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A NEW DIHYDROLIPOAMIDE TRANSACETYLASE IN *ESCHERICHIA COLI* K12

HANS BISSWANGER AND ULF HENNING
Max-Planck-Institut für Biologie, Tübingen (Germany)

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SUMMARY

A new dihydrolipoamide transacetylase with an apparent molecular weight of about $1.5 \cdot 10^5$ has been found in *Escherichia coli* K12. This "transacetylase X" is governed by a genetic locus separate from, and unlinked to, that determining the dihydrolipoamide transacetylase component of the pyruvate dehydrogenase complex. The physiological role of the new enzyme is unknown.

INTRODUCTION

Dihydrolipoamide transacetylase has long been known to be a component of the pyruvate dehydrogenase complex[1,2]. In the oxidative decarboxylation of pyruvate:

$$\text{Pyruvate} + \text{NAD}^+ + \text{CoA} \longrightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad (1)$$

the component effects Reaction 4 in the sequence of reactions leading to the overall Reaction 1:

$$\text{Pyruvate} + \text{TPP–E1} \longrightarrow \text{CO}_2 + \text{hydroxyethyl-TPP–E1} \quad (2)$$

$$\text{Hydroxyethyl-TPP–E1} + \text{lipoyl–E2} \longrightarrow \textit{S}\text{-acetyldihydrolipoyl–E2} + \text{TPP–E1} \quad (3)$$

$$\textit{S}\text{-Acetyl-dihydrolipoyl–E2} + \text{CoA} \longrightarrow \text{acetyl-CoA} + \text{dihydrolipoyl–E2} \quad (4)$$

$$\text{dihydrolipoyl–E2} + \text{NAD}^+ \xrightarrow{\text{(FAD–E3)}} \text{NADH} + \text{H}^+ + \text{lipoyl–E2} \quad (5, 6)$$

(where E1 is the pyruvate dehydrogenase component, E2 is the dihydrolipoamide transacetylase component, and E3 is the dihydrolipoamide dehydrogenase component).

In *Escherichia coli*, as well as in other organisms, no other such transacetylase has been demonstrated that is not part of the pyruvate dehydrogenase complex. We have accidentally found a separate dihydrolipoamide transacetylase, and here we briefly report some properties of the enzyme, although its function *in vivo* remains obscure.

MATERIALS AND METHODS

Bacterial strains, media, and genetic procedures

All *ace* mutants used have been described earlier[3]. KL 16-99 (kindly given to us by Dr W. Maas) is a prototroph Hfr which is *recA* and donates this allele early. Cells for extracts were grown aerobically at 37 °C; individual carbon sources were used at 0.5% in a minimal salts medium[4], and "complete" medium without glucose is Bacto Nutrient Broth (Difco-Lab., Detroit, Mich.). Extracts were prepared as detailed earlier[5].

P1-mediated transductions were performed according to Lennox[6]. For matings, cells were pre-grown in Antibiotic Medium No. 3 (Difco), and mating was allowed to proceed in this medium at 37 °C for 30 min. KL 16-99 was always counterselected with streptomycin (200 μg/ml).

Enzyme assays

Pyruvate dehydrogenase complex activity was measured according to the method of Schwartz and Reed[7], and transacetylase according to the method of Willms *et al.*[8]. Protein was determined with the biuret reaction. Thiols and α-keto acids other than dihydrolipoamide and pyruvate, respectively, were used at the same concentrations as the latter two compounds. Specific activities are expressed as μmoles acetylphosphate or *S*-acetyldihydrolipoamide produced per mg protein per h at 30 °C.

Sucrose density gradient centrifugation

Centrifugations were performed[9] using linear 10–30% (w/v) sucrose gradients. Samples were spun for 15 h in a Spinco SW50L swing bucket rotor at 230 000 $\times$ *g*.

Chemicals, enzymes

Dihydrolipoamide was prepared[10] and DL-lipoic acid was purchased from Carl Roth, Karlsruhe. Alkaline phosphatase was from Worthington Biochemical Corp. (Freehold, N.Y., U.S.A.), and lactate dehydrogenase from Boehringer und Soehne (Mannheim).

RESULTS AND DISCUSSION

Completely polar *amber* mutants of the *aceE* gene (structural gene of the pyruvate dehydrogenase component) do not possess measurable activities in Reactions 1 or 4 (ref. 11). In experiments with such mutants it was found accidentally that in some cases an unusually high specific activity in Reaction 4 was present (about 5-fold higher than in wild type induced for enzyme complex synthesis), although such strains still lacked any activity in Reaction 1. This property was found only when the *amber* mutants had been crossed with strain KL 16-99 in order to obtain *recA* recombinants, and some of the *recA* isolates did while others did not show this unusual transacetylase activity. The high specific activity, however, was not present under all growth conditions. Table I indicates that the synthesis of this enzyme is subject to catabolite repression but has little dependence on carbon sources other than glucose. This behavior is in sharp contrast to the regulation of the syn-

TABLE I

EXPRESSION OF TRANSACETYLASE X

aceE130 recA, a recombinant from a cross between KL 16-99 and *aceE130 his*$^-$ (see text), has inherited high specific activity transacetylase from the KL 16-99 donor. The prototroph HfrH is normally inducible for the synthesis of the pyruvate dehydrogenase complex.

