Gene-Protein Relationships of the α -Keto Acid Dehydrogenase Complexes of *Escherichia coli* K12: Chromosomal Location of the Lipoamide Dehydrogenase Gene

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SUMMARY

Two representative lipoamide dehydrogenase mutants (*lpd1* and *lpd4*) were used to locate the *lpd* gene in the linkage map of *Escherichia coli* by conjugation and detailed transductional analysis with phage P1. Time of entry mapping indicated that the *lpd* gene was between *leu* and *proC* and very close to *leu*. Average cotransduction frequencies between *lpd* and other markers were: *aceF* (97 %), *aceE* (93 %), *aroP* (84 %), *nadC* (71 %), *azi* (64 %), *leu* (8 to 41 %), *ara* (38 %), *pan* (21 %), *tonA* (17 %) and *thr*, *nad* and *gal* (< 1 %). These values, together with the contrasduction frequencies for many other pairs of markers in this region and the results of three-factor crosses, established the gene order *leu-azi-nadC-aroP-aceE-aceF-lpd-pan*. The very close proximity of *aceF* and *lpd* suggested that they may be contiguous and that the *lpd* gene may be the distal gene in the pyruvate dehydrogenase operon. In this case, the expression of the *lpd* gene may be governed by a secondary transcriptional promoter in the *aceF-lpd* region in order to generate lipoamide dehydrogenase components for assembly of the α -ketoglutarate dehydrogenase complex. Other possible mechanisms are also discussed.

INTRODUCTION

The pyruvate and α -ketoglutarate dehydrogenase complexes of *Escherichia coli* each contain three types of enzymic component, the dehydrogenase (E1), dihydrolipoamide transacylase (E2) and lipoamide dehydrogenase (E3) components. Studies with mutants requiring acetate or succinate for aerobic growth on glucose have established the existence of two pairs of closely linked structural genes which determine specific dehydrogenase and transacylase components for the two complexes. These are the *aceE* (E1p) and *aceF* (E2p) genes (Henning & Hertz, 1964), and the *sucA* (E1kg) and *sucB* (E2kg) genes (Herbert & Guest, 1969), which have been located in the 1 to 2 min (*leuAB-mutT-azi-aceEF-nadC*-pan*) and 16.5 to 17.0 min (*gltA-sdh-supL*-sucAB-tolAB*-nadA-aroG-galKTEO*) segments of the *E. coli* linkage map (Taylor, 1970). Studies with polar mutants have also established that expression of the *ace* and *suc* regions is polarized from the dehydrogenase to the transacylase genes (Henning, Dennert, Hertel & Shipp, 1966; Creaghan & Guest, 1972), as indicated by the arrows above. Recently, mutants (*lpd*) requiring acetate *plus* succinate for aerobic growth on glucose have been isolated; these were deficient in lipoamide dehydrogenase activity and also lacked overall pyruvate and α -ketoglutarate dehydrogenase complex activities (Guest & Creaghan, 1973). Studies with these mutants indicated that a single *lpd* gene specifies the lipoamide dehydrogenase components (E3) of both multienzyme complexes. Consistent

Table 1. *Strains of Escherichia coli* K12

Strain	Characteristics	Source or reference
T3A58 <i>lpd</i> 1	F ⁺ , <i>trpE</i> , <i>trpA</i> , <i>lpd</i> 1	Guest & Creaghan (1973)
WGA	F ⁻ , <i>gal</i> , <i>trpA</i> 9761-amber	Herbert & Guest (1969)
WASucA26	F ⁻ , <i>trpA</i> 9761-amber, <i>sucA</i> 26	Herbert & Guest (1969)
AB353	Hfr, <i>thr</i> , <i>ara</i> , <i>leu</i> , <i>pan</i> , <i>lacZ</i> , <i>str</i> , <i>thi</i>	A. L. Taylor
XG3	F ⁻ , <i>leu</i> , <i>ara</i> , <i>lac</i> , <i>proC</i> , <i>tsx</i> , <i>purE</i> , <i>trp</i> , <i>str</i> , <i>xyl</i> , <i>thi</i>	Herbert & Guest (1968)
JP1537	Hfr, <i>thrA</i> , <i>pan</i> , <i>azi</i> , <i>tonA</i> , <i>aroP</i> , <i>str</i> , <i>thi</i>	R. R. B. Russell
E126	F ⁺ , <i>nadC</i> 13	R. K. Gholson
A2T3	<i>aceE</i> 2, <i>trp</i>	U. Henning
A10	<i>aceF</i> 10	U. Henning
AT1371	F ⁻ , <i>pan</i> , <i>proA</i> , <i>lacY</i> , <i>tsx</i> , <i>galK</i> , <i>his</i> , <i>xyl</i> , <i>mtl</i> , <i>argE</i> , <i>thi</i>	Bukhari & Taylor (1971)
K10	Hfr, <i>tonA</i> , <i>rel</i> , <i>tto</i>	B. J. Bachmann
PA-2-18	F ⁻ , <i>lac</i> , <i>nadA</i> , <i>gal</i> , <i>str</i> , <i>mal</i> , <i>xyl</i> , <i>mtl</i> , <i>arg</i> , <i>thi</i>	R. K. Gholson
H	Hfr, <i>thi</i>	W. Hayes

