Acetaldehyde Coenzyme A Dehydrogenase of *Escherichia coli*

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Mutants of *Escherichia coli* (*adh*) in which alcohol dehydrogenase is derepressed under aerobic conditions were also found to overproduce acetaldehyde coenzyme A dehydrogenase. However, acetaldehyde coenzyme A dehydrogenase was induced by ethanol or acetaldehyde and subject to strong catabolite repression, whereas alcohol dehydrogenase was little affected by these conditions. Mutants no longer able to use ethanol as carbon source were isolated from an *adh* strain. Some of these mutants were revertants at the *adh* locus and no longer produced either alcohol dehydrogenase or acetaldehyde coenzyme A dehydrogenase. Others, designated *acd*, were found to lack only acetaldehyde coenzyme A dehydrogenase. The *acd* mutation was located at min 62 of the *E. coli* genetic map, the gene order being *thyA-lysA-acd-serA-fda*. Isolation of Tn10 insertions cotransducible with *acd* greatly simplified the mapping procedure.

In wild-type *Escherichia coli* grown under anaerobic conditions, sugars are fermented to a variety of products (9). One important pathway (5) involves the conversion of acetyl-coenzyme A (acetyl-CoA) to acetaldehyde by means of acetaldehyde CoA dehydrogenase:

The acetaldehyde is then converted to ethanol by alcohol dehydrogenase:

We have recently described mutants of *E. coli* (called *adh*) in which alcohol dehydrogenase is expressed aerobically (4). Further investigation of these mutants showed that high levels of acetaldehyde CoA dehydrogenase were also expressed aerobically. To further investigate the expression of acetaldehyde CoA dehydrogenase activity by *E. coli*, we assayed extracts of a variety of strains grown under different conditions and found that, apart from the joint derepression of alcohol dehydrogenase and acetaldehyde CoA dehydrogenase observed in *adh* mutants, the syntheses of these two enzymes were subject to disparate controls. We also isolated a mutant lacking acetaldehyde CoA dehydrogenase and mapped the genetic lesion responsible for this enzyme deficiency.

MATERIALS AND METHODS

Media, bacterial strains, and genetic techniques. The isolation of the original *adh* mutants, and most of the genetic techniques and media used, have been previously described (4). The ability of bacterial colonies to oxidize ethanol was observed by

using tetrazolium redox-indicator plates (3).

All bacteria were strains of *E. coli* K-12 and are listed in Table 1. A P1 pool of random Tn10 insertions into the *E. coli* chromosome was constructed by Amy Klages of this laboratory as follows: strain W1485 was infected with λ NK370 b221 cI857 cI171::Tn10 *Opa*11261 (from N. Kleckner), and tetracycline-resistant colonies were selected (6). Phage P1 *vir* was then grown on a pool of some 5,000 independent Tn10 insertions.

Enzyme assays. One unit of activity is defined as 1 nmol of product formed per min. Alcohol dehydrogenase was assayed as described (4). Acetaldehyde CoA dehydrogenase was assayed spectrophotometrically by following the reduction of NAD to NADH₂ at 340 nm (10). The assay was run in a total volume of 1.0 ml containing 50 mM CHES [2-(*N*-cyclohexylamino)ethanesulfonate] buffer (pH 9.5), 100 μ M Co-A, 1.0 mM dithiothreitol, 75 μ M NAD, and a volume of cell extract (20 to 200 μ g of protein). The reaction was initiated by adding 10 μ l of 1.0 M acetaldehyde. All enzyme assays were performed on the high-speed supernatants of cell extracts, prepared as previously described (4), since little or no acetaldehyde CoA dehydrogenase activity could be detected in extracts before centrifugation, probably due to interference by particulate NADH₂ oxidase (10).

For product determination, the acetaldehyde CoA dehydrogenase assay was run in 100 mM TAPS [*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonate] buffer (pH 8.4) with the dithiothreitol concentration reduced to 400 μ M and that of NAD increased to 100 μ M. The reaction was followed at either 340 nm (reduction of NAD) or 233 nm (formation of the acetyl-CoA thioester bond).

