Characterization of an *Escherichia coli* Mutant Deficient in Dihydrolipoyl Dehydrogenase Activity

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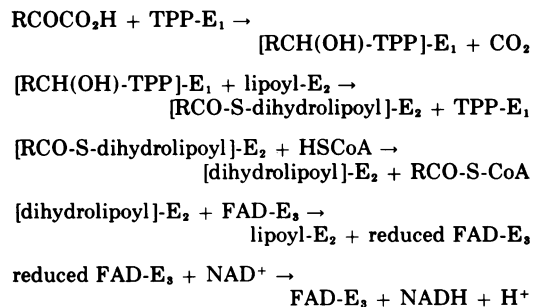
A mutant of *Escherichia coli* deficient in dihydrolipoyl dehydrogenase (DHL) activity has been isolated and its characteristics have been studied. The activities of the pyruvic dehydrogenase (PDC) and α-ketoglutaric dehydrogenase complexes (KDC) are not present in extracts of the mutant unless purified dihydrolipoyl dehydrogenase is added. Experiments with antiserum to DHL have shown that cross-reacting material exists in mutant extracts. This suggests that the dihydrolipoyl dehydrogenase mutation (*dhl⁻*) is a missense structural mutation. The mutation maps very close to, if not adjacent to, the *ace* loci, and is not linked to the *suc* loci. This means the *dhl* locus is grouped with the genes for the other components of the PDC and not with the genes for KDC. The mutation is also transducible into prototrophic strains, demonstrating that no prior mutation is necessary for the DHL activity deficiency to exist. This evidence is consistent with the idea that there is only one gene for DHL and is supported by previous biochemical studies which have shown that DHL preparations from either enzyme complex are electrophoretically and immunochemically indistinguishable. Possible mechanisms for the genetic and metabolic control of DHL, PDC, and KDC are discussed.

The pyruvic (PDC) and α-ketoglutaric (KDC) dehydrogenase complexes catalyze the oxidative decarboxylation of pyruvate and α-ketoglutarate to acetyl-coenzyme A and succinyl-coenzyme A, respectively. The similarity of the two reaction sequences is quite remarkable; however, the most striking similarity between the two complexes in *Escherichia coli* is the third component, dihydrolipoyl dehydrogenase (DHL), which is the only component that is functionally interchangeable for the two complexes in vitro (19). Further, all other parameters which have been studied thus far, such as electrophoretic mobility and immunochemical identity (18), indicate that the DHL derived from either of the two complexes may be structurally identical as well. This presents the interesting genetic possibility of there being only one gene controlling the synthesis of DHL for both enzyme complexes. Previous studies of the genetic constitution of the DHL and consequently its genetic regulation have been severely limited by the lack of mutants for this component (5, 9, 12, 18). Therefore, the goals of the present study were to isolate and characterize such a mutant and to use it to map the location of the gene(s) for dihydrolipoyl dehydrogenase.

The analogous reaction sequences for the two multienzyme complexes are as follows:

$$RCOCO_2H + TPP\text{-}E_1 \rightarrow [RCH(OH)\text{-}TPP]\text{-}E_1 + CO_2$$

$$[RCH(OH)\text{-}TPP]\text{-}E_1 + lipoyl\text{-}E_2 \rightarrow [RCO\text{-}S\text{-}dihydrolipoyl]\text{-}E_2 + TPP\text{-}E_1$$

$$[RCO\text{-}S\text{-}dihydrolipoyl]\text{-}E_2 + HSCoA \rightarrow [dihydrolipoyl]\text{-}E_2 + RCO\text{-}S\text{-}CoA$$

$$[dihydrolipoyl]\text{-}E_2 + FAD\text{-}E_3 \rightarrow lipoyl\text{-}E_2 + reduced\ FAD\text{-}E_3$$

$$reduced\ FAD\text{-}E_3 + NAD^+ \rightarrow FAD\text{-}E_3 + NADH + H^+$$

In the PDC complex, E_1 is pyruvate decarboxylase (PCBX) (EC 1.2.4.1) and E_2 is lipoic transacetylase (LTA) (EC 2.3.1.12). For the

α-ketoglutaric dehydrogenase complex, E_1 is α-ketoglutarate decarboxylase (KCBX) (EC 1.2.4.2) and E_2 is lipoyl transsuccinylase (LTS). E_3 in both complexes is dihydrolipoyl dehydrogenase (DHL) (EC 1.6.4.3).

