A MUTANT OF ESCHERICHIA COLI WITH ALTERED INDUCER SPECIFICITY FOR THE FAD REGULON

W. J. O'Brien and F. E. Frerman

Department of Microbiology, The Medical College of Wisconsin

Milwaukee, Wisconsin 53233

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SUMMARY

A novel regulatory mutant of the fatty acid degradation (fad) regulon of Escherichia coli was isolated. This mutant, D-2, was induced to synthesize the fatty acid β-oxidation enzymes during growth on decanoate and laurate whereas the wild type strain was induced only when fatty acids with a chain length greater than 12 carbon atoms were present in the growth medium. The fatty acid specificity of the acyl CoA synthetase was also changed in strain D-2. The data are consistant with the hypothesis that acyl CoA's themselves are the inducers of the fad regulon and suggest that strain D-2 may synthesize an altered fad regulatory protein. The results also suggest that the acyl CoA synthetase may possess regulatory as well as enzymatic activity.

INTRODUCTION

The enzymes of fatty acid β-oxidation are induced when E. coli is grown in a medium containing a fatty acid with a chain length greater than 12 carbon atoms as the carbon source (1-3). Overath et al. (2) have suggested that acyl CoA's are inducers of the fatty acid degradation (fad) regulon (4) since a mutant of E. coli K12 which lacked the acyl CoA synthetase synthesized the other enzymes of the fad regulon at basal rates in the presence or absence of oleate in the growth medium.

Constitutive fad mutants (fad R^-) of E. coli can be selected during growth on decanoate (C_{10}) since decanoate is catabolized by β-oxidation but decanoate (or decanoyl CoA) is not an inducer of the fad regulon (2, 3, 5). This communication describes the isolation and characterization of novel regulatory mutant in which the fad regulon is induced during growth on decanoate and thus exhibits altered inducer specificity when compared with the wild type strain.

MATERIALS AND METHODS

Organisms and growth conditions. The bacteria used in this study were E. coli and WG4 and the mutants D-2 and D-18 which were derived from WG4. Strain WG4 was the gift of Dr. Aleck Bernstein of this department. Cells were grown at 37° with vigorous aeration in Medium A (6) supplemented with 0.5% Triton X-100. Carbon sources were sterilized separately and added to the culture medium prior to inoculation. Fatty acids were provided at 0.1% in the growth medium; glycerol and acetate were supplied at 0.4% unless otherwise noted. Growth was monitored turbidimetrically at 650 nm with a Spectronic 20 spectrophotometer. The expenontial growth rate constant, k, was calculated from the equation, $k = \frac{\log A_1/A_o}{0.69(t_1-t_o)}$, where A is the optical density of the culture and t is time. The regulatory mutants D-2 and D-18 were selected from strain WG4 after growth on decanoate (2).

Enzyme assays and protein determinations. Cells were harvested at the end of the exponential phase of growth and washed twice at 4° with 50 mM potassium phosphate buffer, pH 7.2, containing 1% Triton X-100 and once with the same buffer without the detergent. Cells were suspended at a density of 100 mg wet weight per ml in the phosphate buffer and disrupted by sonication. The disrupted cell suspension was centrifuged at 20,000 x g for 20 min and the resulting supernatant was assayed for the various enzymatic activities. Enoyl CoA hydratase and 3-hydroxyacyl CoA dehydrogenase were assayed at 22° as described by Overath et al. (1). Acyl CoA synthetase activity was assayed at 30° as described by Overath et al. (2) but in the absence of Triton X-100 since the detergent inhibited synthetase activity of strain WG4. Protein was estimated by the method of Lowry et al. (7) as modified by Miller (8).

Materials. Crotonyl CoA, acetoacetyl CoA, CoA, ATP, NAD and NADH were obtained from P L Biochemicals, Milwaukee, Wisc. Porcine heart 3-hydroxyacyl CoA dehydrogenase was obtained from Calbiochem, La Jolla, Calif. Fatty acids were purchased from the Hormel Lipid Preparation Laboratory, Austin, Minn. All other chemicals were reagent grade and obtained from the usual commercial sources.

TABLE 1. Growth of E. coli strains WG4, D-2 and D-18 grown on acetate, decanoate and oleate.