RESULTS

Characterization of acetaldehyde CoA dehydrogenase activity. An acetyl-CoA-forming acetaldehyde dehydrogenase has been well characterized in anaerobic clostridia (10),

TABLE 1. *Bacterial strains*

Strain	Relevant markers	Source ^a (reference)
W1485	F ⁺ wild type	CGSC 5024
KL96	Hfr, origin at 45 min (2)	K. B. Low
KL228	Hfr, origin as AB313 at 82 min (2)	K. B. Low
PK191	Hfr, origin at 43 min (2)	Laboratory collection
JC1552	F ⁻ <i>argG6 metB1 his-1 leu-6 trp-31 rpsL104</i>	CGSC 4273
PA3306	F ⁻ <i>nadB4 purI66 argH1 thi-1 rpsL9</i>	CGSC 4537
JG123	F ⁻ <i>thyA36 metE70 rpsL151</i>	CGSC 5921
EWB228	F ⁻ <i>metC69 glc-11 hisG1 argG6 rpsL</i>	CGSC 5795
PA360	F ⁻ <i>serA1 thr-1 leu-6 argH1 his-1 thi-1</i>	Laboratory collection
NP315	HfrC <i>fda-2</i> (Ts)	
AT706	HfrH <i>fuc-1</i>	CGSC 4528
AT713	F ⁻ <i>lysA22 argA21 cysC43 rpsL104</i>	CGSC 4529
DC48	<i>aceF10 fadR</i>	(4)
DC81	<i>aceF10 fadR adh-81</i>	(4)
DC271	<i>fadR</i>	P1 (<i>ace</i> ⁺) × DC48
DC272	<i>fadR adh-81</i>	P1 (<i>ace</i> ⁺) × DC81
DC343	<i>adh</i> ⁺ revertant of DC272	See text
DC346	<i>adh</i> ⁺ revertant of DC272	See text
DC349	<i>acd</i> mutant of DC272	See text
DC355	As DC272 but Tn10 near <i>acd</i>	See text
DC356	As DC272 but Tn10 near <i>acd</i>	See text
DC365	As PA360 but Tn10 from DC355	P1 (DC355) × PA360
DC366	As PA360 but Tn10 from DC356	P1 (DC356) × PA360
DC367	As AT713 but Tn10 from DC355	P1 (DC355) × AT713
DC368	As AT713 but Tn10 from DC356	P1 (DC356) × AT713
DC370	<i>thyA</i> of DC272	
DC408	<i>thy</i> ⁺ <i>serA</i> of DC370	P1 (PA360) × DC370
DC409	<i>adh acd serA</i> Tn10 (from DC355)	P1 (DC365) × DC349
DC410	<i>adh acd serA</i> Tn10 (from DC356)	P1 (DC366) × DC349
DC416	<i>thy</i> ⁺ <i>acd</i> of DC370	P1 (DC349) × DC370
DC418	<i>thy</i> ⁺ <i>acd</i> ⁺ of DC370	P1 (DC349) × DC370
DC434	<i>ser</i> ⁺ <i>acd</i> of DC408	P1 (DC349) × DC408
DC436	<i>ser</i> ⁺ <i>acd</i> ⁺ of DC408	P1 (DC349) × DC408
DC437	Spontaneous <i>acd</i> ⁺ revertant of DC349	

^a CGSC indicates that the strain was received from the Coli Genetic Stock Center, Yale University, through the kindness of the curator, Barbara Bachmann.

partially purified from *E. coli* B (8), but only mentioned in passing for *E. coli* K-12 (5). When we found this activity in *adh* mutants in *E. coli* K-12 (see below) we thought it prudent to characterize the reaction. For this purpose we used a high-speed supernatant fraction from strain DC272 grown in minimal salts medium supplemented with glucose (0.4%) and casein hydrolysate (0.1%). The assay described for clostridia (10) was modified, chiefly by using a CHES buffer (pH 9.5) and substituting dithiothreitol for glutathione. The reaction depended absolutely on the presence of NAD, CoA, acetaldehyde, and enzyme and required diethiothreitol for optimum activity as does the enzyme from *E. coli* B (8).