The work in Henning's laboratory (4, 8, 9) has set forth many of the factors involved in the regulation of PDC. Mutants which require acetate, ace^-, may lack PCBX, LTA, or both, the latter resulting from a pleiotropic effect. The genes for these two components ($aceE$ and $aceF$ loci, respectively) are adjacent and map near leu (10); they are expressed coordinately (8, 10) and pyruvate induces their synthesis (4, 9). A map of the $E.\ coli$ genome illustrating the relevant loci is given in Fig. 1.

Mutants of KCBX or LTS require succinate for growth (suc^-). The genes for these two components are also adjacent to each other and are linked to gal, approximately 15 map min away from the ace locus (9-12). Under various growth conditions, the two complexes do not vary either proportionately or inversely (1, 4), strongly suggesting that their synthesis is separately controlled.

Numerous mutants for the complexes have been isolated in different laboratories (over 100 ace^-, 50 suc^- [9, 12, K. N. Murray and U. Henning, unpublished results]), but none of them were dihydrolipoyl dehydrogenase deficient (dhl^-). Therefore, the possibility was considered that the two flavoproteins are identical in each enzyme complex and one dhl gene exists for both enzyme complexes. The existence of a single dhl gene would demand that the two flavoproteins be identical, but the converse is not necessarily true since two substantially identical enzymes catalyzing the same reaction could still be the products of two separate genes. With two genes, the first could be mutated and the second might still provide the enzyme (complementation) so that a phenotypic change would not be observed in selections for single mutants. On the other hand, if a single gene were involved, then alterations should give rise to double mutants (ace^-, suc^-) which would also remain undetected in the individual selection of either marker. By using a selection technique for ace^-, suc^- double mutants, we have isolated a mutant deficient in DHL activity. Studies of this mutant support the idea that there is only one gene which affects the DHL of both complexes, thus requiring that the flavoprotein in both enzyme complexes be identical. This raises the possibility of the existence of coordinate control of both the pyruvic dehydrogenase and the α-ketoglutaric dehydrogenase complexes by the availability of DHL.

MATERIALS AND METHODS

Bacterial strains. $E.\ coli$ K-12 strains W945, AB287, Ymel, and A10 were supplied by Ulf Henning. All other strains were derived from these cultures. Several special strains were constructed for the mapping experiments; they were derived by transduction using the virulent P1 bacteriophage which was supplied by Hideo Ikeda (14).

Media. The minimal medium used was that of Vogel and Bonner (24) with standard trace metals added. All media were made with deionized water. Growth of the DHL mutant (dhl^-) requires the addition of 500 mg of acetate and 380 mg of succinate per liter of medium.

Liquid cultures were started in 10 ml of 1.75% antibiotic medium 3 (Difco) (plus acetate and succinate for dhl^- mutants). These cultures were then used to inoculate a 6-liter flask containing 1 liter of medium with 4 g of glucose, 1 mg of thiamine pyrophosphate, and essential amino acids (plus acetate and succinate, as above, for the dhl^- mutant).

Eosin-methylene blue-arabinose plates were prepared as follows. One liter of medium contained 10 g of arabinose (sterilized separately), 5 g of NaCl, 2 g of K_2HPO_4, 15 g of agar (Difco), 1 g yeast extract (Difco), 8 g of tryptone (Difco), 400 mg of eosine Y (Difco), and 65 mg of methylene blue.

Genetic symbols and abbreviations. Genetic symbols refer to loci concerned with the biosynthesis of leucine (leu) and threonine (thr) and the metabolism of arabinose (ara), lactose (lac), and galactose (gal). The genetic symbol ace is a general symbol for the adjacent $aceE$ and $aceF$ alleles, which are the genes for the pyruvate decarboxylase and lipoic transacetylase, respectively. Likewise, suc is a general symbol for the adjacent $sucA$ and $sucB$ alleles, which code for the α-ketoglutarate decarboxylase and lipoic trans-
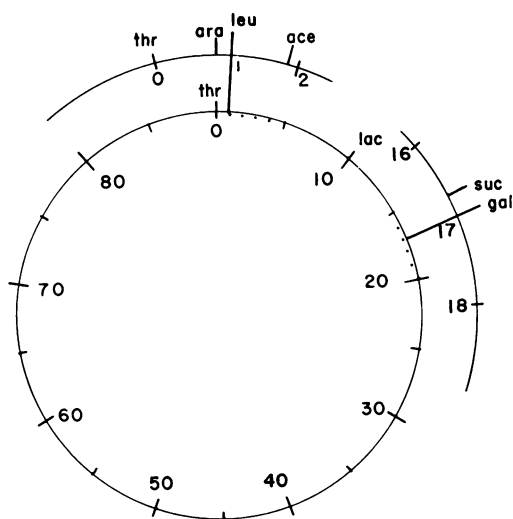

