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Role of Gene *fadR* in *Escherichia coli* Acetate Metabolism

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Mutants of *Escherichia coli* K-12 constitutive for fatty acid degradation (*fadR*) showed an increased rate of utilization of exogenous acetate. Acetate transport, oxidation, and incorporation into macromolecules was approximately fivefold greater in *fadR* mutants than $fadR^+$ strains during growth on succinate as a carbon source. This effect was due to the elevated levels of glyoxylate shunt enzymes in *fadR* mutants, since (i) similar results were seen with mutants constitutive for the glyoxylate shunt enzymes (*iclR*), (ii) induction of the glyoxylate shunt in $fadR^+$ strains by growth on acetate or oleate increased the rate of acetate utilization to levels comparable to those in *fadR* mutants, and (iii) *fadR* and $fadR^+$ derivatives of mutants defective for the glyoxylate shunt enzymes showed equivalent rates of acetate utilization under these conditions. These results suggest that the operation of the glyoxylate shunt may play a significant role in the utilization of exogenous acetate by *fadR* mutants.

Acetyl-coenzyme A (CoA) is a key intermediate in cellular metabolism. Intracellular acetyl-CoA can be derived from pyruvate obtained via glycolytic pathways, from the β-oxidation of fatty acids, or from exogenous acetate. These sources supply the acetyl-CoA needed for fatty acid biosynthesis and the continued functioning of the tricarboxylic acid cycle. During aerobic growth conditions, acetyl-CoA is mainly catabolized by the tricarboxylic acid cycle. However, since with each turn of the cycle two carbon atoms are lost as CO_2, no net assimilation of carbon from acetyl-CoA can occur by this means. Thus, growth on acetate or fatty acids as the sole carbon source requires operation of the anaplerotic pathway, the glyoxylate shunt, to replenish dicarboxylic acids drained from the tricarboxylic acid cycle for cellular biosynthesis (12).

The two enzymes unique to the glyoxylate shunt, isocitrate lyase and malate synthase A, are induced in *Escherichia coli* when acetate or fatty acids are provided as the sole carbon source (12). The structural genes for isocitrate lyase, *aceA*, and malate synthase, *aceB*, map at 90 min on the *E. coli* K-12 linkage map (2, 5) and appear to comprise an operon (S. R. Maloy, unpublished data). The expression of this operon seems to be controlled by an adjacent regulatory gene, *iclR* (5), as well as the *fadR* gene (18), which is also involved in the regulation of the fatty acid degradation (*fad*) regulon (24). Mutations in either the *iclR* or *fadR* gene result in elevated expression of the glyoxylate shunt enzymes under noninducing growth conditions (18).

This paper presents evidence that the operation of the glyoxylate shunt increases the utilization of exogenous acetate by *E. coli* K-12. In addition, we report the size of the intracellular acetyl-CoA pool under several growth conditions and show biochemical evidence that acetyl-CoA is not the inducer of the glyoxylate shunt in *E. coli* in vivo.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The bacterial strains used in this study were all derivatives of *E. coli* K-12. Table 1 gives the genotypes of these strains. Preparation of phage stocks and transductions were performed as previously described (20, 24). Table 1 describes the nomenclature for transposon insertions. The *fadR*::Tn*10* derivatives of strains were obtained by transduction with phage P1 *vir* grown on RS3040 and selection for tetracycline resistance. Strain JRG902 was transduced to tetracycline resistance with phage P1 *vir* grown on SM6001 (*zja*::Tn*10*) in order to place the transposon Tn*10* near the *iclR7* allele. Strains SM6034 and SM6042 were obtained by transducing strains K-12 and RS3010, respectively, with phage P1 *vir* grown on the JRG902 *zja*::Tn*10* isolate.