The partially purified acetaldehyde CoA dehydrogenase of *E. coli* B was reported to have a pH optimum of 7.0 and to be inhibited somewhat by Tris buffer (8). Using soluble cell extracts of our *E. coli* K-12 strain, we found that

the rate of reaction fell off rapidly in Tris. In contrast, dipolar ionic buffers gave sustained reaction rates, and a pH optimum of 8.8 was observed in CHES or TAPS buffer. The same pH optimum was also observed with enzyme purified through the ammonium sulfate step of Rudolph et al. (8).

Identification of product. Since the nucleic acids present in cell extracts absorb strongly at 233 nm, the formation of the thioester bond in acetyl-CoA could be used to follow the reaction only in extracts with high acetaldehyde CoA dehydrogenase activity. The formation of acetyl-CoA was followed at 233 nm in a TAPS buffer at pH 8.4, since acetyl-CoA hydrolyzes spontaneously at a substantial rate above pH 8.5. To positively identify the product as acetyl-CoA, we used the fact that phosphotransacetylase is highly specific for acetyl-CoA and catalyzes its conversion to acetyl-arsenate in the presence of arsenate (11). Acetyl-arsenate hydrolyzes spon-

taneously at the pH used; hence, treatment of acetyl-CoA with arsenate plus phosphotransacetylase results in a specific loss of absorbance at 233 nm. Five identical 1.0-ml reactions, containing 2 μ l of a highly active extract from strain DC272 grown in glucose plus Casamino Acids medium, yielded a final absorbance at 233 nm equivalent to the formation of 35.3 ± 2.7 nmol of thioester. Treatment with sodium arsenate (6 mM final concentration) and 1.6 U of phosphotransacetylase (Sigma) resulted in a loss of absorbance at 233 nm equivalent to 33.8 ± 2.5 nmol of acetyl-CoA. Hence, all the reaction product formed at 233 nm can be accounted for as acetyl-CoA. The average reaction rates observed for these samples were 3.25 and 2.94 nmol of acetyl-CoA and NADH₂ formed per min, respectively. The smaller amount of NADH₂ formed is probably due to the presence of alcohol dehydrogenase in all cell extracts containing acetaldehyde CoA dehydrogenase. Alcohol dehydrogenase catalyzes the reaction $\text{CH}_3\text{CHO} + \text{NADH}_2 \rightarrow \text{C}_2\text{H}_5\text{OH} + \text{NAD}$, and hence reduces the apparent rate of the acetaldehyde CoA dehydrogenase reaction when assayed by the formation of NADH₂.

Production of acetaldehyde coenzyme A dehydrogenase by *adh* mutants. Several wild-type strains and several *adh* mutants were assayed for acetaldehyde CoA dehydrogenase (Table 2). The *adh* mutants produced considerable acetaldehyde CoA dehydrogenase under aerobic conditions, whereas the wild-type strains produced little enzyme. The difference was greatest for cultures grown on rich broth, in which case the enzyme activity of wild-type strains was essentially undetectable. In strain DC272, addition of ethanol, acetaldehyde, or glucose caused a three- to fourfold increase in the level of acetaldehyde CoA dehydrogenase, but little change in the level of alcohol dehydrogenase. In contrast, growth in the presence of α -methyl glucoside caused severe repression of acetaldehyde CoA dehydrogenase, but the level of alcohol dehydrogenase was again scarcely affected. When extracts from cultures grown with and without inducer were mixed, the enzyme activity was additive (data not shown). Hence, the lower enzyme levels in uninduced cultures are not due to the presence of an inhibitor of the enzyme. None of these treatments was able to elicit significant production of acetaldehyde CoA dehydrogenase in strains wild type at the *adh* locus. We presume that the induction of acetaldehyde CoA dehydrogenase by ethanol is due to its conversion to acetaldehyde by alcohol dehydrogenase. However, until mutants lacking the latter enzyme but retaining the former are iso-