Strain	Carbon source	k ($hr.^{-1}$)	lag (hr.)
WG4	Acetate	.24	1.0
	Decanoate	---	---
	Oleate	.21	2.0
D-18	Acetate	.22	1.5
	Decanoate	.15	0.0
	Oleate	.20	0.0
D-2	Acetate	.25	0.8
	Decanoate	.14	3.0
	Oleate	.21	2.2

RESULTS

Growth properties of wild type and regulatory mutants. Cells grown overnight in Medium A containing 0.2% glycerol were inoculated into prewarmed media containing acetate, decanoate or oleate as the principal source of carbon and energy. As shown in Table 1, strain WG4 exhibited a lag when grown with acetate or oleate and did not grow on decanoate-supplemented medium. These are the expected results for a fad R+ strain since in the presence of decanoate, the fad regulon was not induced; the regulon was induced during growth on the long chain length fatty acids. Strain D-18 exhibited the growth properties of the fad R^- mutants previously isolated in other laboratories (2, 3, 5). Unexpectedly, strain D-2 exhibited a lag when grown on decanoate or oleate; this lag phase was considerably shorter than would be expected if fad R^- mutants were being selected. The data suggested that D-2 could be induced to synthesize the β-oxidation enzymes during growth on decanoate and that the inducer specificity of the fad regulon was altered since the fad regulon is not induced during growth of the wild type strain on decanoate.

The three strains were also compared with respect to their capacity to grow on fatty acids of different chain lengths. As shown in Figure 1, strains D-2 and D-18 showed similar growth rates as a function of fatty acid chain

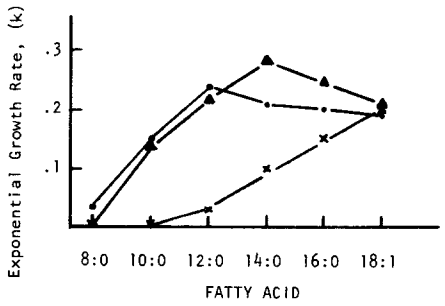

Figure 1. Dependence of exponential growth rate on the fatty acid carbon source. Cells grown overnight in Medium A containing 0.2% glycerol were inoculated into 500 ml Ehrlenmeyer flasks containing 25 ml of prewarmed (37°) Medium A supplemented with the fatty acid indicated. Growth was followed turbidimetrically at 650 nm. (▲) Strain D-2; (●) strain D-18; (x) strain WG4. 18:1 is oleic acid.

length. However, strain D-2 exhibited significantly higher growth rates on myristate and laurate in contrast to the parental inducible strain which grew slowly or not at all on these fatty acids. Strain D-2, unlike fad R^- mutants (2), did not grow on octanoate.

Levels of β-oxidation enzymes. The specific activities of several β-oxidation enzymes from cells grown in the mineral salts medium containing acetate, glycerol, oleate or decanoate were determined. Table 2 shows that the enzymes in WG4 are inducible and those in D-18 are constitutive and catabolite repressed by glycerol. The fad regulon in strain D-2 was induced during growth on decanoate and oleate but the enzymes were synthesized at essentially basal levels by cells grown on acetate and glycerol. The specific activities of all three enzymes were consistantly lower in D-2 cells grown with oleate as the carbon source when compared with the specific activities of the enzymes in this strain grown on decanoate. These data verified the fad R^+ phenotype of WG4, the fad R^- phenotype of D-18 and supported the hypothesis that inducer specificity of the fad regulon is altered in strain D-2 in that D-2 grew rapidly and synthesized the β-oxidation enzymes when the medium was supplemented with decanoate or laurate.

Acyl CoA synthetase in D-2. In addition to activating fatty acids prior to oxidation, the acyl CoA synthetase may play a role in fatty acid uptake (9)

TABLE 2. Specific activities of fatty acid oxidation enzymes in E. coli WG4, D-2 and D-18 grown on various carbon sources.