Bacteria were routinely grown in a New Brunswick gyratory water bath shaker at 37°C. The bacteria were usually grown in medium E (25). Carbon sources and supplements were sterilized separately and added to the culture medium before inoculation. All organic acids were neutralized with potassium hydroxide before sterilization. Acetate was added at 50 mM final concentration. Fatty acids were provided at 5 mM in the presence of Brij 58 (5 mg/ml). All other carbon sources were provided at 25 mM. When necessary, amino acid supplements were added at a final concen-

TABLE 1. *Bacterial strains used*

Strain	Genotype	Source
K-12	Prototrophic	J. Lederberg strain via CGSC[a]
RS3010	*fadR*	Nunn et al. (23)
RS3040	*fadR*::Tn*10*[b]	Nunn et al. (23)
SM6009	*aceA1 zja*::Tn*10*	Maloy et al. (18)
SM6016	*aceA1 zja*::Tn*10 fadR*	Maloy et al. (18)
SM1021	*aceB6 glc-1 thi-1 relA1 lacZ43*	Maloy et al. (18)
SM1022	*aceB6 glc-1 thi-1 relA1 lacZ43 fadR*::Tn*10*	Maloy et al. (18)
SM6034	*iclR7 zja*::Tn*10*	This work
SM6042	*iclR7 zja*::Tn*10 fadR*	This work
SM6001	*metA zja*::Tn*10*	Maloy et al. (18)
Ymel	Prototrophic	CGSC
YmelDT	*fadR*::Tn*10*	This work
K19	*fadE*	Maloy et al. (18)
K19DT	*fadE fadR*::Tn*10*	Maloy et al. (18)
K27	*fadD*	Maloy et al. (18)
K27DT	*fadD fadR*::Tn*10*	Maloy et al. (18)
LS7049	*fadL*	Maloy et al. (19)
LS7050	*fadL fadR*::Tn*10*	Maloy et al. (19)
K1	*fad-5*	Maloy et al. (18)
K1DT	*fad-5 fadR*::Tn*10*	Maloy et al. (18)
JRG902	*trpA9761 menC1 nalA39 rpsL195 iclR7 trpR72 fal-25*	J. R. Guest strain via CGSC
K8-5M	*met-24 aceA3 iclR13 galK2 lacY1 xyl-5 strA159 tsx-6 supE44*	H. Kornberg strain via CGSC
SM1104	K8-5M *fadR*::Tn*10*	This work

[a] CGSC strains obtained from B. Bachmann, Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] Transposon insertions are designated as previously described (7, 19). When an insertion is not within a known gene, it is given a three-letter symbol starting with "z," and the second and third letters indicate the approximate map location in minutes (e.g., *zaf* corresponds to 5 min, and *zbb* corresponds to 11 min).

tration of 0.01%. Cell growth was monitored at 540 nm on a Klett-Summerson colorimeter.

Acetate transport and oxidation. Transport of [1-^{14}C]acetate was determined as previously described (23). Oxidation of [1-^{14}C]acetate was determined as described for long-chain fatty acids (23), but the reaction was stopped by the addition of 0.5 ml of phosphate-citrate buffer (pH 3.9) to the cell suspension as described by Klein et al. (11).

Incorporation of radioactive substrates into macromolecules. Cultures were grown to midexponential phase at 37°C in medium E and the indicated substrate. The cells were harvested by centrifugation, washed twice with medium E, and resuspended in the same medium. The culture was then divided into several portions to which 0.4 mM [1-^{14}C]acetate (0.5 μCi/μmol) or a different labeled substrate was added, and the cultures were returned to a 37°C water bath shaker. At zero time, and at various intervals after the addition of the labeled substrate, 1.0-ml portions were removed, and the incorporation into the lipid and protein fractions was determined. For analysis of incorporation into lipids, portions were directly added to 6 ml of chloroform-methanol (1:2, vol/vol) and blended, and 0.6 ml of concentrated carrier cells (ca. 5×10^9 cells/ml) was added. Lipids were extracted as described by Ames (1) and counted in a liquid scintillation counter (23). Incorporation into protein was determined by precipitation with 10% trichloroacetic acid as described by Glaser et al. (8).

