JOURNAL OF BACTERIOLOGY, Feb. 1974, p. 373-378
Vol. 117, No. 2

Printed in U.S.A.

C_4-Dicarboxylate Transport in *Bacillus subtilis* Studied with 3-Fluoro-L-Erythro-Malate as a Substrate

KLAUS WILLECKE AND RITA LANGE

Institut für Genetik, 5 Köln, 41, Weyertal 121, Federal Republic of Germany

Received for publication 24 October 1973

Bacillus subtilis cells grown in yeast extract medium accumulated 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate more than 30-fold from the surrounding medium. No metabolic products derived from 3-fluoro-L-erythro-malate could be detected in these cells. L-Malate competitively inhibited transport of 3-fluoro-L-erythro-malate. This malate analogue was itself a competitive inhibitor of L-malate uptake. Cells that had been grown in yeast extract supplemented with 5 mM L-malate showed a 10-fold increased affinity towards 3-fluoro-L-erythro-malate relative to cells grown in yeast extract medium with no added malate. Our results suggest that two transport systems for L-malate can be induced in *B. subtilis*. The first of these systems seems to effect uptake of C_4-dicarboxylates (L-malate, succinate, and fumarate) in yeast extract medium. The second transport system (or possibly a modification of the first transport system) seems to be induced by addition of L-malate to this medium and is also functioning in malate minimal medium.

In a preliminary survey on dicarboxylate transport in *Bacillus subtilis*, we found an unusually high uptake rate for malate relative to the maximal uptake of other dicarboxylates and of citrate (4). The high uptake rate might be due to particularly rapid metabolism of malate or to the simultaneous action of two different transport systems (for the same substrate). To answer this question we looked for experimental conditions that would limit or prevent the metabolism of the transported substrate. We wished to synthesize a non-metabolizable radioactive analogue of malate which would be transported by the C_4-dicarboxylate transport system and which would allow us to investigate malate transport independently of the genetic background of the bacterial cell. DL-Fluoro-malate was shown to be a potent competitive inhibitor of malate uptake in *B. subtilis* (4). *Escherichia coli* mutants were characterized that were resistant towards DL-fluoromalate because of a defective C_4-dicarboxylate transport system (9, 13). We decided to synthesize a pure isomer, 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate, to avoid the difficulties of using a mixture of isomers as substrates for transport studies. Skilleter, Dummel, and Kun (14) recently described the synthesis of nonlabeled 3-fluoro-L-erythro-malate which we followed with some modifications for synthesizing the radioactive compound. We found that 3-fluoro-L-erythro-malate was accumulated by *B. subtilis* cells under conditions where no metabolic products of the substrate could be detected in these cells. In this paper we describe kinetic experiments concerning the uptake of 3-fluoro-L-erythro-malate by the C_4-dicarboxylate transport system in *B. subtilis*.

MATERIALS AND METHODS

Bacteria. *B. subtilis* SB-26, auxotrophic for methionine and tryptophan, is a derivative of the transformable 168 strain. Transport of di- and tricarboxylic acids in this mutant has been described (4, 15).

Chemicals. Ethyl fluoroacetate and diethyl oxalate were purchased from Fluka (Neu-Ulm). Anhydrous oxalic acid was bought from Riedel-de-Haen (Seelze-Hannover). All other chemicals were products of Merck (Darmstadt) and usually of highest quality available. Enzymes and cofactors were bought from Boehringer (Mannheim) and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) was purchased from Serva (Heidelberg). All mentioned chemical suppliers are located in the Federal Republic of Germany. *m*-Chlorophenyl carbonyl-cyanide hydrazone (CCCP) was a product of Calbiochem. [U-^{14}C]oxalic acid and [U-^{14}C]malic acid were obtained from Amersham, England.

