, on the low affinity aspartate uptake was examined in the presence of

Table 5. Inhibition of the low affinity uptake of [14 C]aspartic acid

Strain 61507 (*aspH1 aspT13 trpC2*) was grown in S6+10 mM-glycerol+0.2% casein hydrolysate to $A_{600} = 0.5$, centrifuged and resuspended in S6 containing inhibitor. Disodium glycerophosphate (20 mM) was added as energy source and the uptake was initiated by the addition of [14 C]aspartate to a concentration of 5 mM. Antimycin A, HOQNO, CCCP and valinomycin were dissolved in ethanol, dinitrophenol and amytal in dimethylsulphoxide (DMSO), and potassium azide in water. The final concentration of these solvents was less than 1% (v/v) and had no significant effect on aspartate uptake. The percentage inhibition is expressed relative to controls having the same ethanol or DMSO concentrations.

Inhibitor*	Concn (mM)	Inhibition of uptake (%)
Potassium azide	10.0	82.3
CCCP	0.01	95.5
Dinitrophenol	1.0	66.5
Antimycin A	0.5	45.7
HOQNO	0.05	87.9
Amytal	20.0	70.6
Valinomycin	0.1	80.9

* CCCP, Carbonyl cyanide *m*-chlorophenylhydrazone; HOQNO, 2-Heptyl-4-hydroxyquinoline-*N*-oxide.

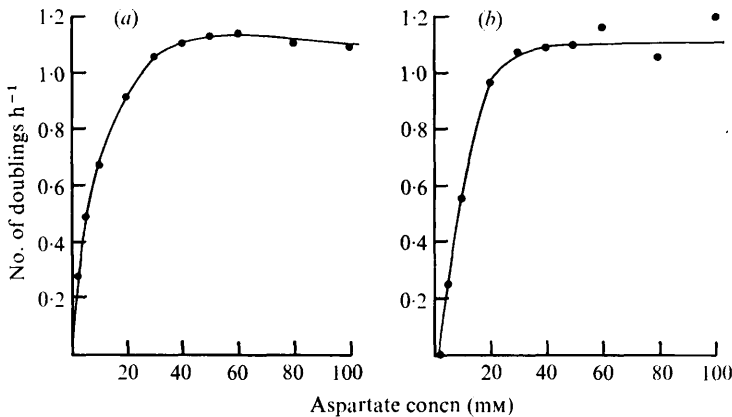


Fig. 8. Aspartate concentration dependence of growth of (a) the *aspH* strain 61501 and (b) the *aspH aspT* strain 61507. The bacteria were grown in S6 containing different concentrations of sodium L-aspartate.

glycerophosphate as energy source. Aspartate uptake was inhibited by all these compounds (Table 5), i.e. it depended on the cellular ability to metabolize and transport substrates. Consequently, the observed aspartate uptake did not result merely from an exchange with a previously accumulated intracellular compound. The observed high rate of aspartate uptake may depend on the immediate metabolism of the aspartate that has entered the organisms. The results do not exclude the possibility that aspartate transport requires the symport or antiport of some compound produced by or actively transported into the organisms.

The *aspH aspT* strain grew as well as the *aspH* strain (61501) in S6 containing high concentrations of sodium aspartate as sole carbon source (Fig. 8). Only at low aspartate concentrations (< 10 mM), at which the contribution of the high affinity transport system to aspartate uptake becomes significant, was the growth rate of the *aspH aspT* strain significantly lower than that of the *aspH* strain. When [14 C]aspartate was added to such a culture growing in S6+25 mM-sodium aspartate, the uptake of aspartate showed an initial rapid rate which changed to a slower constant rate after 2 min. This constant rate of 25 nmol

$A_{600}^{-1} \text{ min}^{-1}$ reflects the rate at which aspartate was metabolized. Since this metabolism occurs rapidly due to the high aspartase activity and since the low affinity transport is active only at high extracellular aspartate concentrations (e.g. 25 mM), growth may not require active transport of aspartate into the cell. In fact, no active transport of aspartate was observed in membrane vesicles of the *aspT* mutant. Facilitated transport may, therefore, suffice to provide aspartate at the necessary rate.

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