Determination of acetyl-CoA pools. The intracellular concentration of acetyl-CoA was determined by a modification of the procedure of Lazarow (14). Cultures (100 to 200 ml) were grown to midexponential phase at 37°C, washed twice with 500 mM Tris-hydrochloride (pH 8.1), and suspended in 0.5 ml of 0.5 N perchloric acid. These samples were vigorously blended and allowed to sit on ice for 15 min. The acid was neutralized by the addition of 0.3 ml of potassium bicarbonate, and the samples were placed on ice for an additional 15 min. The precipitated protein and perchlorate were removed by centrifuging for 2 min in a microfuge. The supernatant was collected, the pellet was washed once with 0.5 ml of ice-cold water, and the supernatants were combined. The acetyl-CoA concentration of the supernatant fraction was then assayed by means of the citrate synthetase reaction coupled to the malate dehydrogenase reaction essentially as described (4). This procedure was found to be accurate and reproducible when controls containing known amounts of acetyl-CoA were treated in an identical manner.

Enzyme assays. Crude extracts were prepared by disrupting midexponential-phase cells in a French press as previously described (18). Isocitrate lyase and malate synthase were assayed as described by Maloy et al. (18). Isocitrate dehydrogenase was assayed by following the formation of NADPH from isocitrate and NADP$^+$ (4). Malate dehydrogenase was assayed by following the formation of NAD$^+$ from oxaloacetate and NADH as described by Bergmeyer (4). Phosphotransacetylase was assayed by following the increase in absorbance at 233 nm due to the formation of acetyl-CoA from acetylphosphate and CoA as described by Bergmeyer (4). Acetate kinase was assayed by the formation of the hydroxamate derivative of acetylphosphate essentially as described (4). All enzyme assays were shown to be linear over the time of assay and directly proportional to the amount of protein added.

Protein and lipid determinations. Protein was usually determined by a microbiuret procedure (21), using bovine serum albumin as a standard. The protein content of extracts prepared in Tris-hydrochloride or triethanolamine buffer was determined by the Bio-Rad method (Bio-Rad Laboratories, Richmond, Calif.). Total lipid phosphate was determined by a micro-Bartlett procedure as described by Kates (10).

Chemicals. Radioactive substrates were purchased from New England Nuclear Corp., Boston, Mass. Unlabeled substrates and enzymes were obtained from Sigma Chemical Co., St. Louis, Mo. All other chemicals used were of reagent grade.

RESULTS

Incorporation of labeled substrates into macromolecules in *fadR*$^+$ and *fadR* strains. We first noted differences in the incorporation of exogenous acetate by *fadR*$^+$ versus *fadR*

strains during studies on lipid biosynthesis in *fadR* mutants. When grown on medium containing succinate as the sole carbon source and [1-^{14}C]acetate incorporation was measured in this medium, the *fadR* strains RS3010 and RS3040 incorporated acetate into phospholipids at about five times the rate of the *fadR*$^+$ parent, K-12 (Table 2). Under these conditions, the rate of acetate incorporation into proteins was also about five times greater in the *fadR* strains than in the *fadR*$^+$ parent (Table 2), indicating that the increased rate of incorporation of acetate by *fadR* strains was not due simply to an effect of the *fadR* mutation on lipid biosynthesis. Furthermore, there was no difference in the rate of turnover in [1-^{14}C]acetate-labeled lipids in *fadR* versus *fadR*$^+$ strains (18), indicating that the increased incorporation of [1-^{14}C]acetate by *fadR* strains was not due to an effect of the *fadR* mutation on lipid degradation.

When pregrown on acetate or oleate, the rate of acetate incorporation into lipids and proteins in the presence of succinate as a carbon source was comparable in *fadR*$^+$ and *fadR* strains (Table 2). The rate of acetate incorporation in both *fadR*$^+$ and *fadR* strains in this case was approximately equal to that of *fadR* mutants pregrown and labeled in medium containing succinate as the carbon source. In addition, when these strains were grown on dextrose and then allowed to incorporate acetate in the medium containing succinate as a carbon source, the rate of acetate incorporation was identical in *fadR*$^+$ and *fadR* strains and was similar to that of the *fadR*$^+$ strain grown on succinate (Table 2).