Synthesis of 3-fluoro-L-erythro-malate. To have reference material available for chromatographic characterization of [^{14}C]fluoromalate, we synthesized the unlabeled product exactly as described by Skilleter et al. (14). Our final yield of L-erythro-fluoromalate was close to the one obtained by these authors. The melting point was 159 C, as expected. For the synthesis of 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate the

procedure of Skilleter et al. (14) was modified to end up with a product of high specific radioactivity and free of other radioactive contaminants.

A 0.5-mCi amount of [U-^{14}C]oxalic acid (87 mCi/mmol) was transferred with 6 ml of absolute ethanol to a 50-ml round-bottomed flask that contained 2.7 g of anhydrous oxalic acid. The mixture was refluxed and subjected to two interrupted vacuum distillations as described by Jewel and Butts (8), thereby removing almost all water from the reaction mixture. Then 2.5 g of diethyl oxalate was added as further carrier compound to the radioactive ester, and the mixture was redistilled yielding 5.5 g (87%) of diethyl-[1,2-$^{14}C_2$]-oxalate.

This product was used for the synthesis of radioactive diethyl fluorooxalacetate, which was achieved by condensation with ethyl-fluoroacetate as described for the unlabeled product by Kun and Dummel (11). For this reaction we found it advantageous to prepare the alcohol-free sodium ethoxide in boiling toluene instead of boiling ether as described in reference 11. After cooling to room temperature, diethyl-[1,2-^{14}C]oxalate and ethyl fluoroacetate were added according to Kun and Dummel (11). All distillations were carried out on a half-micro scale. A 1.7-g amount of diethyl-[1,2-^{14}C]fluorooxalacetate (28%) was obtained. This product was hydrolyzed to the free acid as described by Dummel et al. (1). The final yield after recrystallization from ether-petroleum ether was 0.8 g of [1,2-$^{14}C_2$]fluorooxalacetic acid (55%). The yield of this compound was enzymatically determined with malate dehydrogenase (EC 1.1.1.37) (1).

For the enzymatic conversion of [1,2-$^{14}C_2$]fluorooxalacetic acid to L-erythro-[1,2-$^{14}C_2$]fluoromalate we followed again the method of Skilleter et al. (14). Our reaction mixture contained 600 mg of [1,2-^{14}C]fluorooxalacetic acid, 175 mg of oxidized nicotinamide adenine dinucleotide, 7.2 ml of ethanol, 10 mg of malate dehydrogenase (EC 1.1.1.37) (from pig heart) and 30 mg of alcohol dehydrogenase (EC 1.1.1.1) dissolved in 80 ml of tris(hydroxymethyl)aminomethane-acetate buffer (0.1 M, pH 8.2). The progress of the reaction was estimated by following the disappearance of fluorooxalacetate using the decarboxylation assay as developed by Dummel et al. (1). The reaction mixture was filtered through an Amicon P30 membrane, thereby recovering the enzyme which was reused for the synthesis of L-erythro-fluoromalate. The radioactive filtrate was treated with activated charcoal (Norit A) to remove most of the nucleotides (14). The filtrate was concentrated to about 1 ml in a rotary evaporator.

