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Pantothenate Transport in *Escherichia coli*

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The transport system for pantothenic acid uptake in *Escherichia coli* was characterized. This transport system was specific for pantothenate, had a K_t of 0.4 μM, and had a maximum velocity of 1.6 pmol/min per 10^8 cells (45 pmol/min per mg [dry weight]). Pantothenate uptake was not reduced in osmotically shocked cells or by ATP depletion with arsenate, but was reduced >90% by the dissipation of the membrane electrochemical gradient with 2,4-dinitrophenol. Sodium ions stimulated pantothenate uptake (K_t, 0.8 mM) by reducing the K_t for pantothenate by an order of magnitude. Intracellular pantothenate was rapidly phosphorylated, but phosphorylation of pantothenate was not required for uptake since pantothenate was the only labeled intracellular compound concentrated by ATP-depleted, glucose-energized cells. The data were consistent with the presence of a high-affinity pantothenate permease that concentrates the vitamin by sodium cotransport.

Coenzyme A (CoA) is the predominant acyl group carrier in living systems. Nearly one hundred enzymes require CoA (1), and it also modulates the activity of several other key enzyme activities (10, 13, 17, 18, 25, 35, 36), suggesting that control over the intracellular CoA concentration is an important aspect of metabolic regulation. CoA is formed by a universal series of reactions, beginning with the phosphorylation of the vitamin pantothenic acid (1). Unlike animals, *Escherichia coli* produces its own abundant supply of this vitamin (8, 14, 21) from the condensation of β-alanine and pantoic acid (6). The conversion of pantothenate to CoA is governed by the activity of pantothenate kinase and the transmembrane flux of pantothenate (14, 15). Biochemical evidence indicates that CoA modulates pantothenate kinase activity by an allosteric mechanism (2, 12, 16, 29) and supports the view that feedback control over pantothenate utilization is the key regulatory site in CoA biosynthesis.

Little is known about pantothenate transport in *E. coli*. Most active transport mechanisms in gram-negative bacteria fall into one of two classes (34). Osmotic shock-sensitive systems, typified by glutamine transport (4, 33), are associated with a periplasmic substrate-binding protein and have an obligatory requirement for phosphate bond energy (5). Shock-resistant systems, like proline uptake (4), possess membrane-bound permeases and are coupled to the electrochemical gradient across the membrane (5). Nakamura and Tamura (24) described a saturable pantothenate uptake system in *E. coli* that was sensitive to two uncouplers of oxidative phosphorylation, pentachlorophenol and 2,4-dinitrophenol, consistent with the presence of a membrane-bound pantothenate permease. Studies of pantothenate uptake in *Salmonella typhimurium* (S. D. Dunn, and E. E. Snell, J. Supramol. Struct. **6**:136, 1977) suggested that pantothenate accumulation is coupled to the sodium ion gradient. In contrast, Mantsala (20) reported that pantothenate uptake in *E. coli* U-5/41 is sensitive to osmotic shock and purified a periplasmic pantothenate-binding protein. In light of these conflicting results and the significance of pantothenate transport in the regulation of CoA content (14, 15), we compared the properties of the pantothenate transport system to those of two well-characterized amino acid transport systems.

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MATERIALS AND METHODS

Chemicals and supplies. Sources for supplies were as follows: Amersham Corp., ACS scintillation solution and β-[2,3-^{3}H]alanine (specific activity, 34.0 Ci/mmol); Analtech, 250-μm Silica Gel H plates; Calbiochem-Behring, D-pantethine; Millipore Corp., 0.45-μm-pore filters (type HA); New England Nuclear Corp., β-[3-^{3}H]alanine (specific activity, 38.1 or 40.0 Ci/mmol), L-[U-^{14}C]proline (specific activity, 273 Ci/mol), L-[U-^{14}C]glutamine (specific activity, 263 Ci/mol); Schwarz/Mann, L-[4,5-^{3}H]leucine (specific activity, 55 Ci/mmol); Sigma Chemical Co., β-alanine, D-pantothenate, D-pantothenyl alcohol, D-pantoyl lactone, DL-pantoyltaurine, chloramphenicol, 2,4-dinitrophenol (DNP), and arsenic acid. Sodium pantoate was prepared from D-pantoyl lactone by autoclaving with a 1.1 M excess of sodium hydroxide (19). D-Pantethine was reduced with excess dithiothreitol to yield pantetheine. D-[3-^{3}H]pantothenic acid (specific activity, 5.0 Ci/mmol) was isolated from the medium of a culture of a *panD* strain grown in the presence of β-[3-^{3}H]alanine of the same specific activity by thin-layer chromatography on Silica Gel H plates. The plates were developed with ethanol–28% ammonium hydroxide (4/1, vol/vol). The fraction of the radioactivity corresponding to D-[3-^{3}H]pantothenate was extracted with ethanol, concentrated, and further purified on Silica Gel H layers developed with butanol-acetic acid-water (5/2/4, vol/vol). The radiochemical purity of the D-[3-^{3}H]pantothenate was routinely checked in these solvent systems and was >98%. All other chemicals were reagent grade or better.