TABLE 2. Enzyme activities

Strain	Growth supplement ^a	Sp act (U/mg of protein)	
		ACDH ^b	ADH ^c
DC271	None	1.6	0.1
DC271	Ethanol, 0.5%	0.5	17
DC271	Acetaldehyde, 10 mM	0.5	6.7
DC271	Glucose, 0.4%	1.7	0.7
DC272 <i>adh</i>	None	16	85
DC272 <i>adh</i>	Ethanol, 0.5%	43	110
DC272 <i>adh</i>	Acetaldehyde, 10 mM	72	105
DC272 <i>adh</i>	Glucose, 0.4%	62	27
DC272 <i>adh</i>	α -Methylglucoside, 10 mM	4.2	107
Revertant of DC272			
DC343 <i>adh</i> ⁺	None	0.5	18
DC346 <i>adh</i> ⁺	None	0.9	4.1
DC349 <i>acd</i>	None	0.5	88
DC349 <i>acd</i>	Ethanol, 0.5%	13	156
Transductant			
DC434 <i>acd</i>	None	0.5	115
DC436 <i>acd</i> ⁺	None	43	106
Revertant of DC349			
DC437 <i>acd</i> ⁺	None	35	138

^a Cultures were grown in rich broth. Supplements were as shown. Acetaldehyde was added at the time of inoculation, and again two generation times before harvest, to make up for any loss by degradation or evaporation.

^b ACDH, Acetaldehyde CoA dehydrogenase.

^c ADH, Alcohol dehydrogenase.

lated, this surmise must remain unproven.

Selection of revertants of *adh* mutants. After treatment with ethyl methane sulfonate (4), strain DC272 was grown overnight in rich broth to allow segregation and then plated on tetrazolium indicator plates containing 0.5% ethanol. Colonies able to oxidize ethanol turned red. Revertants no longer able to use the ethanol remained white. Although the screening procedure was performed at 42°C, no temperature-sensitive mutants were obtained. None of the white colonies could grow in ethanol at 30, 37, or 42°C. These revertants fell into three classes: (i) lacking both alcohol dehydrogenase and acetaldehyde CoA dehydrogenase (e.g., strains DC343, DC346); (ii) lacking acetaldehyde CoA dehydrogenase only (e.g., strain DC349); or (iii) retaining both enzymes. No revertants lacking only alcohol dehydrogenase were found. The

lesion in revertants of type I was found to co-transduce with *trp* at the same frequency as the original *adh* mutation (4; data not shown). The first class of revertants are presumably true revertants at the *adh* locus. The second class of revertants did not map near the *adh* locus and still expressed high levels of alcohol dehydrogenase when grown aerobically. The mutation in strain DC349 was designated *acd* and is discussed further below. The third type of revertants has not yet been investigated.

Acetaldehyde CoA dehydrogenase levels in the *acd* mutant. When grown in rich broth, the levels of acetaldehyde CoA dehydrogenase produced by strain DC349 were below the limits of detection of the assay. Under inducing conditions (i.e., supplementation with ethanol or glucose), appreciable levels of this enzyme were observed (Table 2); however, these levels were always less than 30% of the level seen in the parental strain DC272 grown under inducing conditions. In contrast, the amounts of alcohol dehydrogenase found in *acd* strains were identical to those found in the *adh* parent. Phage P1 grown on the *acd* strain DC349 was used to transduce strain DC370 to *thyA*⁺ or DC408 to *serA*⁺. The *acd* transductants resembled strain DC349 in producing no detectable acetaldehyde CoA dehydrogenase when grown on rich broth and levels 10 to 20% those of comparable *acd*⁺ strains when grown under inducing conditions. A spontaneous *acd*⁺ revertant of strain DC349 regained high levels of acetaldehyde CoA dehydrogenase when grown in rich broth (Table 2).