with this view is biochemical evidence pointing to the identity of the E3 components isolated from both complexes (Pettit & Reed, 1967; Burleigh & Williams, 1972; Brown & Perham, 1972).

In the present work genetic studies with representative lipoamide dehydrogenase mutants aimed at locating the *lpd* gene are described. Also reported are the locations of the quinolinate phosphoribosyl transferase (*nadC*) and general aromatic amino acid permease (*aroP*) genes. A preliminary account of some of this work has been published previously (Guest & Creaghan, 1972).

METHODS

Bacterial strains. Characteristics of the strains of *Escherichia coli* K12 used in mapping studies are shown in Table 1. Further details of the lipoamide dehydrogenase mutants T3A58*lpd*1 and XG3*lpd*4 have been described previously (Guest & Creaghan, 1973). Leucine-requiring derivatives of T3A58*lpd*1, the *ace* mutants, A2T3 and A10, and the *nadC* mutant E126 were obtained by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine followed by a penicillin selection. From these a set of *nadC* derivatives was constructed by P1 transduction with E126 as donor. Leu⁺ transductants were selected and screened for Lpd⁻NadC⁻ or Ace⁻NadC⁻ derivatives. Low multiplicities of infection were used and except in the case of the *aceF* product, A10*nadC*(P1), the desired transductants were P1-sensitive. Further tryptophan-independent derivatives, T⁺*lpd*1*nadC*(P1) and A2T⁺*nadC*(P1), necessary for crosses involving the *aroP* marker, were prepared by transduction using a P1.W3110 lysate.

Media. Minimal medium E of Vogel & Bonner (1956) was used throughout. Substrates were glucose (0.2 %), acetate or succinate (50 mM) and galactose or arabinose (0.5 %, plus bromothymol blue at a final concentration of 0.002 %, w/v). Minimal media were supplemented as required with acetate (2 mM), succinate (2 mM), L-amino acids and purines (30 µg/ml), vitamins (5 µg/ml) and streptomycin (200 µg/ml). L-broth (Lennox, 1955) and Bacto Nutrient Broth (Difco) were used as complete media and all media were solidified with 1.5 % agar (Difco), except in the preparation of lysates where the concentrations of agar in top and bottom layers were 0.6 and 1.0 % respectively.

Transduction. Phage P1 lysates of donor strains were prepared by confluent lysis on L-agar plus CaCl₂ (0.25 mM) and titrated with a strain of *Shigella dysenteriae* as indicator. Transduction mixtures contained 10⁹ stationary phase recipient organisms (pre-incubated with CaCl₂, 5 µmol, for 30 min at room temperature) and 2 × 10⁹ phage P1 in a final volume