Bacterial strains and growth conditions. All strains used in this study were derived from *E. coli* K-12. Strains C600 (*thr-1 leuB6 lacY1 thy-1 supE44 tonA21* λ^- F^-), UB1005 (*metB1 relA1* λ^r λ^- *spoT1 gyrA216* F^-), a spontaneous nalidixic acid-resistant derivative of strain W1655F$^-$ (3), and SJ67 (Met$^+$ derivative of strain UB1005) were wild type with respect to pantothenate synthesis. Strains SJ16 (*panD*) (14) and DV39 (*panC*) were derived from strain UB1005 and required pantothenate for growth because of defects in aspartate-1-decarboxylase (*panD*) and pantothenate synthetase (*panC*) (7). Three types of minimal growth medium were used. Medium M9 (22) was used to test the sensitivity of pantothenate uptake to osmotic shock. Minimal medium A (9) was used to examine the energy coupling to pantothenate uptake; in experiments testing the effect of sodium ions on

pantothenate uptake, sodium citrate was omitted from medium A. All other transport studies were performed with cells grown in minimal medium E (32). Cells were grown at 37°C in the appropriate medium containing either glucose or glycerol (0.4%), methionine (0.01%), leucine (0.01%), threonine (0.01%) as required, and thiamine (0.001%). Pantothenate-requiring strains were grown in minimal medium supplemented with 1 μM D-pantothenate unless otherwise indicated. Cell number was monitored during growth by turbidity measurements with a Klett-Summerson colorimeter with a blue filter. The colorimeter was calibrated with strain SJ16 by determining the number of CFU per milliliter in the range of colorimeter readings encountered.

Transport assays. Transport assays were performed by using cultures in exponential growth phase except for assays with osmotically shocked cells. Cells (5×10^8/ml) were harvested by filtration, washed, and resuspended to 10^9/ml in the indicated assay medium. The salt composition of the assay medium was identical to that of the growth medium except when a phosphate-free Tris-hydrochloride assay medium was used in incubations containing arsenate (5) and when the effects of various cations on pantothenate uptake were studied in 100 mM Tris-hydrochloride (pH 7.3). Cells were then incubated for 5 min at 37°C with chloramphenicol (40 μg/ml), and the experiment was started by the addition of radiolabeled substrate. The specific activities of the radiolabeled substrates in the transport assays were 1 to 5 Ci/mmol for D-[3-^{3}H]pantothenate and 50 Ci/mol for L-[U-^{14}C]proline and L-[U-^{14}C]glutamine. Aliquots (50 μl) were removed at various times, filtered, and washed with 10 ml of 37°C assay medium. The filters were then dried and counted in 3 ml of scintillation solution. Pantothenate uptake by strain SJ16 was linear for 10 min and for cell numbers up to 3×10^9/ml. The initial uptake rate was calculated from a linear fit to the data obtained at 10, 30, 60, and 90 s after mixing. For glutamine and proline, uptake was linear for 1 min, and the initial rates were determined from samples removed at 10, 20, 30, and 45 s. Blanks were measured (i) in the absence of cells, (ii) at 0°C, and (iii) at 37°C in the presence of 2% toluene, and all three blank measurements were similar to the values obtained from extrapolation of each time course to 0 s. The initial rate of pantothenate uptake was unchanged for 1 h when the cells were stored at 37°C in the presence of chloramphenicol. Initial uptake rates were expressed as picomoles per minute per 10^8 cells. Cell numbers were measured for each transport experiment by determining the CFU per milliliter immediately before adding chloramphenicol. Intracellular volume was assumed to be 0.437 μl/5×10^8 cells or, equivalently, 2.5 μl/mg (dry weight) (30). The kinetic constants K_t and V_{max} were calculated from a linear fit of the reciprocal of the uptake rate plotted against the reciprocal of the substrate concentration.