FIG. 1. *Genetic map of E. coli genome illustrating the pertinent markers (21).*

succinylase, respectively. The symbol *dhl* is used to designate the allele for dihydrolipoyl dehydrogenase. Phenotypic symbols used are as follows: Leu⁻, leucine-requiring; Ara⁻, unable to grow on arabinose; Ace⁻, requires acetate for growth; Suc⁻, requires succinate for growth; Dhl⁻, requires both acetate and succinate for growth.

Transduction. Transduction with phage P1 was carried out using the method of Lennox (16) with the modification that the cells were washed with minimal citrate. This citrate wash and the citrate on the plates probably prevents re-adsorption of P1 (13).

Testing of transductants. Colonies were picked with sterile toothpicks and mixed into drops of saline in the bottom of a sterile petri dish. Small portions of the suspensions were then replicated to test plates, 25 colonies being tested per plate.

For determining the order of the *ara*, *leu*, and *dhl* loci, Leu⁺ transductants were selected on a plate containing glucose, acetate, and succinate as carbon sources, but lacking leucine. The acetate and succinate had to be present for the Dhl⁻ mutants to grow. Among the Leu⁺ transductants, the Dhl⁺ co-transductants could be detected on plates containing only succinate as carbon source since Dhl⁻ mutants are unable to grow on these plates. The ability or inability to utilize arabinose as a carbon source was checked by spotting on eosin-methylene blue-arabinose plates. Dhl⁺ transductants were selected on plates with succinate as the only carbon source. These plates contain leucine, and the Leu phenotype can be evaluated by spotting on the same type plate minus leucine.

For determining the order of the *leu*, *aceF*, and *dhl* loci, Leu⁺ transductants were selected as above. Colonies were then tested on a plate containing only succinate and acetate as carbon source which allows Dhl⁺ co-transductants to grow regardless of the condition of *aceF*. The colonies were also tested on plates with succinate as the only carbon source; only those transductants which are Ace⁺, Dhl⁺ will grow here; thus with the combination of these plates the numbers of Ace⁺, Dhl⁺ and Ace⁻, Dhl⁺ co-transductants can be determined. When the *dhl* locus is mutant the condition of the *ace* allele cannot be determined by growth characteristics.

When Ace⁺, Dhl⁺ transductants are to be selected, plates with succinate as the only carbon source and supplemented with leucine are used. The Ace⁺, Dhl⁺ transductants are then spotted on the same plate lacking leucine to distinguish between transductants with the *leu⁺ aceF⁺ dhl⁺* and *leu⁻ aceF⁺ dhl⁺* genotypes.

Enzyme assays. The pyruvic dehydrogenase complex can be assayed at all levels of purity by the dismutation assay (20).

The α-ketoglutaric dehydrogenase complex can be assayed in a crude extract by the manometric measurement of CO_2 evolved by the reaction. The method has been described (15).

Pyruvate or α-ketoglutarate decarboxylase (E_1) was assayed by the ferricyanide reduction assay (20).

Dihydrolipoyl dehydrogenase was assayed spectrophotometrically (20). This assay could be performed on diluted crude enzyme preparation under conditions where reduced nicotinamide adenine dinucleotide (NADH) oxidase did not interfere.

Enzyme preparation. After overnight growth in 1 liter of appropriate medium, the cells were gathered, washed, and weighed. The cells were then suspended in two times their wet weight of 0.1 M potassium phosphate buffer (pH 7.0). The suspension was then sonically treated at 4 C in a Sonifer Cell Disruptor for 4 min in 0.5-min intervals, cooling for 0.5 min between each interval.

An alternate cell disruption method was to grind the cells in two times their wet weight of Alumina (grade A-305, Alcoa, Bauxite, Ark.). After being ground thoroughly the paste was taken up in 0.1 M potassium phosphate buffer (pH 7.0).

In either procedure the cell debris was removed by centrifugation at 20,000 × *g* for 20 min. The supernatant fluid represented a crude extract. In these procedures less than 5% of the PDC activity is lost by sedimentation (4).

DHL was purified by the method of Williams et al. (25).