Table 2. Linkage relationships between *lpdI* and other markers

Cross	Donor	Recipient	Selection	Trans- ductants/ 10 ⁶ phage	No. scored	Unselected donor marker	Cotrans- duction frequency (%)
1	AB353	T3A58 <i>lpdI</i>	Lpd ⁺	5	100	Thr ⁻	< 1
						Ara ⁻	38
2 A	XG3	T3A58 <i>lpdI</i>	Lpd ⁺	61	198	Leu ⁻	41
2 B	T3A58 <i>lpdI</i>	XG3	Leu ⁺	15	108	Lpd ⁻	8
3	JP1537	T ⁺ <i>lpdI nadC</i> (P1)	Lpd ⁺	12	289	Azi ^R	64
						AroP ⁻	84
						NadC ⁺	81
			NadC ⁺	11	153	Lpd ⁺	78
						Azi ^R	73
						AroP ⁻	93
4 A	E126	T3A58 <i>lpdI</i>	Lpd ⁺	14	200	NadC ⁻	65
4 B	T3A58 <i>lpdI</i>	E126	NadC ⁺	11	100	Lpd ⁻	58
5	A2T2	T3A58 <i>lpdI</i>	Lpd ⁺	58	500	AceE ⁻	93
6	A10	T3A58 <i>lpdI</i>	Lpd ⁺	54	540	AceF ⁻	97
7 A	AT1371	T3A58 <i>lpdI</i>	Lpd ⁺	7	100	Pan ⁻	24
						Gal ⁻	< 1
7 B	T3A58 <i>lpdI</i>	AT1371	Pan ⁺	13	100	Lpd ⁻	18
8	K10	T3A58 <i>lpdI</i>	Lpd ⁺	8	100	TonA ⁻	17
9 A	PA-2-18	T3A58 <i>lpdI</i>	Lpd ⁺	20	100	NadA ⁻	< 1
						Gal ⁻	< 1
9 B	T3A58 <i>lpdI</i>	PA-2-18	NadA ⁺	4	100	Lpd ⁻	< 1
			Gal ⁺	4	100	Lpd ⁻	< 1
10 A	WGA	T3A58 <i>lpdI</i>	Lpd ⁺	67	230	Gal ⁻	< 0.5
10 B	T3A58 <i>lpdI</i>	WGA	Gal ⁺	4	220	Lpd ⁻	< 0.5

of 1 ml. After incubating for 25 min at 37 °C, unadsorbed phage were removed by centrifuging and appropriate dilutions of the transduced culture were plated on selective media. With the exception of glucose-based media, selective media were enriched with Bacto Nutrient Broth (0.2 %, v/v). Transductant colonies were counted after 2 to 4 days, depending on the selective medium.

Linkage analysis. Transductants were purified on the corresponding selective medium before investigating the segregation of unselected markers by replica-plating to non-permissive and permissive media. Galactose and arabinose minimal media were used for scoring fermentation markers and glucose-based minimal media were used for scoring the other nutritional markers. To obtain clear results with *pan*, *nadA* and *nadC* markers, the size of the inoculum and transfer of vitamins were limited by performing two rounds of replica-plating, in which the first replica-plate was used immediately as the master plate in the second round. The phenotypes affecting the α -keto acid dehydrogenase complexes were also scored on other minimal media: acetate for *lpd*, and *suc* markers and succinate (with and without a supplement of acetate) for *ace* markers. *AroP* was scored on glucose minimal medium containing DL-5-methyltryptophan (20 µg/ml) plus β -2-thienylalanine (20 µg/ml), and azide-resistance was scored on nutrient agar containing sodium azide (3 mM). Since the majority of transductants are P1-lysogens which restrict phage T₁, a modified phage was isolated and propagated on W3110(P1). Resistance to T₁ phage was then tested by replica-plating transductants to plates of nutrient agar spread with 10⁸ p.f.u. of the modified T₁ phage.

Table 3. *Linkage relationships in the leu-pan and sucA-gal region*

Cross	Donor	Recipient	Selection	Trans- ductants/ 10 ⁸ phage	No. scored	Unselected donor marker	Cotrans- duction frequency (%)
11 A	<i>XG3</i>	E126	NadC ⁺	80	113	Leu ⁻	47
11 B	E126	<i>XG3</i>	Leu ⁺	186	112	NadC ⁻	32
12 A	AT137I	E126	NadC ⁺	132	118	Pan ⁻	17
12 B	E126	AT137I	Pan ⁺	88	106	NadC ⁻	15
13 A	<i>XG3</i>	AT137I	Pan ⁺	117	110	Leu ⁻	7
13 B	AT137I	<i>XG3</i>	Leu ⁺	150	100	Pan ⁻	5
14	K10	E126	NadC ⁺	74	177	TonA ⁻	4
15	K10	AT137I	Pan ⁺	40	102	TonA ⁻	59
16 A	E126	A2T3	AceE ⁺	80	100	NadC ⁻	75
16 B	A2T3	E126	NadC ⁺	36	100	AceE ⁻	80
17	JP1537	A2T ⁻ <i>nadC</i> (Pr)	AceE ⁺	11	158	Azi ^R	78
						AroP ⁻	93
						NadC ⁺	87
			NadC ⁺	10	157	AceE ⁻	90
						Azi ^R	87
						AroP ⁻	96
18	E126	A2T3 <i>leu</i>	Leu ⁺	290	96	AceE ⁺	31
19	E126	A10 <i>leu</i>	Leu ⁺	234	96	AceF ⁺	25
20 A	WASucA26	PA-2-18	NadA ⁺	60	142	SucA ⁻	79
20 B	PA-2-18	WASucA26	SucA ⁺	44	136	NadA ⁻	63
21	T3A58 <i>lpdI</i>	PA-2-18	Gal ⁺	4	100	NadA ⁺	79
			NadA ⁺	4	100	Gal ⁺	82

