JOURNAL OF BACTERIOLOGY, Sept. 1992, p. 5533–5539
0021-9193/92/175533-07$02.00/0

Vol. 174, No. 17

Anaerobic Fumarate Transport in *Escherichia coli* by an *fnr*-Dependent Dicarboxylate Uptake System Which Is Different from the Aerobic Dicarboxylate Uptake System

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Received 10 March 1992/Accepted 19 June 1992

***Escherichia coli* grown anaerobically with fumarate as electron acceptor is able to take up C_4-dicarboxylates by a specific transport system. The system differs in all tested parameters from the known aerobic C_4-dicarboxylate transporter. The anaerobic transport system shows higher transport rates (95 μmol/g [dry weight] per min versus 30 μmol/g/min) and higher K_ms (400 versus 30 μM) for fumarate than for the aerobic system. Mutants lacking the aerobic dicarboxylate uptake system are able to grow anaerobically at the expense of fumarate respiration and transport dicarboxylates with wild-type rates after anaerobic but not after aerobic growth. Transport by the anaerobic system is stimulated by preloading the bacteria with dicarboxylates. The anaerobic transport system catalyzes homologous and heterologous antiport of dicarboxylates, whereas the aerobic system operates only in the unidirectional mode. The anaerobic antiport is measurable only in anaerobically grown bacteria with *fnr*$^+$ backgrounds. Additionally, the system is inhibited by incubation of resting bacteria with physiological electron acceptors such as O_2, nitrate, dimethyl sulfoxide, and fumarate. The inhibition is reversed by the presence of reducing agents. It is suggested that the physiological role of the system is a fumarate/succinate antiport under conditions of fumarate respiration.**

Escherichia coli is able to grow under aerobic as well as anaerobic conditions with C_4-dicarboxylic acids as substrates for energy metabolism. In aerobic bacteria, the uptake of dicarboxylic acids appears to be catalyzed by a binding-protein-dependent transport system (14). The periplasmic binding protein, which is probably encoded by the *cbt* gene, was isolated (15). Additionally, mutants affected in the *dctA* and *dctB* genes, which presumably encode membrane components of the transport system, are available (21). Mutants defective in the *dctA*, *dctB*, and *cbt* genes are no longer able to accumulate C_4-dicarboxylic acids and to grow at their expense. From these findings, it is concluded that there is one C_4-dicarboxylate uptake system (*dct* system) in *E. coli* grown aerobically that requires each of the three genes to function. Aspartate can be transported by an additional system with a high affinity (*ast* mutants) (8).

Under anaerobic conditions, fumarate is used by the bacteria as electron acceptor in fumarate respiration. Since the active site of fumarate reductase is orientated to the cytoplasmic space (11, 30), fumarate has to be transported to the cytoplasm. Succinate, the product of fumarate respiration, is not further metabolized in *E. coli* under anaerobic conditions and is excreted by the bacteria. For various reasons, it is unlikely that the aerobic C_4-dicarboxylate transport system is responsible for fumarate uptake in *E. coli* growing anaerobically at the expense of fumarate respiration. The *dct* system, like other binding-protein-dependent transporters, presumably would consume 1 ATP per dicarboxylate for uptake. In fumarate respiration of *E. coli* and other bacteria (e.g., *Wolinella succinogenes*), maximally 1 ADP per 2 electrons or per fumarate can be phosphorylated (2, 16). Consumption of 1 ATP for fumarate transport, however, would prevent growth under conditions of fumarate respiration. Therefore, an energetically more favorable transport system would be required for anaerobic growth. In addition, as a binding-protein-dependent system, the *dct* transport system would be expected to operate unidirectionally in uptake. It should not be able to mediate succinate export. For these reasons, it has to be assumed that *E. coli* contains an anaerobic fumarate (or dicarboxylate) transport system different from the aerobic one that meets all the energetic and mechanistic requirements. Here we address the questions of whether there is a separate anaerobic transport system and in which aspects it differs from the known aerobic C_4-dicarboxylate transport system.