Antiserum preparation. A 1- to 2-mg amount of purified dihydrolipoyl dehydrogenase was mixed with Freund adjuvant (at the rate of 1 mg of DHL per 3 ml of adjuvant). It was found that the adjuvant was necessary to elicit any significant antibody response. The adjuvant-antigen mixture was injected subcutaneously in the neck and intramuscularly in both thighs of white New Zealand rabbits. After 3 weeks, booster shots of 1 to 2 mg of purified DHL were given. After an additional 1 to 2 weeks the blood was drawn, allowed to clot, and centrifuged at 500 × *g* for 10 min. The supernatant serum was used as antiserum to dihydrolipoyl dehydrogenase.

RESULTS

Isolation of dhl⁻ mutant. After ultraviolet irradiation of *E. coli* K-12 strain W945 and standard penicillin selection, the cells were plated on medium containing glucose, succinate, and acetate. The resultant colonies were replicated on glucose medium, glucose plus acetate, and glucose plus succinate. The latter two media were employed to identify mutants which were just Ace⁻ or Suc⁻. Several mutants were isolated which required both acetate and succinate and were considered to be potential *dhl⁻*. However, all grew if lipoic acid replaced the two acids (23), indicating that they were lipoate deficient, not Dhl⁻. One of the mutants isolated, SW28, which appeared to be *suc⁻*, grew distinctly better on glucose, acetate, and succinate than it did on just glucose and succinate. Furthermore, it did not grow on either succinate alone or succinate plus acetate, although a typical *suc⁻* will. Table 1 shows that SW28 behaved like a constructed *ace⁻ suc⁻* double mutant, and, except for the weak growth on glucose plus succinate, has the phenotype

TABLE 1. *Growth of various types of mutants on different carbon sources*

Carbon source	Mutant type			
	ace^-	suc^-	ace^-_{suc}	dhl^-
Glucose, acetate, and succinate	+	+	+	+
Glucose and succinate	+	+	Weak	Weak
Glucose	Weak	−	−	−
Acetate and succinate	+	Weak	−	−
Succinate	−	Weak	−	−

expected for a dhl^- mutant; thus, further investigation was warranted.

Enzyme assays of SW28. Crude extracts were prepared from SW28. Purified DHL was prepared (25) from wild-type *E. coli* K-12. This preparation represents a 400-fold purification of the enzyme. Polyacrylamide gel electrophoresis of the preparation shows one major band with enzyme activity.

Figure 2 shows the effect of added DHL on the activity of the SW28 pyruvic dehydrogenase complex. There is no activity without added DHL; however, upon addition of increasing amounts of the purified enzyme the activity of the complex rises rapidly. The maximum activity is comparable to the activity found in strain W945. Figure 3 shows the effect of purified DHL on the α-ketoglutaric dehydrogenase activity; again there is no activity without added DHL. The apparent small slope of the SW28 activity is not above the usual background that we find in similar assays of suc^- mutants. For the upper curve the amount of DHL added was large enough to bring the α-ketoglutaric dehydrogenase activity to a maximum, comparable to that of strain W945 under similar conditions.

The mutant was also assayed spectrophotometrically for DHL activity. This assay could be performed on diluted crude preparations under conditions where NADH oxidase did not interfere. The specific activity of SW28 was found to be 12.9 μmol of NADH transformed per min per mg of crude protein compared to a specific activity of 251.9 μmol of NADH transformed per min per mg of crude protein or higher (depending on the extract) for wild-type strain W945. It is doubtful that the small activity of SW28 is due to DHL since antiserum to DHL does not affect it. Most likely it is residual NADH oxidase activity.

In addition, the ferricyanide reduction assay showed that the decarboxylase components of each enzyme complex are normal in SW28.

These data demonstrate that the mutation

has caused a DHL activity deficiency and, therefore, that SW28 is dhl^-. The deficiency in enzyme activity could be caused by a lack of active DHL protein or it could be due to a deficiency of the flavine-adenine dinucleotide (FAD) cofactor. Addition of the cofactor to wild-type DHL protein, from which the cofactor was previously removed, causes partial restoration of activity. The addition of the cofactor to
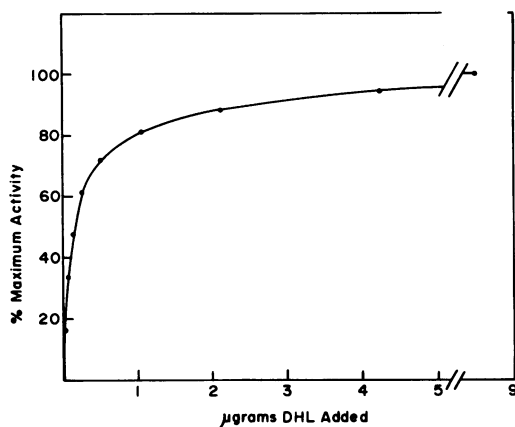