Osmotic shock and binding assays. Osmotically shocked cells were prepared essentially by the procedure of Neu and Heppel (26). Stationary-phase cultures grown in M9-glucose minimal medium were washed three times with 20 mM Tris-hydrochloride (pH 7.5)–30 mM NaCl and suspended in (60 × wet weight) 15% sucrose–20 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA for 10 min at 22°C. The plasmolyzed cells were pelleted and rapidly suspended in an equal volume of ice-cold water. After the cells were in ice-cold water for 10 min, $MgCl_2$ was added to 0.5 mM, and the shocked cells were pelleted and suspended to 2.4×10^9/ml in M9-glucose medium to measure transport activity. Control cells were treated with medium M9 throughout. The viability of the osmotically shocked cells was 85 to 95%.

The supernatant from osmotically shocked cultures was concentrated by lyophilization and dialyzed at 4°C against 20 mM Tris-hydrochloride (pH 7.4)–30 mM NaCl. Binding assays were performed by using a Hoefer eight-sample equilibrium microdialyzer. Wells (0.25 ml) were divided by an EMD103 membrane (molecular weight cutoff, 6,000 to 8,000) and contained either the concentrated protein solution (1 mg/ml) or the radioactive ligand in 20 mM Tris-hydrochloride (pH 7.4)–30 mM NaCl. The specific activities of the ligands were: D-[3-^{3}H]pantothenate, 5 Ci/mmol; β-[3-^{3}H]alanine, 5 Ci/mmol; L-[U-^{14}C]proline, 273 Ci/mol; L-[^{14}C]glutamine, 263 Ci/mol; and L-[4,5-^{3}H]leucine, 10 Ci/mmol. Samples were dialyzed overnight at 4°C. A 50-μl sample was removed from each compartment, and the binding activity was determined from the quantity of radioactivity in the compartment containing the periplasmic proteins minus that in the protein-free compartment. Binding activity was expressed as picomoles of substrate bound per milligram of protein, and protein was determined by the microbiuret method, using bovine serum albumin as a standard (23).

Analysis of intracellular labeled metabolites. Cells (420 μl) were incubated for 90 s at 37°C in Tris-hydrochloride assay medium containing 11 mM glucose and 1 μM D-[3-^{3}H]pantothenate. Aliquots (400 μl) were pelleted by a 20-s spin in an Eppendorf microcentrifuge, and the cell pellet was resuspended and washed twice with 37°C Tris-hydrochloride assay medium containing 0.5 mM Na_2AsO_4 to stop further pantothenate metabolism. Radiolabeled metabolites were extracted by suspension of the cell pellet in 20 μl of 1 N formic acid followed by a 30-min incubation on ice. Extracts were analyzed by thin-layer chromatography on Silica Gel H plates developed with ethanol–28% ammonium hydroxide (4/1, vol/vol) to 14 cm from the origin (14). The distribution of radioactivity was quantitated by scraping 0.5-cm sections of the thin-layer plate into scintillation vials, deactivating the Silica Gel H with 100 μl of water, and counting in 3 ml of scintillation solution.

RESULTS

Affinity and specificity of pantothenate uptake. A high-affinity pantothenate transport system was found in strain SJ16 (Fig. 1). The K_t determined from a Lineweaver-Burk plot (Fig. 1) was 0.4 μM, and the V_{max} was 1.6 pmol/min per 10^8

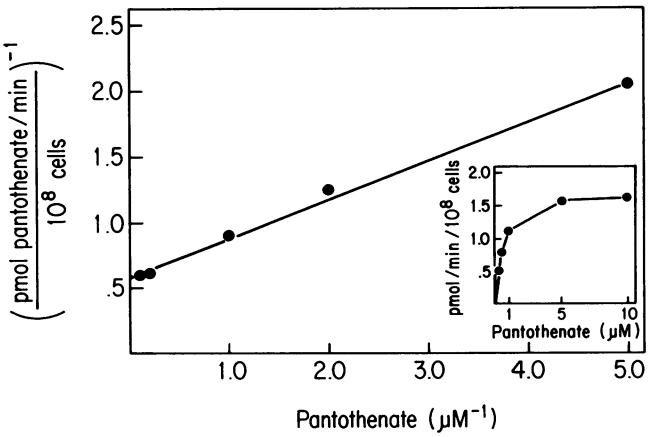

FIG. 1. Kinetics of pantothenate uptake by strain SJ16. The transport assay and growth of strain SJ16 are described in the text.

cells (45 pmol/min per mg [dry weight]). The transport system was saturated at 10 μM pantothenate (Fig. 1, inset).