MATERIALS AND METHODS

Bacteria and growth. For the experiments, the following strains of *E. coli* K-12 were used: AN387 (wild type), CBT38 (*leuB6 lacY1 dctB3 bioA2 rpsL129 thi-1*), CBT312 (*sdh-2 rpsL129 dctA2 thi-1*) (14), PL2024 (*gal trpA9761 iclR rpsL*), JRG861a (as PL2024, but *fnr*) (12), VJS950 {MC4100 with *gyrA219 non-9 narG*::[λ pΦ(*narG-lacZ*)*218*] (*trpEA*)*2*}, VJS957 (as VJS950, but *narL215*::Tn*10*) (27), and CA8306 (*cya-854*). The bacteria were grown at 37°C to an A_{578} of ≈0.8 in 100 ml of mineral salts medium M9 (17) supplemented with HCl-hydrolysate of casein (0.5 g/liter; type C/HSF; Serva, Heidelberg, Germany) and tryptophan (75 mg/liter). Growth substrates were added in the following concentrations as indicated for each experiment: glucose, 10 mM (aerobic growth) or 25 mM (anaerobic growth); Na_2-fumarate, Na_2-succinate, $NaNO_3$, trimethylamine-*N*-oxide (TMAO), dimethyl sulfoxide (DMSO), and glycerol, 50 mM each (31). Aerobic growth (100 ml in 1-liter Erlenmeyer flasks) was on a rotary shaker (180 rpm). For anaerobic

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growth, bacteria were grown in rubber-stoppered infusion bottles under N_2 in degassed media.

Preparation of anaerobic cell suspensions. The anaerobic cultures in stoppered infusion bottles were centrifuged for 15 min at 3,500 × *g* in a rotor with rubber adapters. The supernatant was removed from the tilted bottle by a drain tube in the rubber stopper and by applying an overpressure of N_2 (28). The bacteria were washed once in degassed buffer A (0.1 M Na_2HPO_4-KH_2PO_4, 1 mM $MgSO_4$, 1 mM dithiothreitol; pH 7.4), suspended in buffer A with 50 mM succinate to obtain an A_{578} of 3 to 4, and incubated for 30 min at 37°C under anaerobic conditions. Then the suspension was cooled to 10°C, washed twice with buffer A, and suspended at an A_{578} of 15 to 20 in buffer A (10°C). When no preloading with succinate was intended, succinate was omitted in the incubation step. All steps were performed in degassed buffers under N_2 in the stoppered infusion bottle (5).

Preparation of aerobic cell suspensions. Aerobically grown bacteria were harvested by centrifugation, washed twice in buffer (0.1 M Na_2HPO_4-KH_2PO_4, 1 mM $MgSO_4$; pH 7.4), and incubated for 30 min at 37°C at a ΔA_{578} of 3 to 4. Aerobiosis was maintained by flushing the suspension with air.

Determination of dicarboxylate uptake. For measurement of anaerobic dicarboxylate uptake (4), the anaerobic cell suspension (ΔA_{578} = 15 to 20; see above) was diluted in 4 volumes of anaerobic buffer A in a reaction tube. Anaerobiosis was maintained by flushing the tube with N_2. Uptake was initiated by the addition of [1-^{14}C]fumarate (Amersham Buchler, Braunschweig, Germany; (245 MBq/mmol; final concentration, 5 to 100 μM) or [2,3-^{14}C]succinate (178 MBq/mmol) and terminated by silicone oil centrifugation (10). To that end, 100 μl of the mixture was transferred at different time intervals (beginning after 20 s) to a 400-μl microfuge tube containing a layer of 65 μl of silicone oil (PN200; specific gravity, 1.03 g/ml; Roth, Karlsruhe, Germany) on 30 μl of 20% perchloric acid. The tube was immediately centrifuged in a Beckman Microfuge E for 30 s. The tube was cut at the silicone perchloric acid boundary. The perchloric acid fraction in the tip, which contained the sedimented cells, was transferred to a reaction tube, mixed with 750 μl of H_2O, and sonicated for 5 min to homogenize the bacteria. After removal of the denatured protein by centrifugation, the extracted radioactivity was determined by liquid scintillation counting (scintillator Quicksafe A; Zinsser, Heusenstamm, Germany). Corrections for contaminating cosedimented external medium (periplasmic space and associated water) were done as described below. One unit per gram (dry weight) corresponds to an uptake of 1 μmol of substrate per min per mg (dry weight).