We sought to determine whether the increased rate of acetate incorporation into *fadR* mutants was due to an increase in fatty acid and protein synthesis by *fadR* strains compared with *fadR*$^+$ strains. Thus, we compared the incorporation of other substrates by *fadR* and *fadR*$^+$ strains under different growth conditions. The incorporation of other substrates, including [*U*-^{14}C]glycerol, [*U*-^{14}C]dextrose, [*U*-^{14}C]leucine, [4,5-^{3}H]isoleucine, [^{3}H]H_2O, and [^{32}P]P_i, into lipids and proteins was comparable in *fadR*$^+$ and *fadR* strains under all conditions studied (data not shown). In addition, chemical analysis showed that the total cellular protein and phospholipid contents of *fadR*$^+$ and *fadR* strains were equivalent (data not shown). Similar results were seen with all *fadR* strains studied. These results suggest that the greater rate of acetate incorporation by *fadR* strains is not due to an increase in lipid or protein synthesis.

When the incorporation of [2,3-^{14}C]succinate was studied in cells grown on succinate plus 0.4 mM acetate, *fadR*$^+$ strains incorporated succinate into macromolecules at a rate of approximately 14 nmol/min per mg of protein, whereas *fadR* strains incorporated succinate into lipids at a rate of approximately 7 nmol/min per mg of protein (Table 3). These results suggest that the increased rate of acetate incorporation by *fadR* strains may be accompanied by a decrease in succinate incorporation into macromolecules.

TABLE 2. *Incorporation of [1-^{14}C]acetate into macromolecules by fadR$^+$ and fadR strains*

Carbon source[a]	Phospholipid (nmol/min per mg of protein)			Protein (nmol/min per mg of protein)		
	K-12	RS3010	RS3040	K-12	RS3010	RS3040
Succinate	1.44	8.49	7.29	1.09	5.25	5.13
Acetate	7.03	7.11	7.18	5.82	6.46	6.75
Oleate	8.24	9.40	9.56	5.93	6.59	6.62
Dextrose	1.94	1.96	2.03	1.16	1.85	2.03
Pyruvate	1.08	1.90	2.11	—[b]	—	—

[a] Cultures were grown on the indicated carbon source in medium E, washed twice, and resuspended in the same volume of medium E containing 25 mM sodium succinate. At zero time, 0.4 mM [1-^{14}C]acetate (1 μCi/2 μmol) was added and the samples processed as described in Materials and Methods.

[b] —, Not determined.

Transport of labeled substrates into *fadR*$^+$ and *fadR* strains. One possible explanation for the changes in the rates of acetate and succinate incorporation into macromolecules of *fadR* mutants could be a greater rate of acetate transport by *fadR* strains during growth on succinate. Thus, we examined acetate transport by *fadR*$^+$ and *fadR* strains under different conditions. When grown on succinate and allowed to transport [1-^{14}C]acetate in medium containing succinate as a carbon source, the *fadR* strain transported acetate at about a fivefold greater rate than did the *fadR*$^+$ parent (Table 3). After growth on acetate or oleate, there was no significant difference between the uptake of acetate into the *fadR*$^+$ versus the *fadR* strain in media containing succinate as a carbon source (Table 4).

When *E. coli* is grown on succinate as the sole carbon source, it derives the precursors for lipid and protein synthesis from metabolic by-products of this compound. Since the net protein or lipid synthesis, or both, in *fadR*$^+$ and *fadR* strains is comparable in media containing succinate as a carbon source, the greater rate of incorporation of exogenously labeled acetate into macromolecules of *fadR* strains must be accompanied by a decrease in the rate of incorporation of the succinate by-products into macromolecules. When the rate of succinate transport was measured in *fadR*$^+$ and *fadR* strains growing in media containing succinate as a carbon source and supplemented with acetate, the

TABLE 3. *Incorporation of [2,3-^{14}C]succinate into macromolecules by fadR$^+$ and fadR strains*

Growth condition	Phospholipid[a] (nmol/min per mg of protein)			Protein[a] (nmol/min per mg of protein)		
	K-12	RS3010	RS3040	K-12	RS3010	RS3040
Succinate	13.18	7.69	3.39	16.25	7.53	9.34
Acetate	1.18	0.87	0.88	1.06	0.95	1.04

[a] Rate of incorporation of [2,3-^{14}C]succinate determined as described for [1-^{14}C]acetate incorporation in Table 2.