The pantothenate uptake system was also very specific for pantothenate. Several structurally related compounds were tested for their ability to inhibit pantothenate utilization when present in 100-fold excess over pantothenate. Inhibition by the 2,4-dihydroxy-3,3-dimethylbutyryl portion of pantothenate was tested in incubations containing sodium D-pantoate, D-pantoyl lactone, or DL-pantoyl taurine, but none reduced pantothenate uptake. β-Alanine reduced pantothenate uptake to 55% of the control activity in strain SJ16 (*panD*) but did not inhibit uptake by strain DV39 (*panC*). Therefore, the ability of β-alanine to reduce pantothenate uptake in strain SJ16 was attributed to the synthesis of intracellular pantothenate from β-alanine (14, 19), which depressed the utilization of extracellular pantothenate. Pantetheine was the only inhibitor of pantothenate uptake and reduced transport to 79% of control activity. Pantethine and pantothenyl alcohol did not reduce pantothenate uptake. Pantothenate accumulation was depressed in the presence of vitamin-free casein hydrolysate, but this effect was not investigated further.

Osmotic shock and binding assays. The possible involvement of a pantothenate-binding protein in pantothenate uptake was investigated by testing the sensitivity of transport to osmotic shock. Osmotically shocked cells of strain SJ16 were assayed for uptake of pantothenate, proline, and glutamine, and the initial rates of uptake were compared with those obtained from control cells. Glutamine uptake was the most sensitive to osmotic shock and was reduced by 94% (Table 1). Proline uptake was more resistant to osmotic shock but was reduced by 32% (Table 1). Pantothenate uptake by strain SJ16 was consistently stimulated by the osmotic shock procedure (Table 1) demonstrating that pantothenate uptake did not depend upon the presence of periplasmic components. An identical results was obtained with the prototrophic strain SJ67 (data not shown). This stimulation of pantothenate uptake was attributed to the loss of intracellular pantothenate caused by osmotic shock. The concentrated osmotic shock supernatant from strain SJ67 was assayed for the presence of amino acid- and pantothenate-binding proteins (Table 2), and the results agreed with the sensitivity of the corresponding transport systems to osmotic shock. Leucine- and glutamine-binding proteins were clearly demonstrated, but pantothenate-, proline-, and β-alanine-binding protein activities were not detected (Table 2). Pantothenate binding also was not detected when strain SJ16 or C600 was the source of the periplasmic protein. *E. coli* is not known to degrade pantothenate, and the stability of the D-[3-^{3}H]pantothenate during the binding assay was confirmed by thin-layer chromatography.

Energy coupling to pantothenate uptake. To determine the energy requirements for pantothenate accumulation, the response of pantothenate uptake to glucose, arsenate, and DNP was compared with the effect of these compounds on the uptake of glutamine and proline (Table 3). The addition of 11 mM glucose to the Tris-hydrochloride assay medium stimulated uptake of pantothenic acid by 30% and proline uptake by 61%, but uptake of glutamine was the most sensitive to the addition of an energy source, being stimulated more than 15-fold (Table 3). As expected for a transport system requiring phosphate bond energy (5), the presence of arsenate in this medium resulted in greater than 90% loss of glucose-driven glutamine uptake (Table 3). However, glucose-driven uptake of both pantothenate and proline was resistant to the arsenate inhibition of ATP formation. Pantothenate retained 98% of the maximum activity, and proline retained 82% (Table 3). When the uncoupler DNP was added to the incubation mixture, the arsenate-resistant uptake activities of both pantothenate and proline were reduced by more than 90%, and the residual glutamine uptake was abolished (Table 3). Cells that were depleted of metabolic energy by the procedure of Berger (4) were able to transport pantothenate by facilitated diffusion (K_t, 4 μM, V_{max}, 0.18 pmol/min per 10^8 cells [data not shown]). The initial rate of pantothenate (1 μM) uptake by energy-depleted cells was 0.1 pmol/min per 10^8 cells; this rate increased to 0.6 pmol/min per 10^8 cells after glucose was added to the medium. The similarity in the responses of pantothenate and proline transport to metabolic inhibitors indicated that the primary site of energy coupling in both systems was the membrane potential (5).