For measurement of aerobic dicarboxylate uptake, aerobic cell suspensions were used. During the experiment, aerobiosis was maintained by flushing the tubes with air. The reaction was started by adding labeled dicarboxylic acids as described above.

Determination of the cytoplasmic volume. The cytoplasmic space was determined according to the method of Rottenberg with [U-^{14}C]taurine as nonpermeable marker for the extracellular space (24). Separation of cells from the surrounding space was achieved by silicone oil centrifugation as described above. The volume of the extracellular space was determined by subtracting the volume of the taurine space from total ^3H_2O-accessible volume. The taurine space was 1.12 μl/mg (dry weight), and the water-accessible space was 3.27 μl/mg (dry weight). From this, a cytoplasmic volume of 2.15 μl/mg (dry weight) was calculated for *E. coli* AN387.

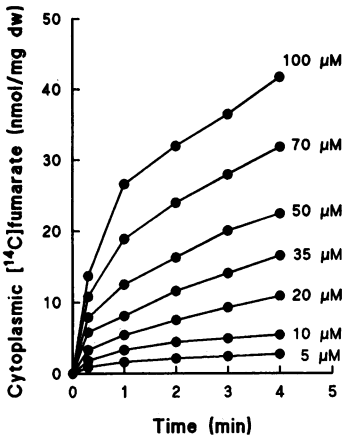

FIG. 1. Uptake of fumarate at different substrate concentrations by cell suspensions of anaerobically grown *E. coli* AN387 (wild type). Bacteria were grown in M9 medium with glycerol plus fumarate. Fumarate uptake was measured with [^{14}C]fumarate (245 MBq/mmol of fumarate) by silicone oil centrifugation.

Other methods. Dry weight of the bacteria was estimated from ΔA_{578} of the bacterial suspensions. A ΔA_{578} of 1 corresponded to 281 mg (dry weight) per liter.

RESULTS

Kinetics of anaerobic fumarate uptake by *E. coli*. In cell suspensions of *E. coli* which was grown anaerobically with glycerol plus fumarate, uptake of [^{14}C]fumarate was measured by the silicone oil centrifugation method. Substrate uptake into the cytoplasmic cell compartment was calculated from the radioactivity associated with the bacteria (cytoplasmic space) after correction for the external volume (attached water and periplasmic space). For optimal uptake rates, the bacteria had to be preloaded by incubation with succinate before measurement of uptake. However, even in the preloaded bacteria, the initial uptake rates slowed down rapidly after the start of the reaction (Fig. 1). After approximately 10 min, the internal radioactivity reached a constant level and no further net uptake could be observed. The internal radioactivity is assumed to consist of the original substrate ([^{14}C]fumarate). This is concluded from the findings that the radioactivity is released specifically by antiport with C_4-dicarboxylic acids (see below and see Fig. 4) and that the uptake kinetics are the same with fumarate reductase (*frd*) and succinate dehydrogenase (*sdh*) mutants (data not shown). Moreover, very similar uptake kinetics were observed with [^{14}C]succinate, which is not metabolized anaerobically by *E. coli*. The uptake resulted in an intracellular accumulation of fumarate; with 10 μM fumarate in the medium, intracellular concentrations of 1.0 mM fumarate were achieved.

In the Lineweaver-Burk plot for anaerobic fumarate uptake, a linear relation was observed over a 15-fold concentration range of [^{14}C]fumarate (Fig. 2). The same was observed for [^{14}C]succinate uptake (data not shown). From the linear range, a K_m of 400 μM and a V_{max} of 95 U/g (dry weight) can be estimated for fumarate uptake. This rate compares with a rate of fumarate respiration of 170 to 200 U/g (dry weight) measured in bacteria grown under the same conditions. The uptake kinetics are completely different from those in bacteria which were grown aerobically with