TABLE 4. *Uptake of acetate and succinate by fadR$^+$ and fadR strains*[a]

Growth condition	Transport condition	Acetate transport (nmol/min per mg of protein)		Succinate transport (nmol/min per mg of protein)	
		K-12	RS3040	K-12	RS3040
Succinate	Succinate + acetate	25	131	196	134
	Succinate			232	221
Acetate	Succinate + acetate	135	138	102	98
	Succinate			115	116
Oleate	Succinate + acetate	138	141	86	83
Dextrose	Succinate + acetate	21	22		

[a] Transport was determined as described for the incorporation of labeled substrates into macromolecules in Table 2. When an unlabeled carbon source was present in addition to the substrate, the culture was allowed to equilibrate with the unlabeled carbon source before addition of the labeled substrate.

fadR strain (RS3040) was found to transport labeled succinate at a slower rate than the *fadR*$^+$ strain (K-12) (Table 4). Both strains transported succinate at the same rate in media containing succinate as the sole carbon source lacking acetate and after pregrowth in acetate (Table 4).

The above data suggested that the differences in acetate and succinate incorporation between *fadR*$^+$ and *fadR* may be due to increased acetate transport in *fadR* mutants. Therefore, we examined the levels of the two key enzymes believed to be involved in acetate transport. However, there was no obvious difference between the activities of the two key enzymes involved in acetate uptake, acetate kinase and phosphotransacetylase (6, 16), in *fadR*$^+$ versus *fadR* strains under the growth conditions studied (Table 5).

Acetyl-CoA pool size in *fadR*$^+$ and *fadR* strains. The increased rate of acetate transport by *fadR* strains could conceivably be due to a smaller acetyl-CoA pool size under these conditions. A smaller acetyl-CoA pool size could result in an increased specific activity of the labeled acetate in the cellular acetyl-CoA pool, causing an apparent increase in the utilization of radioactive acetate (22). This possibility was considered unlikely, since the increased incorporation of acetate into *fadR* versus *fadR*$^+$ strains was observed regardless of the specific activity of the [1-^{14}C]acetate used (22; data not shown). However, the size of the acetyl-CoA pool was directly measured in *fadR*$^+$ and *fadR* strains under several growth conditions (Table 6). There was no dramatic difference in the acetyl-CoA pool size detectable in *fadR*$^+$ or *fadR* strains under any of the growth conditions tested, although the acetyl-CoA pools were somewhat smaller in the *fadR* strains than the *fadR*$^+$ strain.

Incorporation of [1-^{14}C]acetate into macromolecules of mutants with defects in the glyoxylate shunt or fatty acid degradation. The growth of *fadR*$^+$ strains on acetate or oleate was shown to increase the rate of acetate incorporation into macromolecules to levels comparable to those of *fadR* strains grown on succinate, whereas growth on dextrose decreased the rate of acetate incorporation by *fadR* strains to that of the *fadR*$^+$ strain grown on succinate (Table 2). Growth on acetate or oleate is known to induce the glyoxylate shunt enzymes, whereas growth on dextrose represses these enzymes. Furthermore, since previous studies have shown that the glyoxylate shunt enzymes are elevated in *fadR* mutants (18), it was conceivable that the elevated level of these enzymes played a role in the increased rate of acetate utilization.

TABLE 5. *Specific activities of acetate kinase and phosphotransacetylase in fadR$^+$ and fadR strains*

Growth condition	Acetate kinase (nmol/min per mg of protein)		Phosphotransacetylase (nmol/min per mg of protein)	
	K-12	RS3040	K-12	RS3040
Succinate	1,319	1,052	1,838	2,885
Acetate	731	722	1,360	2,400
Oleate	797	1,100	2,503	2,098

TABLE 6. *Acetyl-CoA pools in fadR$^+$ and fadR strains*

Growth condition	Acetyl-CoA (μM)[a]		
	K-12	RS3010	RS3040
Succinate	28.3 ± 8.0	31	42.2 ± 14.6
Acetate	25.0 ± 6.0	19.9	18.4 ± 9.0
Succinate + acetate	28.0 ± 9.2	21.4	30.0 ± 12.4
Oleate	29.4 ± 8.6	28.7	34.6 ± 11.8

[a] The acetyl-CoA concentration was determined as described in Materials and Methods. The acetyl-CoA pool size was based upon an intracellular water volume of 8.71 μl per mg of protein as determined by Maloy et al. (19).