At this point we decided to modify the procedure of Skilleter et al. (14). We purified 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate further by preparative thin-layer chromatography. This step is only feasible with a relatively small amount of material but it assures that the final product is free of radioactive contamination. We applied the concentrated filtrate onto six thin-layer plates (20 by 20 cm) of 0.5-mm cellulose (Macherey and Nagel, Düren, Germany), and chromatographed in the solvent system alcohol-ammonium hydroxide-water (90:17:13). In this system we determined R_f ratios of 0.26 and 0.57 for fluoromalate and fluoropyruvate, respectively. Fluoropyruvate is the main radioactive byproduct of this synthesis. After drying, the thin-layer chromatograms were incubated with Kodak X-ray film for 1 day. The cellulose material containing [^{14}C]-fluoromalate was scraped off the plates. The cellulose particles were suspended in a small amount of water and stirred for 20 min at room temperature. The suspension was filtered through a paper filter (Schleicher and Schüll, no. 5892 - Blue ribbon), washed, and centrifuged in a clinical centrifuge to remove most of the cellulose material. Subsequently the supernatant fluid was filtered through a Sartorius filter (pore size 0.2 μm) and stored frozen. The amount of 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate was determined using a color assay originally published by Hartford (6) and later modified by Fanshier et al. (2). For calibration of this assay we used the unlabeled 3-fluoro-L-erythro-malate. Rechromatography of the purified 3-fluoro-L-erythromalate in the mentioned system of thin-layer chromatography showed only one radioactive spot at the expected position. A second batch of 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate contained a trace amount (<1%) of [^{14}C]fluoropyruvate (cf. Fig. 1), which did not interfere with our experiments. We also subjected the material to thin-layer electrophoresis in 0.1 M ammonium formate-formic acid buffer, pH 2.8, with picric acid as the reference compound (relative migration = 1.0). In this system the migration of fluoromalate was about 1.1 and that of fluoropyruvate was about 1.0. 3-Fluoro-L-erythro-[1,2-$^{14}C_2$]malate showed only one radioactive component at the expected position. The product had a specific radioactivity of 12,000 counts per min per μmol when counted under conditions of transport assays, i. e., on a Sartorius filter (25 mm, 0.6 μm pore size) in a toluene-based scintillation fluid (16).

Growth media. For transport studies the bacteria were grown in a minimal salts medium containing 0.3% yeast extract as carbon source (NYE medium) (15). During our studies we found by enzymatic determination with malate dehydrogenase (7) that this concentration of yeast extract contained about 5μM of L-malate. If maximal induction of malate uptake was desired, the NYE medium was supplemented with 7 mM potassium-L-malate. Other minimal media contained the same amounts of minimal salts plus 25 mM of the appropriate carbon source (ribose, DL-lactate, L-malate, or citrate).

Transport assays. The *B. subtilis* cells were harvested at late log phase of growth from NYE medium by filtration through a Sartorius membrane filter (47 mm, 0.6μm pore size). Then the cells were washed with HEPES medium (15), consisting of 0.1 M K-HEPES buffer, 6 mM $MgCl_2$, and 0.1% glucose. Subsequently the cells were resuspended in HEPES medium containing 50 μg of choroamphenicol per ml and shaken at 37 C at an optical density (650 nm) of about 4.0 (determined in 1:10 diluted samples). For estimating dry weight from OD readings at 650 nm we used the correlation: $A_{650\,nm}$ = 0.44 equals 150 g, dry weight, of cells per ml (15). Samples (0.3 ml) were withdrawn and incubated at 37 C with 20 μliters (0.02 μCi) of 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate (65.8

mM) or with 0.2 ml (0.2 μCi) of L-[U-^{14}C]malate (10 mM). The incubation mixtures were adjusted to 1.1 ml total volume using HEPES medium containing 50 μg of chloroamphenicol per ml. Thus, the extracellular concentrations of fluoromalate or malate were 1.2 mM or 1.8 mM, respectively. After 20 s of incubation (standard time for determination of initial rates), 1.0 ml of the mixture was withdrawn in an automatic pipette (Eppendorf, Hamburg, Germany) and quickly filtered through a Sartorius filter (25 mm, 0.6 μm pore size). The filter was washed with 5 ml of HEPES medium, placed immediately in 4 ml of scintillation fluid (16), and counted in a small scintillation vial (15).

Studies of [^{14}C]fluoromalate metabolism in vivo. *B. subtilis* SB-26 was grown to mid-log phase in 70 ml of NYE medium, supplemented with 5 mM L-malate. The cells were washed, concentrated fivefold, and resuspended at 37 C in NYE medium, free of additional malate. 3-Fluoro-L-erythro-[1,2-$^{14}C_2$]malate (0.1 μCi) was added to give a final concentration of about 1 mM. Incubation was carried out for 13 min at 37 C. Afterwards, the cells were filtered and washed in 0.1 M HEPES buffer, pH 6.8. The filter was transferred to a test tube containing 3 ml of ice-cold toluene-water (5:95, vol/vol) and shaken vigorously (10). Then the debris was removed by filtration through a Sartorius membrane filter (25 mm, pore size 0.25 μm). The filtrate was lyophilized, dissolved in 0.1 ml of water, and applied to a thin-layer plate.