The intracellular products of pantothenate uptake were analyzed by thin-layer chromatography. Within 90 s, the majority of intracellular pantothenate was phosphorylated,

TABLE 1. Effect of osmotic shock on pantothenate transport[a]

Compound (concn, μM)	pmol/min per 10^8 cells		% Control
	Control	Shocked	
Pantothenate (0.5)	0.273	0.319	117
Proline (10)	60	41	68
Glutamine (10)	113	6.3	5.6

[a] Transport assays and osmotic shock were performed as described in the text, using strain SJ16.

TABLE 2. Absence of a periplasmic pantothenate-binding protein[a]

Labeled ligand	dpm_i/dpm_o[b]	Sp act (pmol/mg of protein)
Patothenate	0.96	<0.8
β-Alanine	0.99	<0.8
Proline	1.01	<3.5
Glutamine	1.96	293
Leucine	1.77	499

[a] Osmotic shock supernatant was prepared from strain SJ67 to avoid repression of glutamine- and leucine-binding proteins by the methionine supplement required for strain SJ16. Specific binding activity was determined as described in the text.

[b] Ratio of disintegrations per minute on the protein side (dpm_i) to those on the buffer side (dpm_o) of the equilibrium dialysis chamber. The concentration of labeled ligands was 0.5 μM in all cases.

TABLE 3. Effect of arsenate and DNP on pantothenate uptake[a]

Tris-hydrochloride assay medium[b]	Uptake (pmol/min per 10^8 cells) of (concn, μM):		
	Pantothenate (1)	Proline (10)	Glutamine (10)
No addition	0.50	138	7
+ Glc	0.65	223	118
+ Glc + AsO_4^{2-}	0.63	183	10
+ Glc + AsO_4^{2-} + DNP	0.04	10	ND[c]

[a] Transport assays were performed with strain SJ16 as described in the text.

[b] Cells were preincubated for 10 min in Tris-hydrochloride medium (no addition), Tris-hydrochloride medium plus 11 mM glucose (+ Glc), 0.5 mM sodium arsenate followed by an additional 10-min incubation in 11 mM glucose (+ Glc + AsO_4^{2-}), or 0.5 mM sodium arsenate plus 1 mM DNP followed by an additional 10-min incubation with 11 mM glucose (+ Glc + AsO_4^{2-} + DNP).

[c] ND, Not detected.

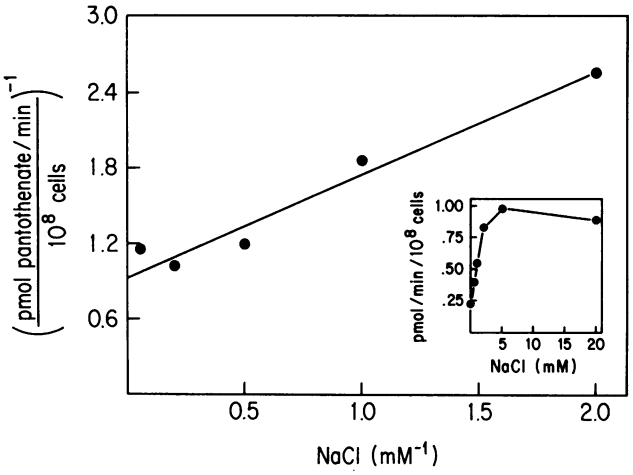

FIG. 2. Stimulation of pantothenate uptake by sodium. Transport assays were performed with strain SJ16 in minimal medium A minus sodium citrate containing 1 μM pantothenate. An exogenous energy source was not present.

but in the presence of arsenate, phosphorylation and subsequent metabolism of pantothenate were blocked, and pantothenic acid accumulated in the cells. Since the initial rate of pantothenate uptake was not reduced by arsenate (Table 3), it was clear that neither phosphorylation of pantothenate nor ATP hydrolysis was necessary for pantothenate uptake. The rate of pantothenate uptake by arsenate-treated cells dropped after 90 s, but after 10 min, pantothenate was concentrated 56-fold (4.8 pmol/10^8 cells) over the medium.