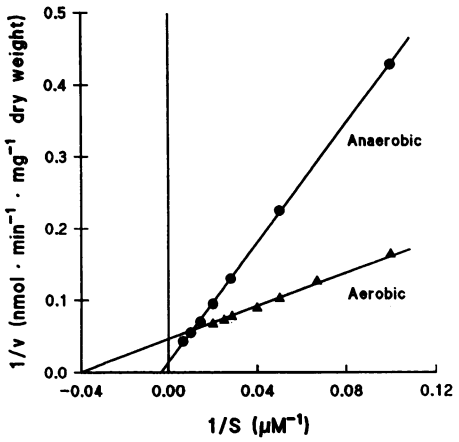

FIG. 2. Comparison of [^{14}C]fumarate uptake at different substrate concentrations (Lineweaver-Burk plot) by cell suspensions of aerobic (▲) and anaerobic (●) *E. coli* AN387 (wild type). Bacteria were grown in M9 medium with succinate (aerobic growth) or with glycerol plus fumarate (anaerobic growth). Initial uptake rates were determined by silicone oil centrifugation.

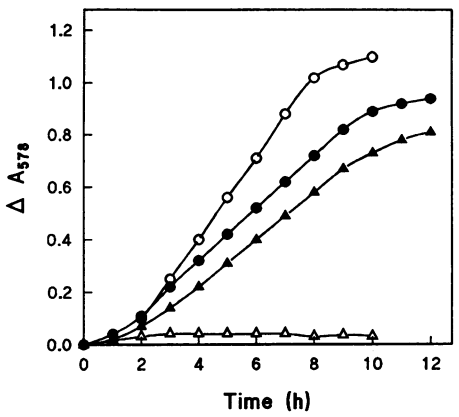

FIG. 3. Aerobic and anaerobic growth of *E. coli* AN387 (○, ●) and CBT38 (*dctB3*) (△, ▲) in M9 medium with succinate (open symbols, aerobic growth) or glycerol plus fumarate (closed symbols, anaerobic growth).

succinate or fumarate (Fig. 2). In aerobic bacteria, optimal uptake did not require preloading of the bacteria. The Lineweaver-Burk plot gave a linear relation, and the kinetic parameters V_{max} (25 U/g [dry weight]) and K_m (30 μM for fumarate) are distinctly different from the values of anaerobic bacteria. Very similar values for fumarate transport were found for bacteria pregrown in fumarate and succinate. Additionally, aerobic succinate uptake (V_{max} = 35 U/g [dry weight]; K_m = 25 μM succinate) closely resembled fumarate uptake.

Anaerobic dicarboxylate uptake in *E. coli* CBT38 (*dctB3*). In wild-type *E. coli* and a *dctB3* mutant, which is defective in aerobic C_4-dicarboxylate uptake, the kinetic parameters of fumarate and succinate uptake were compared (Table 1). The anaerobically grown wild type is able to take up succinate with rates and K_ms similar to those of its uptake of fumarate. In the anaerobically grown *dctB* mutant, the rates and K_ms for fumarate and succinate uptake were comparable to those of the wild type. Therefore, the *dctB* mutation obviously does not affect uptake of C_4-dicarboxylates in anaerobically grown bacteria. In aerobically grown bacteria, the situation is different. In the wild type, the uptake rates and K_ms for fumarate and succinate were very similar to those of the known aerobic C_4-dicarboxylate uptake system (9). The aerobic system is clearly differentiated from the anaerobic one by its >10-fold-lower K_m. In the *dctB* mutant, the aerobic uptake is strongly reduced by lack of the aerobic C_4-dicarboxylate system. From the results, it becomes clear that there are different C_4-dicarboxylate uptake systems functional in *E. coli* under aerobic and anaerobic growth conditions and that anaerobic uptake does not depend on *dctB*.

TABLE 1. Dicarboxylate uptake in wild-type *E. coli* AN387 and CBT38 (*dctB3*) after anaerobic and aerobic growth

Type of growth and strain	Transport[a]		
	Substrate	V_{max} (U/g [dry wt])	K_m (μM)
Anaerobic[b]			
AN387	Fumarate	95	400
	Succinate	82	480
CBT38 (*dctB3*)	Fumarate	78	470
	Succinate	72	490
Aerobic[c]			
AN387	Fumarate	25	30
	Succinate	33	25
CBT38 (*dctB3*)	Fumarate	2	50
	Succinate	3	40

[a] Measured with preloaded (anaerobic growth) or not preloaded (aerobic growth) bacteria.
[b] Growth substrate was glycerol plus fumarate in M9 medium.
[c] Growth substrates were succinate for AN387 and lactose plus fumarate for CBT38 in M9 medium.