FIG. 2. *Effect of purified dihydrolipoyl dehydrogenase (DHL) on the pyruvic dehydrogenase complex activity of SW28. Varying amounts of purified DHL were added to 135 μg of crude SW28 protein.*
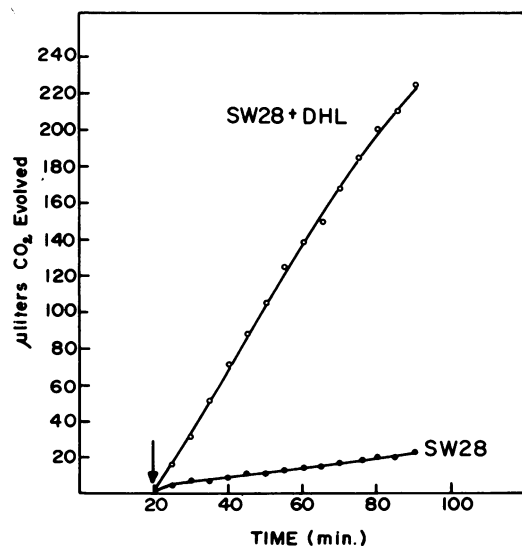

FIG. 3. *Effect of purified dihydrolipoyl dehydrogenase (DHL) on the α-ketoglutaric dehydrogenase complex activity of SW28. An 11.6-mg amount of crude SW28 protein was assayed alone (●) and with 0.68 mg of purified DHL (O). The arrow indicates when the α-ketoglutarate was added to the assay mixture.*

mutant extracts causes no such activation. Therefore we conclude that it is highly unlikely that a deficiency of the cofactor is causing the deficiency in DHL activity. The next objective was to locate the SW28 mutation on the genetic map and to determine if it is a single or a double mutation.

Map position of the dhl locus. Conjugation experiments using F$_2$ *gal* and Hfr donor cultures indicated that the *dhl* locus was not linked to *suc* or *gal* but rather maps about 16 min counterclockwise from *gal*. This locates *dhl* in the vicinity of *leu* on the chromosome map (see Fig. 1). To map the *dhl* locus more precisely, SW28 (*ara⁻ leu⁻ dhl⁻*) was transduced using a P1 lysate made on the Yme1 (*ara⁺ leu⁺ dhl⁺*) strain. Transductants were selected for either Leu⁺ or Dhl⁺, and then scored for the unselected markers. Table 2 shows the results of these experiments. When *leu⁺* was the selected marker (Table 2A) it was found that both *ara⁺* and *dhl⁺* could be frequently and independently co-transduced with *leu⁺*. However, when *dhl⁺* was the selected marker (Table 2B) almost all of the Ara⁺, Dhl⁺ co-transductants (27/28) also received the donor *leu⁺* allele, whereas among the Leu⁺, Dhl⁺ co-transductants only 66% (27/41) received the donor *ara⁺* allele. These data establish the gene order *ara leu dhl*.

Since the *dhl* locus is found to map on the same side of *leu* as *ace* (see Fig. 1), P1 transductions were done to determine the position of *dhl* with respect to *leu* and *ace*. For these experiments appropriate strains were constructed using the virulent P1 phage (14). A virulent P1 lysate, made on the A10 strain which has the *aceF⁻ dhl⁺* genotype, was used to infect SW28 and an *aceF⁻ dhl⁺* derivative was prepared. This strain still carried the original *leu⁻* allele of

SW28, and *leu⁺* was introduced with one additional transduction. Using these strains along with the SW28 (*leu⁻ aceF⁺ dhl⁻*), the necessary reciprocal three-factor crosses could be done. The results of these experiments are shown in Table 3. When Ace⁺, Dhl⁺ recombinants are selected it can be seen that in the first cross (Table 3A), where *aceF⁺* is a donor allele, 34% of the Ace⁺, Dhl⁺ transductants received the donor *leu⁺* allele, whereas in the second cross (Table 3C), where *dhl⁺* is a donor marker, only 10% received the donor *leu⁺* allele. These results establish the gene order *leu aceF dhl*. Since *aceE* lies between *leu* and *aceF* and is adjacent to *aceF* (10), the order would be *leu aceE aceF dhl*.