Mapping of the *acd* mutation. Since wild-type *E. coli* cannot grow on ethanol, the inability to use ethanol caused by the *acd* mutation is only visible in an *adh* background. To simplify the mapping of the *acd* locus, we therefore infected strain DC349 with P1 grown on a pool of Tn10 insertions. After joint selection for *acd*⁺ and tetracycline resistance, we found several Tn10 inserts that were cotransducible with *acd*. P1 grown on strains DC355 and DC356, which carried two such Tn10 insertions, was used to transduce several Hfr strains to Tet^r. The Tet^r Hfr strains were then crossed with appropriate streptomycin-resistant recipient strains, and recombinants were selected at a variety of known loci and scored for Tet^r. From the cross Hfr KL96 *tet* × JC1552 we obtained 27% linkage between *tet* and *argG* (68 min) but <5% linkage with *his*, *trp*, *metB*, or *leu*. The crosses of Hfr KL228 *tet* with PA 3306 and Hfr PK191 *tet* with strains JG123 and EWH228 yielded linkages of *tet* to *purI* (55 min) of 38%, to *thyA* (61 min) of 68%, and to *met C* (64 min) of 70%.

P1 grown on DC355 and DC356 was then used

TABLE 3. Cotransduction experiments^a

P1 donor	Recipient	Marker selected	Colonies scored	% Cotransduction of donor marker
DC355 <i>tet</i>	DC349 <i>acd</i>	<i>tet</i>	200	25
DC355 <i>tet</i>	JG123 <i>thyA</i>	<i>tet</i>	200	12
DC355 <i>tet</i>	JG123 <i>thyA</i>	<i>thy</i> ⁺	200	15
DC355 <i>tet</i>	AT713 <i>lysA</i>	<i>tet</i>	100	11
DC355 <i>tet</i>	AT713 <i>lysA</i>	<i>lys</i> ⁺	100	37
DC355 <i>tet</i>	PA360 <i>serA</i>	<i>tet</i>	100	12
DC355 <i>tet</i>	NP315 <i>fda</i>	<i>tet</i>	100	3
DC365 <i>tet</i> <i>serA</i>	DC272 <i>ser</i> ⁺	<i>tet</i>	100	10
DC367 <i>tet</i>	DC349 <i>acd</i>	<i>tet</i>	200	31
DC367 <i>tet</i>	DC349 <i>acd</i>	<i>acd</i> ⁺	100	26
DC349 <i>acd</i>	DC370 <i>thyA</i>	<i>thy</i> ⁺	66	67
DC349 <i>acd</i>	DC408 <i>serA</i>	<i>ser</i> ⁺	223	35
DC370 <i>thyA</i>	DC349 <i>acd</i>	<i>acd</i> ⁺	200	56
CY151 <i>serA</i>	DC349 <i>acd</i>	<i>acd</i> ⁺	100	45
PA360 <i>serA</i>	DC370 <i>thyA</i>	<i>thy</i> ⁺	100	2

^a Although tested, no cotransduction was found between the Tn10 inserts near to *acd* and the markers *eno*, *pyrG*, *cysC*, *metC*, and *fuc*. A parallel series of cotransductions was performed using another, independently isolated Tn10 insertion near *acd*. Although the cotransduction frequencies obtained were different for the two Tn10 insertions, both occupied the same position relative to other markers (see Fig. 1).

to test for cotransduction of their Tn10 insertions with markers in the 60-min region of the genetic map (Table 3). A further series of cotransduction experiments (Table 3) and three-point crosses (Table 4) established the cotransduction of *acd* with *argA*, *thyA*, *lysA*, and *serA*. The gene order was *argA-thyA-lysA-tet-acd-serA-fda* (see Fig. 1).