RESULTS

Two representative lipoamide dehydrogenase mutants, T3A58*lpdI* and *XG3lpd4* which is a recipient strain with several other nutritional markers, were chosen for determining the chromosomal location of the *lpd* gene using transduction and conjugation techniques respectively.

Conjugation

Close proximity between the *lpd* gene and the *ace* region was first indicated by time of entry mapping using strain H as the Hfr donor and *XG3lpd4* as recipient. Lpd⁺ recombinants started to appear 0.8 min after Leu⁺ recombinants and approximately 8 min before entry of the donor ProC⁺ marker. The gradients of transmission averaged for five samples taken at different times in the 40 to 60 min period of the same experiment were: Leu⁺, 90 % of donor input; Lpd⁺, 73 %; and ProC⁺, 27 %. This confirms the order *leu...lpd...proC*.

Transduction

Two-factor crosses. The results of linkage studies in which T3A58*lpdI* served as donor or recipient with a variety of strains carrying markers in or near the *ace* and *suc* genes are shown in Table 2. These indicate that the *lpdI* site is linked to *aceF*, *aceE*, *aroP*, *nadC*, *azi*, *leu*, *ara*, *pan* and *tonA* (in decreasing order of cotransduction frequency). No linkage with *thr* was observed nor was there any evidence of cotransduction with *nadA* or *gal*, markers which are readily cotransduced with the α -ketoglutarate dehydrogenase complex genes, *sucA* and *B*. Glucose minimal media were used as the selective media in all these transductions and supplements of acetate and succinate were added when *lpd* was a non-selective marker. In addition, many of the Lpd⁺ selections were made with enriched acetate medium, which selects for a functional α -ketoglutarate dehydrogenase complex, and the results were virtually the same

as those shown in Table 2. The linkage between *lpd* and *leu* was invariably greater when Lpd^+ was selective than when Lpd^- was the non-selective marker (crosses 2A and B). This apparent tendency for selection against the donor Lpd^- marker was also observed to a lesser degree, in similar crosses involving *lpd* and *nadC* (crosses 4A and B) or *pan* (crosses 7A and B). Also, in cross 3, where the donor strain carries both Nad^+ and Lpd^+ markers, Nad^+Lpd^+ transductants appear to be preferentially selected and cotransduce at higher frequencies than were observed in cross 4. The same applies to *nadC* and *aceE* in crosses 16 and 17 (Table 3).

Linkage between *lpd* and *aceE* and *F* was examined by selecting Lpd^+ transductants on acetate-supplemented glucose medium and enriched acetate medium (crosses 5 and 6) and the results indicate the gene order: *aceE-aceF-lpd*. Direct selections for Ace^+Lpd^+ transductants on glucose and enriched succinate media in the same crosses gave recombination frequencies of 11 % between *aceE2* and *lpd1* and 3.3 % between *aceF10* and *lpd1*, with respect to the total number of Lpd^+ transductants. These values also indicate that the *lpd* gene is closer to *aceF* than *aceE*. No procedure for selecting Ace^+ transductants which is not automatically selective for Lpd^+ could be devised, nor was any medium found which permitted either Suc^- or Lpd^- to be used as unselective markers for direct tests for possible linkage between *lpd* and the *sucA* and *B* genes. Normal frequencies of Lpd^+ abortive transduction were observed in all the crosses, including those with *aceE* and *F* markers, indicating that the *lpd* mutation affects an independent functional unit.

The results of the two-factor crosses involving the *lpd* marker are summarized in the upper part of the linkage map shown in Fig. 1. The cotransduction frequencies are entirely consistent with the gene order shown, but the relative position of all six genes in the *leu* to *pan* area cannot be considered conclusive on the basis of the two-factor data alone.