Anaerobic growth of *E. coli* CBT38 (*dctB3*) with fumarate. The significance of the *dct* system for fumarate respiration was tested by growing strain CBT38 (*dctB3*) with glycerol plus fumarate (Fig. 3). The mutant showed growth and growth rates ($t_d \approx$ 2.5 h for CBT38 and ≈2.0 h for the wild type) similar to those of the wild type. The slightly decreased growth of CBT38 was also found for anaerobic growth on glucose and therefore is not specific for growth with fumarate. As expected, the *dctB3* mutant was not able to grow with succinate or fumarate under aerobic conditions (Fig. 3), whereas growth with glucose was comparable to that of the wild type (not shown). Therefore, the *dctB3* mutant apparently can use dicarboxylic acids as growth substrates anaerobically but not aerobically. Similarly, strain CBT312, which is defective in *dctA2*, grew anaerobically with fumarate as electron acceptor but not aerobically (data not shown).

Export of intracellular C_4-dicarboxylates by the anaerobic fumarate transport system. Anaerobically grown *E. coli* was preloaded by incubation in 100 μM [^{14}C]fumarate. By such preloading, nearly constant levels of [^{14}C]fumarate (40 nmol/mg [dry weight], corresponding to a cytoplasmic concentration of 19 mM) were obtained within the bacteria after 10 min (Fig. 4). A 100-fold excess of unlabeled dicarboxylic acids or other anions was then added to the external medium, and export of cytoplasmic [^{14}C]fumarate was determined by measuring the residual cytoplasmic [^{14}C]fumarate over a period of 6 min. The C_4-dicarboxylic acids fumarate, succinate, malate, maleinate, aspartate, and oxaloacetate caused rapid extrusion of 84 to 91% of the internal [^{14}C]fumarate (Fig. 4 and Table 2). In a rough estimate from Fig. 4, the initial rate of fumarate export was between 60 and 90 U/g (dry weight) and therefore was comparable to that of fuma-

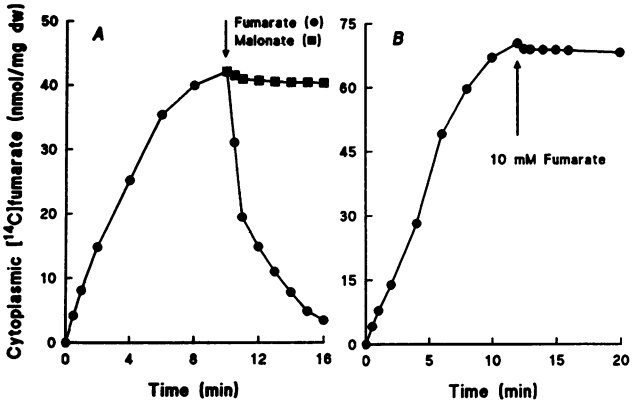

FIG. 4. Release of intracellular [^{14}C]fumarate by addition of 10 mM external anions in anaerobically (A) and aerobically (B) grown bacteria. Cell suspensions of *E. coli* AN387 (not preloaded) were incubated with 100 μM [^{14}C]fumarate. At the times indicated, antiport substrates (10 mM) were added. The cytoplasmic fumarate contents were determined by the silicone oil centrifugation technique. dw, dry weight.

rate uptake (Table 1). Of the effective dicarboxylic acids, fumarate caused the most rapid extrusion, and succinate followed fumarate. Oxaloacetate and maleinate were the least effective. Other anions such as malonate, citrate, pyruvate, acetate, and sulfate did not provoke release of [^{14}C]fumarate (Fig. 4 and Table 2). In aerobically grown *E. coli*, neither dicarboxylic acids nor other anions resulted in an export of internal fumarate (≤ 1 U/g [dry weight]) (Fig. 4 and Table 2). This demonstrates that under anaerobic conditions, transport of the dicarboxylates occurs by antiport across the cytoplasmic membrane, whereas aerobic transport operates only as an uptake system.