After electrophoresis in 0.1 M ammonium formate-formic acid buffer, pH 6.8, and autoradiography (2 weeks) only one spot was observed on the autoradiograph. Its relative position of migration was slightly different from the one of co-chromatographed unlabeled fluoromalate, probably owing to some salt in the cell extract. Therefore, the entire area was scraped off and re-chromatographed in the previously mentioned ethanol-ammonium hydroxide-water system. Figure 1 shows the autoradiograph of this chromatogram.

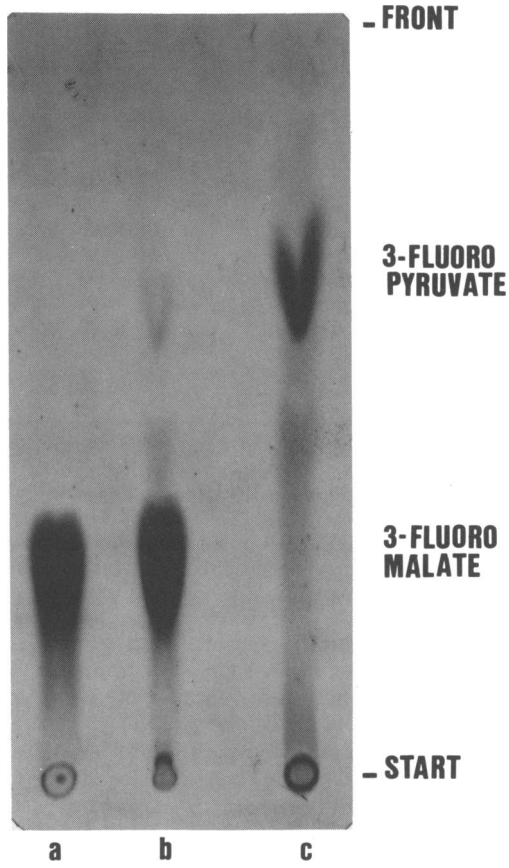

FIG. 1. *Autoradiography of ^{14}C-dissimilation products from B. subtilis SB-26. After growth in NYE medium supplemented with 5 mM L-malate, the bacteria were incubated for 13 min in the presence of 1.2 mM 3-fluoro-L-erythro-[1,2-^{14}C]malate, filtered, washed, and extracted with an ice-cold toluene-water mixture (10). The extract was subjected to a thin-layer electrophoresis. Then the radioactive material was removed from the plate and chromatographed in the solvent system ethanol-ammonium hydroxide-water (90:17:13). For further details see Materials and Methods. The thin-layer plate was exposed to Kodak X-ray film for 3 weeks. (a) Strain SB-26 + 3-fluoro-L-erythro[1,2-^{14}C]malate; (b) 3-fluoro-L-erythro-[1,2-^{14}C]malate as reference, (c) 3-fluoro-[1,2-^{14}C]pyruvate as reference.*

RESULTS

Transport of L-erythro-fluoromalate in B. subtilis. As shown in Fig. 1, no metabolic products derived from L-erythro-fluoromalate were found in chromatographed extracts of *B. subtilis* cells that had been incubated with this substrate. In addition we tried to trap $^{14}CO_2$ on hyamine-soaked paper strips (Whatman 1) during incubations of *B. subtilis* cells with 3-fluoro-L-erythro-[1,2-^{14}C]malate in Warburg vessels (data not shown). We did not find any $^{14}CO_2$ evolution under these conditions above the level measured in samples without bacteria. Therefore, we concluded that L-erythro-fluoromalate is not significantly metabolized under conditions of our transport assays.