The *fda* locus was found to be clockwise of *serA* rather than counterclockwise as indicated in the 1976 version of the genetic map (2). The clockwise location is supported by the presence of *fda* on the same plasmid-linked chromosomal fragment as *pgk* (D. F. Fraenkel, personal communication via Barbara Bachmann), and this position is given in the latest version of the map (1). Both of the Tn10 inserts lay between *lysA* and *acd* but were distinct with regard to their cotransduction frequencies with outside markers.

DISCUSSION

Growth by the fermentation of sugars requires the disposal of excess reducing equivalents. This is usually achieved by the excretion of highly reduced organic compounds such as lactate or ethanol. In *E. coli* lactate is produced from pyruvate under acidic conditions, whereas in more alkaline media, acetyl-CoA is converted to ethanol (9). The pathway between ethanol and acetyl-CoA involves two steps, mediated by the enzymes alcohol dehydrogenase and acetaldehyde CoA dehydrogenase. We have previously

TABLE 4. Three-factor crosses

P1 donor	Recipient	Selected marker	Recombinant classes				Implied order
DC365 Tet ^r <i>serA</i> DC366 Tet ^r <i>serA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	<i>acd ser</i> ⁺	<i>acd ser</i>	<i>acd</i> ⁺ <i>ser</i> ⁺	<i>acd</i> ⁺ <i>ser</i>		
		Tet ^r	151	1	18	30	<i>tet-acd-ser</i>
DC365 Tet ^r <i>serA</i> DC366 Tet ^r <i>serA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	Tet ^r	64	2	19	15	<i>tet-acd-ser</i>
DC365 Tet ^r <i>serA</i> NP315 <i>fda</i>	NP315 <i>fda</i>	<i>fda ser</i> ⁺	<i>fda ser</i>	<i>fda</i> ⁺ <i>ser</i> ⁺	<i>fda</i> ⁺ <i>ser</i>		
		Tet ^r	57	43	0	4	<i>tet-ser-fda</i>
DC81 DC81	DC409 <i>acd serA</i> DC410 <i>acd serA</i>	<i>acd Tet</i> ^r	<i>acd Tet</i> ⁺	<i>acd</i> ⁺ Tet ^r	<i>acd</i> ⁺ Tet ⁺		
		<i>ser</i> ⁺	98	17	178	89	<i>tet-acd-ser</i>
DC367 Tet ^r <i>argA</i> DC368 Tet ^r <i>argA</i>	DC370 <i>thyA</i> DC370 <i>thyA</i>	<i>ser</i> ⁺	33	22	77	68	<i>tet-acd-ser</i>
		<i>ser</i> ⁺					<i>tet-acd-ser</i>
DC367 Tet ^r <i>argA</i> DC368 Tet ^r <i>argA</i>	DC370 <i>thyA</i> DC370 <i>thyA</i>	<i>arg</i> ⁺ Tet ⁺	<i>arg</i> ⁺ Tet ⁺	<i>arg</i> Tet ⁺	<i>arg</i> Tet ⁺		
		<i>thy</i> ⁺	114	43	38	5	<i>arg-thy-tet</i>
DC367 Tet ^r <i>argA</i> DC368 Tet ^r <i>argA</i>	DC370 <i>thyA</i> DC370 <i>thyA</i>	<i>thy</i> ⁺	125	29	34	2	<i>arg-thy-tet</i>
		<i>thy</i> ⁺					
DC367 Tet ^r <i>lysA</i> DC368 Tet ^r <i>lysA</i>	DC370 <i>thyA</i> DC370 <i>thyA</i>	<i>lys</i> ⁺ Tet ⁺	<i>lys</i> ⁺ Tet ⁺	<i>lys</i> Tet ⁺	<i>lys</i> Tet ⁺		
		<i>thy</i> ⁺	88	19	64	29	<i>thy-lys-tet</i>
DC367 Tet ^r <i>lysA</i> DC368 Tet ^r <i>lysA</i>	DC370 <i>thyA</i> DC370 <i>thyA</i>	<i>thy</i> ⁺	27	2	54	7	<i>thy-lys-tet</i>
		<i>thy</i> ⁺					
DC367 Tet ^r <i>lysA</i> DC368 Tet ^r <i>lysA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	<i>acd lys</i> ⁺	<i>acd lys</i>	<i>acd</i> ⁺ <i>lys</i> ⁺	<i>acd</i> ⁺ <i>lys</i>		
		Tet ^r	95	62	43	0	<i>lys-tet-acd</i>
DC367 Tet ^r <i>lysA</i> DC368 Tet ^r <i>lysA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	Tet ^r	102	28	48	2	<i>lys-tet-acd</i>
		Tet ^r					
DC367 Tet ^r <i>argA</i> DC368 Tet ^r <i>argA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	<i>acd arg</i> ⁺	<i>acd arg</i>	<i>acd</i> ⁺ <i>arg</i> ⁺	<i>acd</i> ⁺ <i>arg</i>		
		Tet ^r	136	21	43	0	<i>arg-tet-acd</i>
DC367 Tet ^r <i>argA</i> DC368 Tet ^r <i>argA</i>	DC349 <i>acd</i> DC349 <i>acd</i>	Tet ^r	123	7	49	1	<i>arg-tet-acd</i>
		Tet ^r