The degree of linkage of *aceF* and *dhl* can be estimated from the data in Table 3B. Of the 144 Leu⁺ transductants tested, 40 also received the donor *aceF dhl* region (i.e., the five *leu⁺ aceF⁺ dhl⁺* and the *leu⁺ dhl⁻* transductants). Since only 5 of these 40 had a crossover between *aceF* and *dhl*, we estimate that *aceF* and *dhl* are 87.5% co-transducible. This high frequency of co-transduction, although based on small numbers, suggests close linkage between *aceF* and *dhl*.

Transduction of the dhl⁻ mutation. To reinforce the concept that *dhl* is a single locus and to definitely eliminate any linkage with the *suc* locus, the following experiment was done. A

TABLE 2. *Ordering of dhl with respect to ara and leu*[a]

Selected allele	Transductant class[b]			No. observed	Frequency (%)
	ara	leu	dhl		
A. *leu⁺*	+	+	+	44	54
	+	+	−	12	15
	−	+	+	17	21
	−	+	−	8	10
B. *dhl⁺*	+	+	+	27	32
	−	+	+	14	16
	−	−	+	44	51
	+	−	+	1	1

[a] Donor (P1 lysate), *ara⁺ leu⁺ dhl⁺*; recipient (SW28), *ara⁻ leu⁻ dhl⁻*.

[b] Selection procedures are given in Materials and Methods.

TABLE 3. *Ordering of dhl with respect to leu and aceF*

Selected allele(s)	Transductant class[a]			No. observed	Frequency (%)
	leu	aceF	dhl		
Cross 1[b]					
A. *aceF⁺ dhl⁺*	+	+	+	47	34
	−	+	+	92	66
B. *leu⁺*	+	+	+	5	4
	+	+	+	104	72
	+	−[c]	−	35	24
Cross 2[d]					
C. *aceF⁺ dhl⁺*	+	+	+	15	10
	−	+	+	129	90
D. *leu⁺*	+	+	+	1	0.5
	+	−	+	70	37.7
	+	−[c]	−	115	61.8

[a] Selection procedures are given in the Materials and Methods section.

[b] Donor, *leu⁺ aceF⁺ dhl⁻*; recipient, *leu⁻ aceF⁻ dhl⁺*.

[c] When *dhl* is mutant the condition of *ace* cannot be determined by growth characteristics.

[d] Donor, *leu⁺ aceF⁻ dhl⁺*; recipient, *leu⁻ aceF⁺ dhl⁻*.

P1 lysate of a *leu⁺ dhl⁻* derivative of SW28 was used to infect the parental strain, W945, and AB287, another *leu⁻ ace⁺ dhl⁺ suc⁺* strain. If the transduced marker behaves the same way in AB287 as in W945, it greatly reduces the possibility that some additional but unknown genetic alteration, originally present in W945, combines with the *dhl⁻* mutation in SW28 to give the observed properties. Selection was made for Leu⁺, and these colonies were checked for the Dhl⁻ phenotype.

The *dhl⁻* mutation was transferred successfully to both strains with normal frequencies. Two new *dhl⁻* strains were thus created, DHL10 from W945 and DHL54 from AB287. The growth of these two strains was found to be like that of SW28 (see Table 1). Enzymological studies also indicated that the new strains lacked dihydrolipoyl dehydrogenase activity. Figure 4 shows that, like SW28, both DHL10 and DHL54 are without pyruvic dehydrogenase activity unless purified dihydrolipoyl dehydrogenase is added. Figure 5 shows that the α-ketoglutaric dehydrogenase complexes of DHL10 and DHL54 are also inactive without addition of purified DHL to the reaction mixture. Overall the activation of the two enzyme complexes in either of the new strains is quite similar to that of SW28 (Fig. 2 and 3).