Table 1 shows uptake rates of L-erythro-fluoromalate into cells which had been grown in a yeast extract medium in the presence of different additions. L-Malate, fumarate or succinate in the medium led to the highest uptake rates of L-erythro-fluoromalate. This suggested that the malate analogue is a substrate of the C_4-dicarboxylate transport system in *B. subtilis*. The uptake of L-erythro-fluoromalate is partially suppressed in cells that had been grown on ribose, DL-lactate, citrate, or glucose. This is in accord with published results, which described catabolite repression of malate (4) or succinate (5) uptake in *B. subtilis*. Using cells

TABLE 1. *Uptake of L-erythro-fluoromalate in Bacillus subtilis SB-26 from different media*[a]

Carbon source in growth medium	Transport (μmol/min/g, dry wt)
Yeast extract	58
Yeast extract + tartrate	41
Yeast extract + succinate	66
Yeast extract + L-malate	85
Yeast extract + fumarate	83
Yeast extract + glucose	4
Ribose	23
DL-Lactate	25
Citrate	21
L-Malate	59

[a] *B. subtilis* SB-26 was grown for about three generations in the listed media. The final concentrations of compounds added to yeast extract medium (NYE medium) was 5 mM. The bacteria were filtered, washed, and resuspended in HEPES medium. Transport was measured as described in Materials and Methods.

grown in minimal medium with 25 mM L-malate as the carbon source, we reproducibly found somewhat less uptake of L-erythro-fluoromalate than in cells grown in yeast extract medium, supplemented with 5 mM L-malate.

Figure 2 shows time dependencies of L-erythro-fluoromalate transport in *B. subtilis* cells. The initial transport rate was higher in cells grown in the presence of L-malate than in cells grown in the absence of L-malate, although the equilibrium level of uptake was similar in both cases. It was calculated from the equilibrium level of L-erythro-fluoromalate transport that these cells concentrated the malate analogue more than 30-fold over its concentration in the surrounding medium. The energy dependence of this transport system is shown by the 95% inhibition caused by 50 μM CCCP. In addition, Fig. 2 illustrates that there was very little incorporation of label from 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate into trichloroacetic acid-insoluble material during our transport assays. This further supports our conclusion that L-erythro-fluoromalate was not significantly metabolized under our assay conditions.

Results (Table 2) indicate that the uptake of L-erythro-fluoromalate could be severely inhibited by malate, succinate, and fumarate but not by L-tartrate. Furthermore, malate, succinate, and fumarate inhibited the transport of L-erythro-fluoromalate equally well by more than 75% when they were present at the same concentration as the malate analogue. This can be explained by assuming that malate, succinate, and fumarate are substrates of higher affinity for the dicarboxylate uptake system than L-erythro-fluoromalate.

Figure 3 shows the concentration dependency of L-erythro-fluoromalate transport in cells grown in yeast extract with no added L-malate and with cells grown in yeast extract plus 5 mM L-malate. The two curves are significantly different. When the data were replotted by the method of Lineweaver and Burk (12), we found that cells from NYE medium plus additional malate showed an almost 10-fold higher affinity towards L-erythro-fluoromalate than cells grown