Growth conditions and anaerobic fumarate uptake. Expression of the enzymes of fumarate respiration is regulated by the available growth substrates and the electron acceptors present. The effect of the relevant substrates and acceptors on anaerobic fumarate uptake was tested in the experiment

TABLE 2. Export of cytoplasmic [^{14}C]fumarate by addition of external substrates to aerobically or anaerobically grown *E. coli* (wild type)

External antiport substrate (10 mM)	Release of cytoplasmic [^{14}C]fumarate (% of total)[a]	
	Aerobic	Anaerobic
Fumarate	<1	91
Succinate	<1	88
Malate	<1	86
Aspartate	<1	85
Oxaloacetate	<1	85
Maleinate	<1	84
Butyrate	<1	5
Malonate	2	5
Pyruvate	2	4
Citrate	2	5
Na_2SO_4	2	2
Acetate	2	3

[a] Bacteria were preloaded for 10 min with [^{14}C]fumarate (100 μM) before the addition of antiport substrate (10 mM each) as described in the legend to Fig. 3. Residual cytoplasmic [^{14}C]fumarate was measured by silicone oil centrifugation 6 min after addition of the antiport substrate. Aerobic growth was on succinate, and anaerobic growth was on glycerol plus fumarate.

TABLE 3. Effect of growth conditions (carbon source and electron acceptors) on anaerobic fumarate transport activities in *E. coli* AN387 (wild type)

Growth substrate	Fumarate uptake	
	V_{max} (U/g [dry wt])	K_m (μM)
Glucose	54	350
Glucose + fumarate	86	390
Glucose + succinate	77	390
Glycerol + fumarate	95	400
Glycerol + fumarate + TMAO	90	400
Glycerol + fumarate + DMSO	90	380
Glycerol + fumarate + NO_3^-	22	380
Glycerol + fumarate + O_2	28	30

reported on in Table 3. With glucose as the sole substrate for growth, rather high activities were measured, but presence of dicarboxylates (fumarate or succinate) in addition to glucose caused a significant increase in activity. Growth in rich medium (Luria broth; data not shown) resulted in similar activities. Maximal activities were found in bacteria grown anaerobically with glycerol plus fumarate. Presence of nitrate in addition to fumarate, but not of other acceptors of anaerobic respiration such as DMSO or TMAO, caused a decrease to 23% of the original activities. From the similar K_ms found under all conditions of anaerobic growth, it can be concluded that the same system was operating in all instances. In the presence of O_2, however, only the aerobic system was active. Under these conditions, the uptake kinetics were monophasic, with a K_m typical for the aerobic *dct* system, and the anaerobic system with a high K_m was missing.

Reversible inhibition by oxidizing agents. A cell suspension of anaerobically grown bacteria was incubated with various electron acceptors or oxidizing agents (Table 4). Bacteria incubated anaerobically without oxidizing agents showed optimal uptake rates. Aerobic incubation of the cell suspension resulted in strongly reduced activities (experiment 2, Table 4). By subsequent anaerobic incubation in the presence of a reducing agent such as dithiothreitol or glucose, the original high activities could be approached again (experiment 3). Inactivation could also be achieved by anaerobic incubation of the bacteria with acceptors of anaerobic respiration such as fumarate, TMAO, DMSO, and nitrate or with hexacynaoferrate(III) (experiments 4 to 9). In any case, the inactivation was not observed when glucose was present additionally. It appears, therefore, that anaerobic fumarate uptake can be reversibly inactivated by oxidizing agents and reactivated by reducing agents. The effect obviously is achieved not only by chemicals which could react directly with the transporter [dithiothreitol, O_2, or hexacyanoferrate(III)] but also by agents which have to act by enzymatically mediated oxidation (fumarate, DMSO, TMAO, nitrate, and possibly O_2) or reduction (glucose). The inactivation and reactivation can also be achieved in the presence of translational inhibitors such as chloramphenicol (experiment 5) and therefore appeared to be posttranslational. The low levels of fumarate uptake which were caused by the presence of nitrate and O_2 during growth, on the other hand, could not be reversed by reductive incubation (experiment 10 and 11).