Sodium dependency of pantothenate uptake. The pantothenate transport system was dependent on the presence of sodium ions in addition to an energy source for maximum activity. The Na^+ requirement was most pronounced when glycerol was substituted for glucose as the carbon source for growth and when the assay was performed in the absence of an exogenous energy source (Fig. 2), since the presence of glucose resulted in higher basal uptake activity in the Na^+-free assay medium. Maximum stimulation of pantothenate uptake was observed at above 5 mM NaCl (Fig. 2, inset), and the K_t for NaCl was 0.8 mM (Fig. 2). NaCl (5 mM) also stimulated glycerol-driven uptake of 0.2 μM pantothenate fivefold, but did not stimulate pantothenate uptake activity in energy-depleted cells, suggesting the involvement of a membrane gradient of sodium ions. This idea was supported by the lack of DNP inhibition when sodium was a component of the assay medium. DNP (1 mM) reduced glucose-driven pantothenate uptake 60 to 70% in sodium- and phosphate-free Tris-hydrochloride medium and in minimal medium A when sodium citrate was omitted, but had no effect in medium M9 or E. The stimulation of pantothenate uptake was specific for Na^+ and to a lesser extent for Li^+ (approximately 80% as effective as Na^+), but equivalent concentrations of ammonium, potassium, magnesium, and calcium ions did not stimulate pantothenate transport. The V_{max} for pantothenate uptake was identical in the presence or absence of sodium ions (Fig. 3), whereas the K_t for pantothenate was lowered from 2 to 0.2 μM when NaCl was substituted for KCl (Fig. 3).

Expression of pantothenate uptake activity. Strain SJ16 (*panD*) was used in the majority of experiments and exhibited 25 to 35% higher pantothenate uptake activity than its Pan^+ counterparts SJ67 and UB1005. The rate of uptake by strain SJ16 growing in minimal medium supplemented with 1 μM pantothenate was similar to the rate found in pantothenate-starved cells. The higher uptake activity observed with strain SJ16 was consistent with its inability to synthesize intracellular pantothenate. When strain SJ16 was grown in the presence of higher pantothenate supplements (10 μM), the rate of pantothenate uptake was reduced to the level seen in the wild-type strains. High-specific-activity D-[3-^{3}H]pantothenate was required to compare pantothenate transport with proline and glutamine transport since both amino acid uptake systems were at least 200-fold more active than pantothenate uptake.

DISCUSSION

E. coli possesses a high-affinity transport system for the concentration of pantothenic acid. The K_t for pantothenate was 0.4 μM, and the V_{max} was 1.6 pmol/min per 10^8 cells (45 pmol/min per mg [dry weight]) (Fig. 1). These data agree with the kinetic properties of the pantothenate transport system described by Nakamura and Tamura (K_t, 0.3 μM; V_{max}, 13 pmol/min per mg [dry weight]) (24). The *E. coli* pantothenate uptake system was also highly specific for the vitamin. Of several compounds structurally related to pantothenate, only a large excess of pantetheine could reduce pantothenate uptake. Uptake activity was sensitive to the intracellular pantothenate pool as evidenced by the inhibition of pantothenate uptake by β-alanine in *panD* but not *panC* strains and by the decreasing rate of pantothenate accumulation with time in arsenate-treated cells. Pantothenate transport activity was 0.5% of those of proline and glutamine (Tables 1 and 3) but supplied enough vitamin to pantothenate auxotrophs for CoA and acyl carrier protein biosynthesis (14).

Pantothenate transport is accomplished by a membrane-bound permease coupled to the electrochemical gradient. We compared pantothenate uptake to two well-characterized amino acid transport systems that typify membrane-bound permease (proline) and periplasmic binding protein-dependent (glutamine) transport systems (4, 5). Pantothenate transport resembled proline transport as opposed to glutamine transport in its response to glucose and its insensitivity to ATP depletion by arsenate (Table 3). Pantothenate uptake was not reduced in osmotically shocked cells (Table 1), and a pantothenate-binding protein was not de-