During the course of this work linkages between other pairs of markers were determined with the same strains and results are outlined in Table 2 (cross 3) and Table 3 (crosses 11 to 21). The cotransduction frequencies, averaged where applicable, are also included in the lower section of the linkage map (Fig. 1). One aim of these crosses was to establish the positions of the *nadC* and *aroP* genes relative to each other and to the *azi* and *ace* genes which served as reference points in the *leu* to *pan* region. The *nadC* gene had been placed in this region by conjugation (Gholson, Tritz, Matney & Andreoli, 1969) and this was supported by the linkage data obtained from crosses 11, 12 and 14 (Table 3). The *aroP* gene had also been placed in this region by transductional analysis (Brown, 1970). Furthermore, Russell (1970) has presented evidence that *aroP* lies between *azi* and a temperature-sensitive, osmotic remedial mutation in strain JP1433. This mutant responds to acetate at 42° C (Russell, 1972) and it probably affects the *aceF* gene (J. R. Guest and I. T. Creaghan, unpublished observations). Clearly *azi*, *nadC*, *aroP* and *aceE* are all very close, with cotransduction frequencies greater than 78 % for any pair (crosses 3, 16, 17). Furthermore, this two-point data is best interpreted in terms of the gene order illustrated in Fig. 1. The cotransduction frequencies observed for *leu* with *aceE*, *aceF* and *pan* (Table 3) agree with those reported by Henning & Hertz (1964), Russell (1970) and Bukhari & Taylor (1971). The results are also consistent with values observed for other pairs of markers, 47 to 77 % for *leu-azi* (Lennox, 1955; Yura & Wada, 1968; Beelem, Feldmann & Wijsman, 1973) and 30 % for *leu-aroP* (Brown, 1970). The linkages of *sucA* with *nadA* and *nadA* with *gal* were evaluated in cross 20 (Table 3) and these are consistent with values of 32 to 54 % for *sucA* with *gal* (Herbert & Guest 1968).

Three-factor crosses. Knowledge of the position of the *lpd* gene relative to the *aceE* and *aceF* genes is clearly important for postulating mechanisms for the regulation of the genes specifying components of the α -keto acid dehydrogenase complexes. Therefore the order of

Fig. 1. Linkage map showing position of *lpd* with respect of other loci. Cotransduction frequencies are given as the number of recombinants inheriting the unselected marker, expressed as a percentage of the number of selected transductants examined. For each cross, the appropriate cotransduction frequency is placed near the head of the arrow which points to the selected marker. Average values are given for linkages between pairs of markers which were determined in more than one of the crosses recorded in Tables 2 and 3.

Table 4. Three-factor reciprocal crosses between *lpd*, *ace* and *nadC* mutants with *leu* as outside marker

The non-selective donor markers (NSM) were either *Leu*⁺ (crosses I to III) or *Nad*⁺ (crosses IV to VI) and the map distances represent the number of transductants expressed as a percentage of the total number of *Leu*⁺ or *Nad*⁺ transductants selected from a corresponding sample of the same transduction mixture.

Cross	Donor	Recipient	Selection	Trans- ductants/ 10 ⁶ phage	Map distance	No. scored	Distri- bution of donor NSM (%)	Order indicated
IA	A2T3	T3A58 <i>lpd</i> <i>leu</i>	AceE ⁺ Lpd ⁺	3.04	10.7	236	9.7	<i>leu</i> ... <i>aceE</i> . <i>lpd</i>
IB	T3A58 <i>lpd</i>	A2T3 <i>leu</i>	AceE ⁺ Lpd ⁺	0.81	8.8	162	35.2	
IIA	A10	T3A58 <i>lpd</i> <i>leu</i>	AceF ⁺ Lpd ⁺	0.41	1.7	458	17.2	<i>leu</i> ... <i>aceF</i> . <i>lpd</i>
IIB	T3A58 <i>lpd</i>	A10 <i>leu</i>	AceF ⁺ Lpd ⁺	0.27	1.9	254	30.0	
IIIA	E126	A2T3 <i>leu</i>	AceE ⁺ NadC ⁺	24.5	18.0	238	9.7	<i>leu</i> ... <i>nadC</i> . <i>aceE</i>
IIIB	A2T3	E126 <i>leu</i>	AceE ⁺ NadC ⁺	5.2	12.7	259	47.9	
IVA	A2T3	T3A58 <i>lpd</i> <i>nadC</i>	AceE ⁺ Lpd ⁺	0.24	6.7	211	9.0	<i>nad</i> ... <i>aceE</i> . <i>lpd</i>
IVB	T3A58 <i>lpd</i>	A2T3 <i>nadC</i>	AceE ⁺ Lpd ⁺	0.41	14.9	235	66.4	
VA	A10	T3A58 <i>lpd</i> <i>nadC</i>	AceF ⁺ Lpd ⁺	0.27	1.3	170	18.2	<i>nad</i> ... <i>aceF</i> . <i>lpd</i>
VB	T3A58 <i>lpd</i>	A10 <i>nadC</i> (Pr)	AceF ⁺ Lpd ⁺	0.46	2.4	190	56.3	
VIA	A2T3	A10 <i>nadC</i> (Pr)	Ace ⁺	2.7	5.9	211	8.1	<i>nad</i> ... <i>aceE</i> . <i>aceF</i>
VIB	A10	A2T3 <i>nadC</i>	Ace ⁺	3.0	7.9	227	63.0