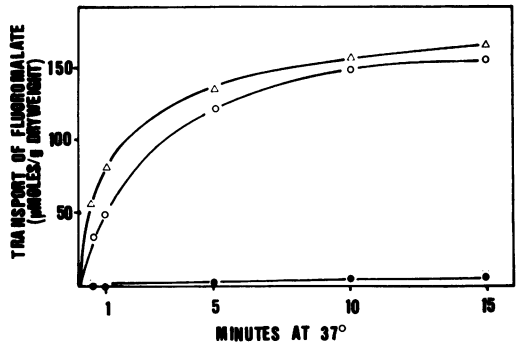

FIG. 2. *Time dependency of L-erythro-fluoromalate transport. Transport was measured by our standard assay conditions in B. subtilis SB-26 cells grown in NYE medium in the presence (△) and absence (○) of 5 mM L-malate. In addition, cells which had been grown in the absence of L-malate were preincubated with 50 μM CCCP for 5 min at 37 C. Uptake assays were also carried out in the presence of 50 μM CCCP (□). To measure incorporation of isotope into trichloroacetic acid-insoluble material (●), cells were incubated with labeled fluoromalate for the indicated times, filtered, and washed with 5 ml of HEPES medium, 10 ml of 5% trichloroacetic acid, and 5 ml of HEPES medium. Then the filters were counted as usual.*

TABLE 2. *Inhibition of L-erythro-fluoromalate transport by different C_4-dicarboxylic acids*[a]

Concentration of inhibitor (mM)	Inhibition (%) of L-erythro-fluoromalate transport caused by:			
	L-Malate	Succinate	Fumarate	L-Tartrate
0.23	52	50	56	0
0.46	60	66	61	0
0.92	73	76	74	0

[a] *B. subtilis* SB-26 was harvested in HEPES medium after growth in NYE medium. The transport incubations were carried out in the presence of the listed inhibitor concentrations. The concentration of 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate in the incubation mixtures was 0.6 mM. A transport rate of 62 μmol/min/g (dry weight) was determined in the absence of any inhibitor.

in yeast extract (Table 3). We investigated the competetive inhibition of L-erythro-fluoromalate transport by L-malate and determined a K_i of 0.055 mM using *B. subtilis* SB-26 grown in NYE medium. L-Erythro-fluoromalate inhibited the uptake of L-malate competetively with a K_i of 0.04 mM in SB-26 cells which had been harvested from NYE medium containing 5 mM L-malate.

Table 4 presents results of L-erythro-fluoromalate transport into cells which were grown in NYE medium with different concentrations of L-malate. Previously we had found that unsupplemented NYE medium (0.3% yeast extract) contained about 5 μM L-malate (see Materials and Methods). The transport of L-erythro-fluoromalate increased gradually with increasing concentration of L-malate in the medium and reached a maximum above 1 mM L-malate. It was interesting that growth of *B. subtilis* SB-26 in NYE medium in the presence of 1 mM L-erythro-fluoromalate maximally induced uptake of L-erythro-fluoromalate. The corresponding result is included in Table 4. It is possible that fluoromalate acted as inducer for the C_4-dicarboxylate transport system. On the other hand L-erythro-fluoromalate might have caused an accumulation of L-malate inside the cells perhaps by inhibition of malate catabolism. This would have led to indirect induction of the dicarboxylate transport system. 3-Fluoro-L-erythromalate is known to be a strong inhibitor of malate dehydrogenase (14).

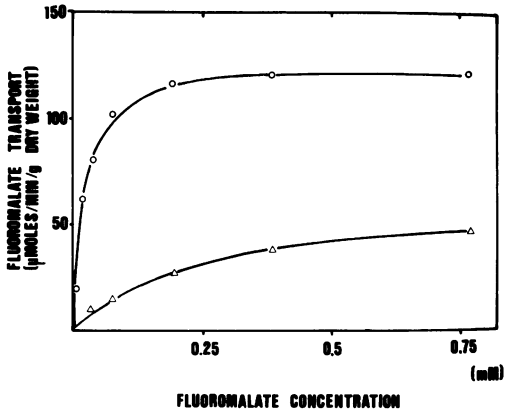

FIG. 3. *Transport of L-erythro-fluoromalate as a function of fluoromalate concentration. Transport assays were carried out as described in Materials and Methods. Cells of B. subtilis SB-26 were used which had been grown in NYE medium in the absence* (Δ) *and in the presence* (○) *of 7.5 mM L-malate.*

TABLE 3. *Comparison of kinetic constants for uptake of L-erythro-fluoromalate into cells grown in different media*

Growth conditions	Transport[a] of L-erythro-fluoromalate
NYE medium	K_m = 0.23 mM V_{max} = 68 μmol/min/g (dry wt)
NYE medium plus 7.5 mM L-malate	K_m = 0.02 mM V_{max} = 125 μmol/min/g (dry wt)
Malate minimal medium (25 mM L-malate)	K_m = 0.016 mM V_{max} = 86 μmol/min/g (dry wt)

[a] Transport was measured as described in Materials and Methods using *B. subtilis* SB-26.