Effect of regulatory mutations on anaerobic fumarate transport. The effects on anaerobic fumarate transport of different regulatory genetic loci which are known to affect expression

TABLE 4. Posttranslational inactivation and activation of fumarate transport in *E. coli* AN387 by oxidizing and reducing agents

Expt no. and incubation condition (37°C) for bacteria[a]	Fumarate uptake (V_{max}, U/g [dry wt]) in buffer A[b]	
	Without glucose	With glucose
Anaerobically grown (glycerol + fumarate)		
1. N_2	95	95
2. O_2	10	ND
3. O_2 (60 min) followed by N_2 (60 min)	75	ND
4. N_2 + fumarate (50 mM)	10	84
5. N_2 + fumarate (50 mM) + CAP (200 mg/liter)	10	80
6. N_2 + TMAO (50 mM)	22	90
7. N_2 + DMSO (50 mM)	24	89
8. N_2 + nitrate (50 mM)	10	85
9. N_2 + $Fe(CN)_6^{3-}$ (5 mM)	20	85
Anaerobically grown (glycerol + fumarate + nitrate)		
10. N_2	15	15
Aerobically grown (succinate + O_2)		
11. N_2	10	10

[a] Cell suspensions of anaerobically or aerobically grown *E. coli* were incubated for 60 min at 37°C under aerobic (O_2) or anaerobic (N_2) conditions with the additions given for each experiment and then used for measurement of fumarate uptake. CAP, chloramphenicol.

[b] For anaerobic incubation, buffer A also contained 1 mM dithiothreitol. ND, not determined.

of anaerobic respiratory genes in *E. coli* were studied with the help of the respective mutant strains (Table 5). With all strains, the K_ms of transport are somewhat lower than with strain AN387 (Table 1) but are characteristic for the anaerobic fumarate uptake system. In a mutant deficient in FNR (encoded by the *fnr* gene), the transcriptional activator of anaerobic respiratory genes, fumarate uptake was decreased by a factor of 5.3 compared with that of the parental strain. Presence of nitrate in the growth medium resulted in diminished fumarate transport rates in the wild type and the *narL* mutant. Therefore, in the case of fumarate uptake, nitrate possibly does not act via the nitrate regulator NarL. Synthesis of the transport system appears to be regulated not at all or only slightly by catabolite control, since a *cya* mutation (deficient in adenylate cyclase) with or without cyclic AMP in the medium showed fumarate uptake activities similar to those of the respective wild-type strains. This is in agreement with the finding that glucose did not repress fumarate uptake (Table 3).

TABLE 5. Effect of regulatory mutants on anaerobic fumarate uptake in anaerobically grown *E. coli* strains

Strain (relevant genotype)	Growth substrate	Fumarate uptake	
		U/g (dry wt)	K_m
PL2024 (wild type)	Lactose + fumarate	63	280
JRG861a (*fnr*)	Lactose + fumarate	12	270
VJS950 (wild type)	Glycerol + fumarate	54	250
	Glycerol + fumarate + NO_3^-	11	230
VJS957 (*narL*)	Glycerol + fumarate	53	250
	Glycerol + fumarate + NO_3^-	15	240
8306CA (*cya*)	Glucose + fumarate	55	340
	Glucose + fumarate + 2 mM cyclic AMP	69	350

DISCUSSION

An anaerobic dicarboxylate transport system different from the aerobic dicarboxylate transport (*dct*) system. The anaerobic fumarate transport system differs in all tested parameters from the aerobic dicarboxylate transport system (9, 14). The aerobic system shows a rather high affinity for the substrates combined with relatively low activities, which is characteristic of binding-protein-dependent transport systems. The anaerobic system differs by having significantly higher activities and a high K_m (95 U/g [dry weight] and 400 μM, respectively, for fumarate). The aerobic transport system corresponded to the known dicarboxylate transport system (*dct*); the anaerobic system has not been described so far. The two systems have different physiological functions. The *dct* system obviously is responsible for aerobic dicarboxylate uptake, and the anaerobic system appears to function in fumarate respiration. For growth by fumarate respiration, a fumarate turnover of approximately 200 μmol/min/g (dry weight) is necessary for growth, as calculated from the specific growth rate and the growth yield as described in reference 13. This calculation is in agreement with fumarate respiration rates (H_2 → fumarate) of 170 to 200 U/g (dry weight), which were measured in cell extracts. The fumarate uptake rates determined here account for approximately 50% of the theoretical values. The slower rates can be explained by the facts that the measurement of the initial rates is difficult (Fig. 1) and that resting cells were used. According to its presumed function, the system is most active under conditions of fumarate respiration and requires the *fnr* gene product for activity. Under the same growth conditions, high activities for other C_4-dicarboxylic acids (succinate, malate, maleinate, aspartate, and oxaloacetate) are also present. Presumably, transport of the different C_4-dicarboxylic acids is effected by the same transport system. However, this assumption has to be confirmed by the characterization of mutants with mutations in the anaerobic transport in future. The system therefore could also be suited to the uptake of malate or aspartate, which are used by the bacteria as precursors of fumarate in fumarate respiration. Malate and aspartate have to be transported to the cytoplasm, too, since fumarase and aspartase, which produce fumarate from their substrates, are cytoplasmic enzymes. Tricarboxylic, monocarboxylic, and C_3- or C_2-dicarboxylic acids are not accepted by the anaerobic transport system.