Therefore, the incorporation of acetate into macromolecules by mutants with defects in the glyoxylate shunt enzymes was studied (Table 7). After pregrowth on succinate, both the *fadR*$^+$ and *fadR* derivatives of mutants with an *aceA* or *aceB glc* mutation showed equivalent low levels of incorporation of acetate into phospholipids with succinate as a carbon source (Table 8). The *fadR*$^+$ *iclR* (SM6034) strain incorporated acetate into phospholipids at about a threefold-greater rate than the *fadR*$^+$ *iclR*$^+$ (K-12) strain under these conditions. However, both the *fadR* (RS3040) and the *fadR iclR* (SM6042) strains showed rates of acetate incorporation about twice as high as the rates for the *fadR*$^+$ *iclR* strain (Table 8). These rates may reflect the greater activity of the glyoxylate shunt enzymes in *fadR* and *fadR iclR* strains during growth on succinate compared with the *fadR*$^+$ *iclR* strains (Table 7).

The rate of acetate incorporation into phospholipids of *fadR*$^+$ and *fadR* derivatives of *fad* mutants was also studied to determine whether fatty acid degradation is required in order to see this effect. After growth on succinate, *fadR fad* mutants showed about fivefold-greater incorporation of acetate into phospholipids in the presence of succinate than *fadR*$^+$ *fad* strains (Table 7). This indicates that fatty acid degradation did not cause the increased incorporation of acetate by *fadR* strains.

Activity of the tricarboxylic acid cycle in *fadR* and *fadR*$^+$ strains. The increased incorporation of acetate into macromolecules by strains with an operative glyoxylate shunt could also be due to a coordinate decrease in acetate oxidation by the complete tricarboxylic acid cycle, thereby making more acetyl-CoA available for lipid and protein synthesis. After growth on succinate, the rate of $^{14}CO_2$ evolution from acetate in the *fadR* strain was about fivefold greater than that seen in the *fadR*$^+$ strain (Table 9). Under these conditions, the rate of $^{14}CO_2$ release from succinate was less in the *fadR* strain than the *fadR*$^+$ strain (Table 9). However, the latter results could simply reflect the differences in acetate and succinate transport into *fadR* strains under these conditions. Thus, we also determined the activities of the tricarboxylic acid

TABLE 8. *Incorporation of [1-^{14}C]acetate into mutants with defects in the glyoxylate shunt or fatty acid degradation*

Strain	Relevant genotype	Incorporation into lipids[a] (nmol/min per mg of protein)
K-12	Prototrophic	1.44
RS3040	*fadR*::Tn*10*	7.29
SM6009	*aceA*	0.67
SM6016	*aceA fadR*::Tn*10*	0.51
SM1021	*aceB glc*	0.88
SM1022	*aceB glc fadR*::Tn*10*	1.45
K8-5M	*aceA iclR*	0.99
SM1104	*aceA iclR fadR*::Tn*10*	0.93
SM6034	*iclR*	3.60
SM6042	*iclR fadR*::Tn*10*	7.24
Ymel	Prototrophic	1.74
YmelDT	*fadR*::Tn*10*	8.01
K19	*fadE*	1.04
K19DT	*fadE fadR*::Tn*10*	7.72
K27	*fadD*	1.13
K27DT	*fadD fadR*::Tn*10*	7.84
LS7049	*fadL*	0.74
LS7050	*fadL fadR*::Tn*10*	6.03
K1	*fad-5*	1.23
K1DT	*fad-5 fadR*::Tn*10*	7.88

[a] Incorporation of [1-^{14}C]acetate into phospholipids after growth on succinate determined as described in Table 2.