Strain	Carbon source	[a]Enzyme activity (nmoles x min^{-1} x mg^{-1}) Oleyl CoA Synthetase	Enoyl CoA Hydratase	3-Hydroxyacyl CoA Dehydrogenase
WG4	Oleate	3.02	4640	1900
	Acetate	0.82	188	238
	Glycerol	0.46	146	200
D-18	Oleate	3.10	5304	1890
	Decanoate	3.35	5679	2086
	Acetate	2.68	5269	1833
	Glycerol	1.38	1799	466
D-2	Oleate	1.69	1819	998
	Decanoate	2.60	5229	2248
	Acetate	0.44	340	421
	Glycerol	0.25	110	195

[a]The specific activities indicated are the average of at least two determinations.

and may catalyze the synthesis of the actual inducer of the fad regulon (2). Figure 2 illustrates the dependence of acyl CoA synthetase activity on the fatty acid substrate. Acyl CoA synthetase activity with short, medium and long chain length saturated fatty acids was comparable in the three strains; however, the activity of the D-2 synthetase with oleate as the substrate was reduced by about 50% when compared with the activities determined in strains D-18 and WG4 when the cells were grown on oleate. When strains D-2 and D-18 were grown on decanoate, the oleyl CoA synthetase activity of D-2 was about 77% of that determined in D-18. The specific activity of the synthetase with linoleate as the substrate in oleate-grown D-2 (0.82 nmoles x min^{-1} x mg^{-1}) was only 23% of the linoleyl CoA synthetase specific activity of strain D-18 grown on decanoate.

DISCUSSION

Inducible systems normally exhibit a high degree of specificity for inducing substrates (9). Our data inducate that E. coli D-2 is a novel regulatory

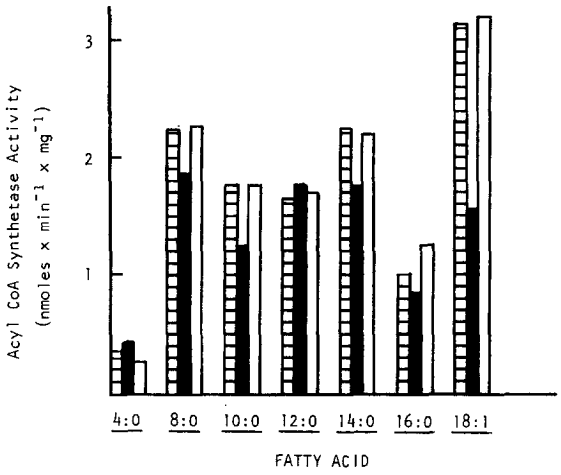

Figure 2. Fatty acid substrate specificity of the acyl CoA synthetases from E. coli WG4, D-18 and D-2. Acyl CoA synthetase activity was assayed by hydroxamate formation by the procedures described in Materials and Methods using the fatty acid substrates indicated at a concentration of 1 mM. The open bars | | indicate WG4 activity; the solid bars ▮ indicate D-2 activity and the striped bars ▤ indicate D-18 activity. 18:1 is oleic acid. The specific activities in the figure represent the average of at least two determinations.

mutant of the fad regulon. Although the exact nature of the mutation is presently unknown, the observations described here suggest that strain D-2 synthesizes an altered regulatory protein which results in a change of inducer specificity.

The results are consistant with the hypothesis of Overath et al. (2) that acyl CoA's are the inducers of the fad regulon. During growth on oleate, D-2 oleyl CoA synthetase specific activity was about 50% that of strains WG4 and D-18 grown on oleate; the specific activities of the hydratase and dehydrogenase were similarly reduced in oleate-grown D-2. However, during growth on decanoate, strain D-2 exhibited near normal oleyl CoA, hydratase and dehydrogenase specific activities. These results would be expected if the hypothetical fad regulatory protein had an increased affinity for decanyol CoA and decreased affinity for oleyl CoA. The results are also consistant with the idea that the synthesis of acyl CoA synthetase and other β-oxidation enzymes are controlled by the same regulatory protein although the enzymes map genetically at separate loci (2, 9). Strain D-2 exhibited altered inducer specificity and altered acyl CoA synthetase

activity with long chain unsaturated fatty acid substrates. It is reasonable to assume that the phenotype of D-2 is the result of a single mutational event since D-2 is a spontaneous mutant. Unless the altered region of the DNA overlaps both the synthetase locus and a hypothetical regulatory protein locus in D-2, it may be that the synthetase has regulatory as well as enzymatic activity. Further studies on the purified synthetases from WG4 and D-2 and determination of genetic locus responsible for the altered inducer specificity in strain D-2 are being undertaken to clarify this point. Lastly, the data clearly indicate that during growth on oleate, acyl CoA synthetase activity is not rate-limiting; therefore, oleate translocation catalyzed by the synthetase (9) is not the rate-limiting step during the growth of *E. coli* on oleate.

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