TABLE 4. *Dependence of fluoromalate transport on malate concentration in the growth medium*[a]

Concentration in yeast extract medium (mM)	Transport of L-erythro-fluoromalate (μmol/min/g, dry wt)
L-Malate	
0.005	63
0.01	70
0.05	86
0.3	85
1.0	103
5.0	107
L-Erythro-fluoromalate	
1.0	119

[a] *B. subtilis* SB-26 was grown for at least four generations in NYE medium in the presence of the indicated L-malate concentrations. Samples were used for transport studies as described in Materials and Methods. In one experiment the bacteria were grown in NYE medium in the absence of additional L-malate but in the presence of 1 mM 3-fluoro-L-erythro-malate. The result of a transport assay using these cells is included in the table.

DISCUSSION

Our results show that 3-fluoro-L-erythro-[1,2-$^{14}C_2$]malate can be used as a nonmetabolized malate analogue to study the C_4-dicarboxylate transport system in *B. subtilis*. We have preliminary evidence that this malate analogue is also taken up by *E. coli* Hfr H cells grown in succinate minimal medium, i.e., under conditions which provide maximal induction of the C_4-dicarboxylate transport system in *E. coli* (13). As in *B. subtilis*, glucose minimal medium leads to repression of L-erythro-fluoromalate transport in *E. coli* Hfr H (K. Willecke and R. Lange, unpublished experiments). Therefore, this malate analogue may be of general use for

investigation of C_4-dicarboxylate transport in bacteria. This is in constrast to the situation found in mitochondria. Skilleter et al. (14) have reported that L-erythro-fluoromalate did not penetrate the inner mitochondrial membrane although it acted as a powerful activator of the mitochondrial tricarboxylate carrier. These results confirm conclusions from other experiments that the uptake systems for C_4-dicarboxylates have widely different specificities in bacteria and in mitochondria (4, 5).

Mutants of *B. subtilis* and *E. coli* defective in fumarase, succinate dehydrogenase (5, 13), or malate dehydrogenase (4) have been used for studies of C_4-dicarboxylate transport in order to limit metabolism of the transported substrate. Recently Ghei and Kay (5) found, however, that L-malate is extensively metabolized in a malate dehydrogenase mutant of *B. subtilis*. A nonmetabolized analogue of malate like L-erythro-fluoromalate may provide a useful experimental tool for studying C_4-dicarboxylate transport independently of the genetic background of the bacterium. In this context it should be mentioned that 2-fluoro-L-erythro-citrate can be used as a nonmetabolized analogue for investigation of the citrate-Mg^{2+} transport system in *B. subtilis* (P. Oehr and K. Willecke, J. Biol. Chem., in press).

When *B. subtilis* cells were grown in yeast extract plus malate or in malate minimal medium, they showed a considerably increased affinity towards L-erythro-fluoromalate. The easiest explanation for this finding is that new binding sites are formed by the cells in response to the induction signal triggered by malate addition to the growth medium. We suggest that the new binding sites represent most likely uptake sites of a second transport system or of an additional transport component whose affinity towards L-erythro-fluoromalate is higher than the affinity of the preexisting transport system. The high affinity uptake as well as the low affinity uptake of L-erythro-fluoromalate can both be inhibited by 50 μM CCCP to more than 95%.