TABLE 7. *Specific activities of isocitrate lyase and malate synthase in fadR*$^+$ *and fadR derivatives of mutants affecting the glyoxylate shunt*

Strain	Relevant genotype	Sp act (nmol/mg of protein)					
		Isocitrate lyase			Malate synthase		
		Succinate	Acetate	Succinate + acetate	Succinate	Acetate	Succinate + acetate
K-12	Prototrophic	21	244	70	103	410	186
RS3040	*fadR*::Tn*10*	209	320	276	267	594	330
SM6009	*aceA*	<1	NG[a]	<1	98	NG	196
SM6016	*aceA fadR*	<1	NG	<1	276	NG	311
SM1021	*aceB glc*	22	NG	198	32	NG	32
SM1022	*aceB glc fadR*	102	NG	224	33	NG	31
K8-5M	*aceA iclR*	<1	NG	<1	205	NG	274
SM1104	*aceA iclR fadR*	<1	NG	<1	293	NG	338
SM6034	*iclR*	172	272	204	371	566	389
SM6042	*iclR fadR*	383	396	386	396	592	402

[a] NG, No growth on this carbon source.

TABLE 9. *Oxidation of acetate and succinate by fadR$^+$ and fadR strains*

Growth condition	Rate of $^{14}CO_2$ release from acetate[a]		Rate of $^{14}CO_2$ release from succinate		Isocitrate dehydrogenase (nmol/min per mg of protein)		Malate dehydrogenase (nmol/min per mg of protein)	
	K-12	RS3040	K-12	RS3040	K-12	RS3040	K-12	RS3040
Succinate	22	112	173	111	276	295	2,125	2,180
Acetate	90	91	52	53	238	224	2,395	2,544
Oleate	125	124	—[b]	—	125	137	3,642	3,917
Dextrose	20	21	—	—	—	—	—	—

[a] Oxidation of [1-^{14}C]acetate and [2,3-^{14}C]succinate determined in medium containing 25 mM succinate and 0.4 mM acetate as described in Table 2.
[b] —, Not determined.

cycle enzymes isocitrate dehydrogenase and malate dehydrogenase. Isocitrate dehydrogenase is a prime candidate for regulation since it is the first enzyme of the tricarboxylic acid cycle after the glyoxylate shunt branches off. Malate dehydrogenase, on the other hand, is required for both the glyoxylate shunt and the complete tricarboxylic acid cycle. Holms and Bennett have proposed that the accumulation of glyoxylate causes the allosteric inhibition of isocitrate dehydrogenase when *E. coli* ML308 adapts from growth on glucose to acetate (9), although they failed to observe this result with *E. coli* K-12 (3). We observed that the activity of isocitrate dehydrogenase was somewhat lower in *fadR*$^+$ and *fadR* strains after growth on acetate or oleate than after growth on succinate (Table 9). However, there was no significant difference between the activity of isocitrate dehydrogenase in *fadR*$^+$ and *fadR* strains under any growth condition studied (Table 9). In addition, the activity of malate dehydrogenase was also equivalent in *fadR*$^+$ and *fadR* strains under each condition (Table 9).

DISCUSSION

The results presented in this paper indicate that the expression of the glyoxylate shunt increases the rate of utilization of exogenous acetate by *E. coli* K-12. The glyoxylate shunt enzymes are normally induced in wild-type strains during growth on acetate or fatty acids as the sole carbon source (12). Both *fadR* and *iclR* mutants express these enzymes constitutively (5, 18). When the glyoxylate shunt enzymes are expressed during growth on succinate, the rate of acetate transport, oxidation, and incorporation into macromolecules is increased. However, there is no change in the specific growth rate or net protein and lipid synthesis under these conditions. These results could be due to either (i) a diminished intracellular acetyl-CoA pool under these conditions or (ii) a preferential use of exogenous acetate over succinate under these conditions.