Anaerobic fumarate uptake is an antiport system. The aerobic and anaerobic uptake systems are completely different with respect to their transport mechanisms. Aerobic uptake operates unidirectionally, as is expected for a binding-protein-dependent system. Anaerobic transport is an antiport system which transports dicarboxylic acids in homo- and heterologous antiports. The export of succinate and fumarate can be estimated to occur at rates comparable to uptake and is driven by concomitant uptake of external dicarboxylic acids. Therefore, uptake and export are likely to operate in an antiport mechanism. In this system, export of one substrate (e.g., succinate) along a concentration gradient could drive accumulation of another substrate (e.g., fumarate). In contrast to the aerobic *dct* system, the antiport would not depend on ATP hydrolysis as the driving force.

With these properties, the anaerobic transport system would be suitable for fumarate respiration with respect to the transport mechanism (fumarate/succinate antiport) and energetics. The observed coupling of uptake and export argues for catalysis of both processes by one transport system and against the operation of separate uptake and export carriers. In recent years, it has been shown that transport systems operating in the antiport mode are quite common in bacteria. The known examples include various phosphate-linked antiport systems (glycerol-3-phosphate/phosphate; sugar phosphate/phosphate; phosphate/phosphate) (19) but also antiport of carboxylates such as oxalate/formate in *Oxalobacter formigenes* (1) and malate/lactate in lactic acid bacteria (19, 20, 22).

Regulation of synthesis and activity. The reduced uptake rates in an *fnr* background and under aerobic conditions suggest that the synthesis is under (direct or indirect) transcriptional control of FNR, the transcriptional regulator of anaerobic respiratory genes (reviewed in references 25 and 29). This indicates that the physiological role of the uptake system lies in fumarate respiration. Unlike the repression of the fumarate reductase genes (6, 7, 27) and DMSO reductase genes (3), the nitrate-dependent inhibition of fumarate transport apparently is not exerted at the transcriptional level in response to the *narL* gene product (7, 26, 27). The posttranslational inhibitory effects of O_2, nitrate, DMSO, TMAO, and hexacyanoferrate(III) might be caused by an oxidative inactivation of the transport system by the respective respiratory enzymes or electron transport systems. Oxidative inactivation of this type has also been observed for other transport systems (23). For the nitrate uptake system, too, a posttranslational inhibition by O_2 was described (18). The physiological significance of inhibition by nitrate and O_2 could reside in an exclusion of fumarate by the preferred substrates of respiration (O_2 and NO_3^-). Such a regulation at an early metabolic step (uptake) would be physiologically sensible. This type of regulation could also be essential for the shift from anaerobic to aerobic metabolism. The shift in *E. coli* is connected with derepression and operation of the citric acid cycle in the aerobic bacteria (6). Inactivation of the antiport by O_2 would be required to prevent leakage of citric acid cycle intermediates such as succinate, fumarate, malate, and oxaloacetate by the anaerobic antiport system in the shifted bacteria.

ACKNOWLEDGMENTS

We are grateful to V. Stewart (Ithaca, N.Y.) and B. Bachmann (New Haven, Conn.) for supplying bacterial strains.

This work was supported by grants from the Deutsche Forschungsgemeinschaft and by the Fonds der Deutschen Chemie to G.U.

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