That the *dhl⁻* character is transducible with P1 signifies that it is carried on a segment of deoxyribonucleic acid (DNA) no greater than 1.5 to 2 map min, the maximum amount of DNA which can be carried by P1. Consequently, should two mutations be required to make the cell *dhl⁻*, they must be very closely linked. This again demonstrates the important point that the *dhl* mutation is not linked to the *suc* locus.
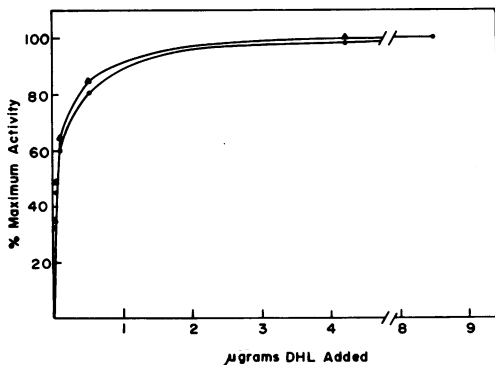

FIG. 4. *Effect of purified dihydrolipoyl dehydrogenase (DHL) on the pyruvic dehydrogenase complex activity of strains DHL10 and DHL54. Varying amounts of purified dihydrolipoyl dehydrogenase were added to 110 μg of crude DHL10 protein (▲) and to 90 μg of crude DHL54 protein (●).*
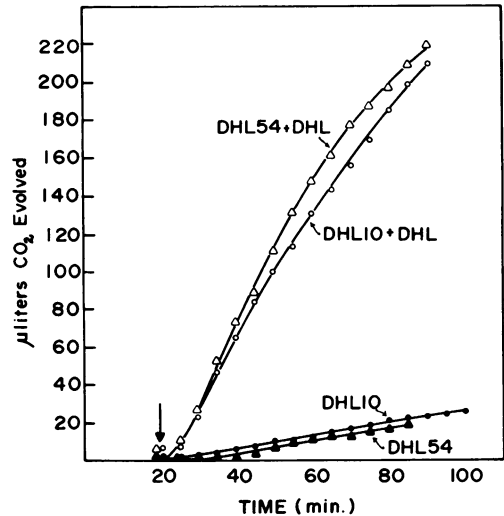

FIG. 5. *Effect of purified dihydrolipoyl dehydrogenase (DHL) on the α-ketoglutaric dehydrogenase complex activity of strains DHL10 and DHL54. A 10.86-mg amount of crude DHL10 protein was assayed alone (●) and with 0.425 mg of purified dihydrolipoyl dehydrogenase (○). Likewise, 8.1 mg of crude DHL54 protein was assayed alone (▲) and with 0.425 mg of purified dihydrolipoyl dehydrogenase (△).*

Immunochemical assays using the dhl mutant. If the *dhl⁻* mutation is a missense structural mutation, then cross-reacting material should be detectable in extracts of the mutant cells.

Antiserum to purified dihydrolipoyl dehydrogenase was prepared as described in the Material and Methods section. Various dilutions of the antiserum were prepared; 0.025 ml of each dilution was mixed with an equal volume of either saline or crude extract from *dhl⁻* mutant DHL10. These samples were allowed to incubate on ice for 2 h after which they were centrifuged. A 0.02-ml amount of the supernatant fluid from each sample was then mixed with an equal volume of purified dihydrolipoyl dehydrogenase. After incubation for 2 h on ice followed by centrifugation, the samples were assayed for dihydrolipoyl dehydrogenase activity.

If cross-reacting material is present in the *dhl⁻* mutant, then the serum preincubated with it should have less of an inactivating effect on dihydrolipoyl dehydrogenase than the antiserum preincubated with saline. Figure 6 shows that this is the case: the antiserum preincubated with mutant extract (open circles) does have less of an inactivating effect. It should be noted that the addition of purified dihydrolipoyl dehydrogenase to a mutant extract

results in a 40% higher activity of the enzyme than that found in the same amount of dihydrolipoyl dehydrogenase assayed alone. This is probably due to favorable association of the enzyme with the rest of the PDC or KDC complexes present in the mutant. This phenomenon should not alter the results of Fig. 6 since percentages of total activities are being compared. The objection might be raised that the antiserum preincubated with the mutant extract (Fig. 6) could have been lost by entrapment in nonspecific immune precipitates. We feel that this is highly unlikely since the antigen used to prepare the antiserum was highly purified. In addition, the conclusions drawn from Fig. 6 are supported by Ouchterlony double-diffusion tests which show one identical band with either purified DHL, wild-type extract, or mutant extract as antigens. In summary, our evidence demonstrates the presence of cross-reacting material. This strongly suggests a missense structural mutation.