Fig. 2. Genetic map showing the position of *lpd* relative to *nadC*, *aceE* and *aceF* markers. The distances are averaged from values based on Leu^+ and NadC^+ transduction in the crosses outlined in Table 4.

Table 5. Detailed analysis of Lpd^+ and AceE^+ transductants from crosses 3 and 17

Lpd^+ and AceE^+ transductants obtained with JP1537 as donor and $\text{T}^+ \text{lpd} \text{nadC}(P_I)$ (cross 3) and $\text{A2T}^+ \text{nadC}(P_I)$ (cross 17) as recipients, respectively, were scored for the segregation *nadC*, *aroP* and *azi* markers.

Classes of recombinant	Number of transductants in each class after selection for	
	Lpd^+	Ace^+
$\text{NadC}^+ \text{AroP}^- \text{Azi}^R$	181	121
$\text{NadC}^+ \text{AroP}^- \text{Azi}^S$	49	16
$\text{NadC}^+ \text{AroP}^+ \text{Azi}^R$	2	0
$\text{NadC}^+ \text{AroP}^+ \text{Azi}^S$	1	0
$\text{NadC}^- \text{AroP}^- \text{Azi}^R$	2	2
$\text{NadC}^- \text{AroP}^- \text{Azi}^S$	11	2
$\text{NadC}^- \text{AroP}^+ \text{Azi}^R$	1	0
$\text{NadC}^- \text{AroP}^+ \text{Azi}^S$	42	17
Total	289	158

these genes was investigated by reciprocal three-factor crosses (Table 4). Initially *leu* was used as the outside marker and for this purpose a series of independent *leu* mutants of T3A58-*lpd1*, A2T3, A10 and E126 were isolated. Later, *nadC* was used as the outside marker and a corresponding series of *nadC* mutants was constructed from the *leu* mutants by transduction with E126 as donor. The results show that *nadC* lies between *leu* and *aceE* (cross III) and not between *aceF* and *pan* where it had been arbitrarily placed (Taylor, 1970). Clearly also, *aceE* and *aceF* lie between *leu* and *lpd* (crosses I and II) and between *nadC* and *lpd* (crosses IV and V). The order of *aceE* and *aceF* relative to *nadC* (cross VI) confirms the order established by Henning & Hertz (1964) who used *leu* as the outside marker. Taken together, these results establish the gene order: *leu-nadC-aceE-aceF-lpd*; the relative distances based on the frequency of Leu^+ and NadC^+ transduction are summarized in Fig. 2. It should be noted that high frequencies of Ace^+ and $\text{Ace}^+ \text{Lpd}^+$ abortive transduction were observed in all crosses between pairs of *aceE*, *aceF* and *lpd* mutants.

The relative positions of *nadC* and *aroP* were confirmed by a detailed analysis of the Lpd^+ and AceE^+ transductants arising in crosses 3 and 17 (Table 5). Of the possible orders, *azi-nadC-aceE/lpd* and *nadC-azi-aceE/lpd*, the former would give rise to $\text{Azi}^R \text{NadC}^-$ recombinants by quadruple crossing over whereas double crossing-over would suffice in the latter case. This class of recombinants was in fact rare (3/289 and 2/158, Table 5) compared

with the complementary class, Azi^sNad⁺ (50/289 and 16/158), and the results favour the first alternative, *azi-nadC-aceE/lpd*.