FIG. 1. Genetic map of the *argA* to *serA* region. The linkages given are average cotransduction frequencies, taken from Tables 3 and 4.

isolated mutants (*adh*) which have abnormally high levels of alcohol dehydrogenase under aerobic conditions (4). We here show that *adh* mutants also overproduce acetaldehyde CoA de-

hydrogenase. Thus, the *adh* mutation appears to switch on the fermentation pathway from acetyl-CoA to ethanol in aerobically grown cells. Since the *adh* mutation also depresses the levels

of nitrate reductase (4), the *adh* locus cannot merely code for a repressor for an operon producing both enzymes. The presence of enzymes able to interconvert ethanol and acetyl-CoA allows *adh* mutants to grow aerobically on ethanol as sole carbon and energy source. We took advantage of this property to isolate revertants no longer able to grow on ethanol. Most of these proved to be true *adh*⁺ revertants, having lost the ability to produce both alcohol dehydrogenase and acetaldehyde CoA dehydrogenase. We also found *acd* mutants which retained the former enzyme but had lost the ability to produce the latter. The *acd* mutation was distinct genetically from *adh* mapping close to *serA* at min 62 on the genetic map (1).

Apart from their joint derepression in *adh* mutants, we found that acetaldehyde CoA dehydrogenase and alcohol dehydrogenase were disparately controlled by alterations of the culture medium. Alcohol dehydrogenase was relatively unaltered in level by a variety of treatments, whereas levels of the other enzyme varied considerably. Acetaldehyde CoA dehydrogenase was markedly induced by acetaldehyde and also by ethanol (presumably after its intracellular conversion to acetaldehyde by alcohol dehydrogenase). Two explanations for this phenomenon seem plausible. First, acetaldehyde CoA dehydrogenase may play a secondary role in the detoxification of aldehydes. The failure of acetaldehyde to induce this enzyme in wild-type strains under aerobic conditions suggests that some other detoxification mechanism may be available under these conditions. Second, under anaerobic conditions the citrate cycle no longer functions and acetyl-CoA tends to accumulate. This would cause a buildup of pyruvate and of the α -hydroxyethyl-thiamine pyrophosphate derivative of enzyme I of the pyruvate decarboxylase complex (7). The α -hydroxyethyl-thiamine pyrophosphate slowly breaks down non-enzymatically, releasing acetaldehyde. This possibility has the advantage of explaining why glucose, a precursor of pyruvate, induces acetaldehyde CoA dehydrogenase although α -methyl glucose,

which is not metabolized, has the opposite effect.

The observation that induction of strain DC349 with ethanol or glucose results in the production of low but significant quantities of acetaldehyde CoA dehydrogenase suggests that the *acd* mutation may not actually be in the structural gene. Further investigation of the control of the pathway for ethanol production in *E. coli* will require the isolation of unambiguous structural gene mutants of both enzymes.

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