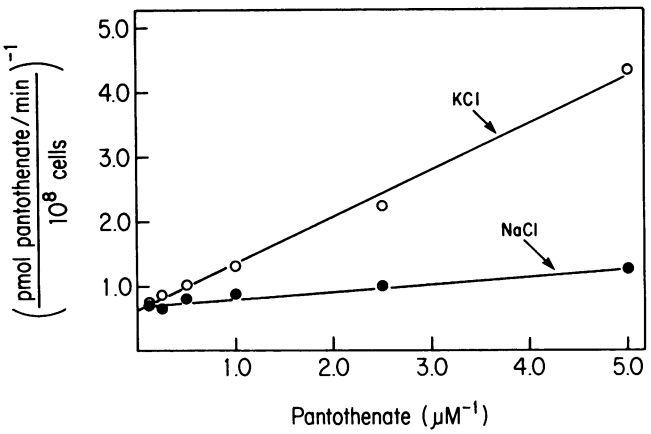

FIG. 3. Effect of sodium on the kinetics of pantothenate uptake. Transport assays were performed with strain SJ16 in medium A minus sodium citrate containing 0.4% glycerol and either 10 mM NaCl or 10 mM KCl.

tected in osmotic shock supernatants (Table 2). Although pantothenate was rapidly utilized for CoA biosynthesis, pantothenate phosphorylation was not required for transport since pantothenate was concentrated from the medium when its metabolism was blocked with arsenate. The arsenate-resistant uptake of both pantothenate and proline was greatly reduced when the electrochemical gradient was eliminated by DNP (Table 3).

Our data suggest that the pantothenate permease uses an Na^+ cotransport mechanism to drive pantothenate uptake. Pantothenate transport was markedly stimulated by sodium ions (Fig. 2; K_t, 0.8 mM), and with the exception of lithium ions, other cations were without effect. The cation specificity for pantothenate uptake was the same as the specificity of established bacterial Na^+ cotransport systems (31). Sodium ions activated pantothenate uptake by lowering the K_t for pantothenate in both *E. coli* (Fig. 3) and *S. typhimurium* (Dunn and Snell, J. Supramol. Struct. **6**:136, 1977). An increase in the affinity, but not the capacity, of the transport system by sodium ions is also characteristic of the sodium-dependent glutamate (11) and melibiose (31) permeases. Although our data were consistent with an Na^+ cotransport mechanism for pantothenate, further experiments that impose artificial proton and sodium ion gradients in energy-depleted cells or vesicles and a demonstration of pantothenate-dependent sodium transport are needed to determine whether the transport system is absolutely specific for the sodium gradient.

Our conclusion that the pantothenate transport system is a membrane-bound permease differs fundamentally from the binding protein-dependent transport system described by Mantsala for *E. coli* U-5/41 (20). Mantsala reported greater than 75% reduction of pantothenate uptake activity after osmotic shock (20). However, this experiment cannot be interpreted since the effect of osmotic shock on established transport systems and the effect of the osmotic shock procedure on cell viability were not investigated (26, 28). Mantsala also reported the partial purification of a pantothenate-binding protein from the concentrated osmotic shock supernatant of strain U-5/41 (20). We were repeatedly unable to demonstrate pantothenate-binding activity in osmotic shock supernatants concentrated from three different strains of *E. coli* K-12 (Table 2). D-[3-^{3}H]pantothenate was used as the ligand in our experiments (Table 2) to increase the sensitivity of the binding protein assay nearly 1,000-fold over the D-[^{14}C]pantothenate-binding assay used by Mantsala (20). The glutamine-binding (33) and leucine-binding (27) activities present in the same preparation of an osmotic shock supernatant assayed for pantothenate binding confirmed our methodology (Table 2).

Previous work has shown that CoA biosynthesis in *E. coli* is controlled by the activity of pantothenate kinase coupled with the expulsion of excess pantothenate produced by the cell (14, 15). Since *E. coli* produces 15 times more pantothenate than is utilized, pantothenate efflux is essential to the regulation of CoA biosynthesis (14, 15). The simplest model describing the role of pantothenate transport in the regulation of CoA content is that a single pantothenate facilitator catalyzes the transmembrane movement of pantothenate and that intracellular pantothenate is trapped by its phosphorylation catalyzed by pantothenate kinase. The results of this study showed that a transport system is specifically designed for the concentrative uptake of pantothenate. Although pantothenate was transported by facilitated diffusion in energy-depleted cells, it is unlikely that the sodium-dependent uptake system could efficiently catalyze pantothenate efflux. Therefore, a more tenable hypothesis is that *E. coli* has separate transport systems for the uptake and efflux of pantothenate. We are currently testing this idea by isolating mutants lacking the high-affinity pantothenate uptake system.

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