The linkage data (Fig. 1) indicated that *nadC* is closer to *aroP* than to *aceE* or *lpd*, and a similar analysis of the distribution of NadC and AroP in Lpd⁺ and Ace⁺ transductants (Table 5) showed that the NadC⁻AroP⁻ class is more frequent than the NadC⁺AroP⁺ (13/289 and 4/158 compared with 3/289 and 0/158); this confirms the order *nadC-aroP-aceE-lpd*. NadC⁺ transductants were selected in the same crosses (3 and 17) and analysis of the segregation of the *azi*, *aroP* and *lpd* or *aceE* markers supported the gene orders established above.

DISCUSSION

The genetic analysis with two representative lipoamide dehydrogenase mutants shows that the gene which determines the E3 components of both α -keto acid dehydrogenase complexes is very closely linked to the pyruvate dehydrogenase (E1p) and dihydrolipoamide transacetylase (E2p) genes (*aceE* and *F*). No evidence for linkage with genes in the region of the α -ketoglutarate dehydrogenase (E1kg) or the dihydrolipoamide transsuccinylase (E2kg) genes (*sucA* and *B*), nor for the existence of another *lpd* gene, was found. The distance between the *aceF* and *lpd* mutations is sufficiently small to suggest that these two genes are contiguous. This immediately raises questions concerning the mechanism of expression for the *lpd* gene with respect to the *ace* and *suc* genes. If the *lpd* gene is contiguous with *aceF* then it would become the distal gene of the pyruvate dehydrogenase operon. However, for several reasons it seems unlikely that expression of the *lpd* gene could be tightly coupled to that of the *ace* genes in a completely co-ordinate fashion. For example, under many physiological conditions both enzyme complexes co-exist, so a direct coupling of E3 synthesis to the synthesis of E1p and E2p would mean a wasteful excess of these components in order to supply the α -ketoglutarate complex with E3 components. In fact, an excess of both types of E1 and E2 components would be produced if these components compete equally for available lipoamide dehydrogenase. Furthermore, the coupling of E3 synthesis with the metabolism of only one α -keto acid would present problems during growth on acetate, where synthesis of the pyruvate complex is repressed but the α -ketoglutarate complex is derepressed. Such problems could be circumvented during growth on glucose in the event of sequential metabolism of pyruvate via acetate in such a way that E3 components used at the first step could be salvaged and reassembled into α -ketoglutarate dehydrogenase complex for acetate metabolism. The simplest solution would be to postulate the existence of a secondary transcriptional promoter in the *aceF-lpd* region, of the type found in the tryptophan operons of *Escherichia coli* and *Salmonella typhimurium* (Morse & Yanofsky, 1968; Wuesthoff & Bauerle, 1970). This semi-coordinate expression could generate an excess of E3 components to satisfy the requirements of α -ketoglutarate dehydrogenase complex formation. It would also explain why the most polar *ace* mutations only partially block *lpd* expression (Henning *et al.* 1966). A development of this would be to include in the same *aceF-lpd* region a transcribable operator, which could respond to the same regulatory factors controlling the *suc* region without interfering with transcription generated by regulatory elements at the proximal end of the *ace* operon. More complex regulatory mechanisms could be postulated, in which the *lpd* gene is independently expressed under the control of completely separate regulatory elements, for example, by induction with pyruvate and/or α -ketoglutarate, or with one or both E1-E2 partial complexes, or by self-repression by uncomplexed E3 component. All these possibilities are currently under investigation.

The remaining linkage data is consistent with the map shown in Fig. 1 and some of this information has been incorporated in the recent linkage map of *Escherichia coli* K12 (Taylor & Trotter, 1972). It is interesting that a mutant possessing a temperature-sensitive pyruvate dehydrogenase complex can grow at the restrictive temperature if provided with either acetate or an environment of high osmotic pressure. Also, L-agar cultures of *ace* mutants often clear in crowded regions and growth on glucose minimal medium with limiting or no acetate can result in the production of curious star-shaped microcolonies containing very long filamentous organisms. Thus defects in pyruvate metabolism may create metabolic imbalances, presumably in the provision of precursors for lipid synthesis, which in turn affect formation of the cell envelope.

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