FIG. 6. *Decrease in dihydrolipoyl dehydrogenase activity upon treatment with antiserum which had been preincubated with either saline (●) or dhl⁻ mutant extract (O).*

DISCUSSION

This report describes the isolation and characteristics of a mutation leading to a deficiency in dihydrolipoyl dehydrogenase activity in *E. coli*. We have shown that extracts of mutant cells contain dihydrolipoyl dehydrogenase cross-reacting material which strongly suggests a missense structural mutation. Our data show that the *dhl⁻* mutation maps very close to the *aceE* and *aceF* genes (the structural genes for the other two components of the pyruvic dehydrogenase complex) in the order *aceE aceF dhl* and is not at all linked to the *suc* loci. The fact that the *dhl* mutation is transducible into two different prototrophic strains supports the argument that no prior mutation is needed for the DHL activity deficiency to exist, further supporting the idea that a single locus is involved. The possibility exists that the inactivity of the mutant is caused by a deficiency of the FAD cofactor of the enzyme; this is doubtful since addition of the cofactor to mutant extracts causes no activation where addition to wild-type DHL protein, from which the cofactor was removed, caused partial restoration of activity. The simplest conclusion at this point is that one gene exists which affects the dihydrolipoyl dehydrogenase of both complexes.

We find at least 87.5% co-transduction of the *aceF* and *dhl* loci. Taylor (22) states that markers separated by 0.5 min or less are cotransduced at a frequency ranging from 35 to 90%. Our co-transduction frequency of 87.5% indicates that *aceF* and *dhl* are probably much
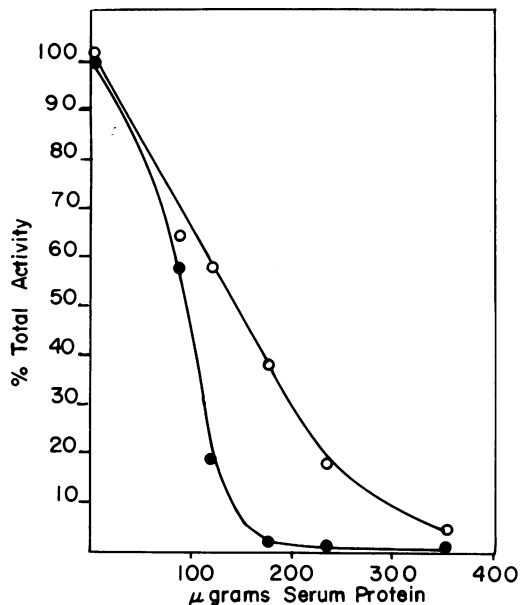
less than 0.5 min apart. Using the equation given by Wu (26) which can be used to convert co-transduction frequencies to map minutes, we calculate (assuming the total length of the P1-transducing DNA particle is 2 min) that *aceF* and *dhl* are less than 0.1 map min apart. From considerations discussed by Taylor (21) one can assume 35 "average" genes per map minute; thus we estimate that *aceF* and *dhl* are within 3 genes of each other and quite possibly are adjacent.

The fact that the gene for dihydrolipoyl dehydrogenase is closely linked or adjacent to the genes for the pyruvic dehydrogenase complex and not to the genes for α-ketoglutaric dehydrogenase complex is very interesting since the dihydrolipoyl dehydrogenase is common to both enzyme complexes. This raises the question of how the *dhl* gene is controlled. Henning (8) has shown that certain polar mutations in the pyruvic dehydrogenase complex (*ace* locus) cause a partial pleiotropic effect on the dihydrolipoyl dehydrogenase; thus the expression of *dhl* may be under partial coordinate control with the *ace* locus. However, the lack of strong polar effects suggest that *dhl* may be under some type of noncoordinate control. An interesting possibility is that there may be a low-efficiency promoter between *aceF* and *dhl* similar to the internal low-efficiency promoter reported

by Bauerle and Margolin (2, 3) in the *trp* operon of *Salmonella typhimurium* and by Morse and Yanofsky (17) in the same operon of *E. coli*. In other words, under some conditions the *dhl* may be transcribed as a polycistronic message which includes the *aceE* and *aceF* genes; however, under conditions where the *ace* locus is repressed, expression of *dhl* may be independent of the expression of *ace*. If this were the case, one could explain how dihydrolipoyl dehydrogenase can be supplied for the α-ketoglutaric dehydrogenase complex under conditions when the *ace* locus is repressed.

The existence of one gene for dihydrolipoyl dehydrogenase demands that the flavoprotein be identical in both enzyme complexes. This leads to the interesting proposal that the pyruvic dehydrogenase complex and the α-ketoglutaric dehydrogenase complex can be coordinately controlled at both the genetic and metabolic levels. At the genetic level, *dhl* may be controlled by the mechanism discussed above. At the metabolic level, the distribution of dihydrolipoyl dehydrogenase between the two enzyme complexes may change under different metabolic conditions.

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