It is obvious that only genetic dissection of the malate transport system(s) in *B. subtilis* can verify or dismiss our conclusions. Recently Ghei and Kay (5) mentioned the isolation of a *B. subtilis* mutant that was apparently unable to grow on L-malate, succinate, or fumarate as single carbon source and that could not take up these substrates from the medium. The authors did not report any data to characterize this mutant. It would be interesting to see whether or not this mutant is also unable to grow on high concentrations of L-malate. Under this condition malate may be taken up by a second transport system. Alternatively one could explain the high affinity of malate-induced cells towards L-erythro-fluoromalate by assuming that an additional transport component is functionally coupled in malate-induced cells to the preexisting transport system for C_4-dicarboxylates.

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 74).

LITERATURE CITED

1. Dummel, R. J., M. N. Berry, and E. Kun. 1971. Studies with specific enzyme inhibitors. XIII. Kinetics of nonenzymatic decarboxylation of fluorooxalacetic acid. Mol. Pharmacol. 7:367-374.
2. Fanshier, D. W., L. W. Gottwald, and E. Kun. 1962. Enzymatic synthesis of monofluorocitrate from β-fluoro-oxaloacetate, J. Biol. Chem. 237:3588-3596.
3. Fanshier, D. W., L. K. Gottwald, and E. Kun. 1963. Studies on specific enzyme inhibitors. VI. Characterization and mechanism of action of the enzyme-inhibitory isomer of monofluorocitrate J. Biol. Chem. 239:425-434.
4. Fournier, R. E., M. N. McKillen, A. B. Pardee, and K. Willecke. 1972. Transport of dicarboxylic acids in *Bacillus subtilis*. Inducible uptake of L-malate. J. Biol. Chem. 247:5587-5595.
5. Ghei, O. K., and W. W. Kay. 1973. Properties of an inducible C_4-dicarboxylic acid transport system in *Bacillus subtilis*. J. Bacteriol. 114:65-79.
6. Hartford, C. G. 1962. Rapid spectrophotometric method for the determination of itaconic, citric, aconitic, and fumaric acids. Anal. Chem. 3:426-428.
7. Hohorst, H. J. 1962. L-Malat, Bestimmung mit Malat Dehydrogenase und DPN, p. 328-332. *In* H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim.
8. Jewel, P. W., and J. S. Butts. 1931. A method for the preparation of diethyl oxalate. J. Amer. Chem. Soc. 53:3560.
9. Kay, W. W., and H. L. Kornberg, 1969. Genetic control of uptake of C_4-dicarboxylic acids by *Escherichia coli*, Fed. Eur. Biochem. Soc. Lett. 3:93-96.
10. Kay, W. W., and H. L. Kornberg. 1971. The uptake of C_4-dicarboxylic acids by *Escherichia coli*. Eur. J. Biochem. 18:274-281.
11. Kun, E., and R. J. Dummel. 1969. Chemical properties and synthesis of fluoro analogs of compounds related to substrates of the citric acid cycle, p. 623-672. *In* J. M. Lowenstein (ed.), Methods in enzymology, vol. 13. Academic Press Inc., New York.
12. Lineweaver, H., and D. J. Burk. 1934. The determination of enzyme dissociation constants. J. Amer. Chem. Soc. 56:658-666.
13. Lo, T. C. Y., M. K. Rayman, and B. D. Sanwal. 1972. Transport of succinate in *Escherichia coli*. I. Biochemical and genetic studies of transport in whole cells. J. Biol. Chem. 247:6323-6331.
14. Skilleter, D. N., R. J. Dummel, and E. Kun. 1972. Studies with specific enzyme inhibitors. XIV. The effects of enzymatically synthesized (-)-erythro-fluoromalic acid on malate dehydrogenase and on anion carriers of liver mitochondria. Mol. Pharmacol. 8:139-148.
15. Willecke, K., E. M. Gries, and P. Oehr, 1973. Coupled transport of citrate and magnesium in *Bacillus subtilis*. J. Biol. Chem. 248:807-813.
16. Winkler, H. H., and T. H. Wilson. 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli* J. Biol. Chem. 241:2200-2211.