Since *E. coli* lacks a detectable pool of free acetate (15), we measured the size of the intracellular acetyl-CoA pool to determine whether the increased acetate incorporation by *fadR* mutants was due to a pool effect. A decrease in the acetyl-CoA pool size could result in an increased specific activity of the labeled acetate taken up by the cell, causing an apparent increase in the metabolism of radioactive acetate. The acetyl-CoA pool appeared to be maintained at a relatively constant concentration (ca. 25 μM) in either *fadR*$^+$ or *fadR* strains after growth on succinate, acetate, or oleate (Table 5), although the acetyl-CoA pools were somewhat lower in *fadR* than *fadR*$^+$ strains. The acetyl-CoA pool was about one-tenth to one-fifth the size of the pools of other tricarboxylic acid intermediates (13, 17). The acetyl-CoA pool determined is compatible with the reported K_m values for acetyl-CoA with citrate synthase (2 to 500 μM) and malate synthase (40 μM) (4). These data suggest that the acetyl-CoA pool size may be highly regulated, an observation which is not surprising in view of the limited supply of cellular CoA and the central role of acetyl-CoA as a cellular intermediate. Thus, the increased rate of incorporation of radioactive acetate in *fadR* strains does not seem to be simply a consequence of changes in the acetyl-CoA pool size. In addition, since the intracellular concentration of acetyl-CoA did not increase during growth on acetate or fatty acids, these results imply that acetyl-CoA is not the inducer of the glyoxylate shunt in vivo, in agreement with the genetic data of Kornberg (12).

On the other hand, the increased rate of acetate utilization by *fadR* mutants seemed to correlate well with the decreased utilization of exogenous succinate (Table 10). In media containing both succinate and acetate, the *fadR* strain transported 106 nmol/min per mg of protein

TABLE 10. *Comparisons of the rate of metabolism of [1-^{14}C]acetate and [2,3-^{14}C]succinate by fadR and fadR^{+} strains pregrown on succinate or acetate*

Pregrowth condition	Label[a]	Difference in the rate of utilization: *fadR* minus *fadR*$^{+}$ (Δ[nmol/min per mg of protein])[b]				
		Transport	Lipid	Incorporation		Total[c]
				Protein	Oxidation	
Succinate	[1-^{14}C]acetate	106	5.74	12.57	90	108.31
	[2,3-^{14}C]succinate	−62	−3.48	−7.82	−62	−73.30
Acetate	[1-^{14}C]acetate	3	2.33	1.02	1	4.35
	[2,3-^{14}C]succinate	−4	−0.30	−0.07	1	0.63

[a] Strains were grown on the indicated carbon source, and the metabolism of the labeled substrate was measured in medium containing succinate and acetate as described in Table 2.

[b] Values represent the average rate of metabolism of the labeled substrate by *fadR* strains minus the average rate of metabolism of isogenic *fadR*$^{+}$ strains.

[c] Sum of the rates of incorporation into lipid and protein and the rate of oxidation of the labeled substrate.

more acetate than *fadR*$^{+}$ strains and 62 nmol/min per mg of protein less succinate than *fadR*$^{+}$ strains. Therefore, on a carbon-for-carbon basis, the greater utilization of acetate by *fadR* strains was counterbalanced by the lesser utilization of succinate. Similarly, succinate oxidation and incorporation into macromolecules in *fadR* mutants decreased proportionally to the increased acetate utilization. These results suggest that the supply of precursors for lipid and protein synthesis obtained from acetate is increased under conditions in which the glyoxylate shunt is operative. As a consequence, the cell reduces its use of exogenous succinate for these processes. Thus, constitutive expression of the glyoxylate shunt enzymes enables the cell to utilize exogenous acetate in preference to succinate.

The increased rate of acetate transport and metabolism by *fadR* strains was not seen when either of the glyoxylate shunt enzymes, isocitrate lyase or malate synthase, was defective. Therefore, the increased utilization of acetate by *fadR* and *iclR* mutants is dependent on the constitutive expression of a fully functional glyoxylate shunt. The increase in acetate transport does not seem to be due to an increase in the two key enzymes believed to be involved in the uptake of acetate, acetate kinase, and phosphotransacetylase. However, since evidence for this comes solely from in vitro enzyme assays, this may not reflect the regulation of these enzymes in vivo under conditions in which the glyoxylate shunt is expressed. It is also conceivable that another, as yet uncharacterized, acetate transport component is induced coordinately with the glyoxylate shunt enzymes. Nevertheless, since the activities of acetate kinase and phosphotransacetylase are considerably greater than the rates of acetate transport observed, the greater rate of acetate transport by *fadR* mutants could be completely accounted for by the greater flux of acetate metabolized when the glyoxylate shunt is operative.

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