Strain	*Carbon source*	*Specific activity*	
		Transacetylase	*Pyruvate dehydrogenase complex*
aceE130 (recA)	Pyruvate	67	< 0.1
	Acetate	60	< 0.1
	Glucose	5	< 0.1
	Glycerol	44	< 0.1
	Succinate	51	< 0.1
	"Complete" (no glucose)	56	< 0.1
HfrH	Pyruvate	12	60
	Acetate	3	12
	Glucose	6	30

thesis of the pyruvate dehydrogenase complex[5]. Relevant data are shown for comparison in Table I.

In searching for the cause of the phenomenon we found that the high transacetylase activity is a property of the KL 16-99 parent. Transacetylase specific activity normally ranges from about 2 (synthesis of pyruvate dehydrogenase complex not induced) to about 20 (fully-induced synthesis), and, since it is measured with a non-physiological model reaction, amounts to about 1/5 of the specific activity of the enzyme complex (Reaction 1). When grown on succinate, strain KL 16-99 had a Reaction 1 specific activity of 15 but a transacetylase specific activity of 50.

The allele(s) responsible for the high specific activity is located somewhere near the *pps* locus on the *E. coli* chromosome, *i.e.* far away from the *ace* locus[12]. When KL 16-99 *(his*$^+$ *pps*$^+$*)* was crossed into a *his*$^-$ *pps*$^-$ recipient, almost all of the *his*$^+$ *pps*$^+$ recombinants (9/10) inherited the high specific activity transacetylase, while only 2/8 *his*$^+$ *pps*$^-$ recombinants possessed this activity.

The allele in question does not in some manner increase the expression of the *aceF* gene (structural gene of the dihydrolipoamide transacetylase component). An *aceF* mutant *(aceF10)* which was *thy*$^-$ *trp*$^-$ was crossed with KL 16-99, and *thy*$^+$ *trp*$^+$ recombinants were selected (all remained *ace*$^-$ (phenotype)). Several were found possessing high specific activity transacetylase (and, of course, no activity in Reaction 1). Expression of the *aceE* gene in *aceF* mutants is normal; thus the experiment at the same time showed that the pyruvate dehydrogenase component produced in *aceF10* does not complement with the high specific activity transacetylase to yield enzymatically-active pyruvate dehydrogenase complex. All these data indicated that we were dealing with a transacetylase other than that of the enzyme complex, and we have designated it transacetylase X.

Properties of transacetylase X

Transacetylase X does not behave as a non-specific thioltransacetylase. Table II shows that it has a specificity for dihydrolipoamide very similar to that of the trans-

TABLE II

SPECIFICITY OF DIHYDROLIPOAMIDE TRANSACETYLASE

For an explanation of the strains see Table I. The carbon source was pyruvate (0.5%) *plus* acetate (0.05%). The activities were measured in crude extracts, and the data found for cysteine, cysteamine, and glutathion, therefore, do not imply that dihydrolipoamide transacetylase can acetylate these thiols.

Substrate	*Specific activity of transacetylase in strain*	
	aceE130 recA	*HfrH*
Dihydrolipoamide	41	9.4
Dihydrolipoate	4.5	1.6
Cysteine	0.31	0.21
Cysteamine	0.26	0.29
Glutathion	0.06	0.03

acetylase from the pyruvate dehydrogenase complex. All other properties of transacetylase X, however, are quite different from those of the other transacetylase. We have shown[13] that the latter enzyme exhibits cooperativity for the substrate dihydrolipoamide. Transacetylase X, under the same conditions of assay, follows Michaelis–Menten kinetics (K_m for dihydrolipoamide: $4.2 \cdot 10^{-3}$ M). Rabbit antiserum against the sub-complex dihydrolipoamide transacetylase-dihydrolipoamide dehydrogenase (subcomplex prepared according to the method of Koike *et al.*[2]) precipitates the dihydrolipoamide transacetylase component of the enzyme complex (unpublished experiments). Such antiserum was found to have no influence on transacetylase X activity.

In sucrose density gradient centrifugation, transacetylase X moved as a perfectly symmetrical peak, and the apparent molecular weight (reference enzymes: *E. coli* alkaline phosphatase, mol. wt $8 \cdot 10^4$ (ref. 14); lactate dehydrogenase, mol. wt $1.36 \cdot 10^5$ (ref. 15)) was calculated to be about $1.5 \cdot 10^5$. The subunit of the normal transacetylase has a mol. wt of about $8 \cdot 10^4$ (ref. 16), and the enzyme component in the pyruvate dehydrogenase complex according to our studies[17] consists of 16, and according to investigations by Eley *et al.*[18] of 24, such polypeptide chains. In any case, transacetylase X, as judged from the centrifugation experiments alone, could only be a previously unknown enzyme or a dimer of the normal transacetylase subunit.

Transacetylase X is a hitherto unknown enzyme

The results described so far are not entirely incompatible with transacetylase X being a dimeric form of the normal transacetylase subunit. With well-established precedence (*e.g.* ref. 19), we could argue that the KL 16-99 locus in question has arisen by a duplication translocation of the normal transacetylase gene; the translocated gene has become fused to another (catabolite-repression sensitive) unit of expression. During the genetic rearrangement, the locus may have lost a small segment with the result that while enzymatic activity is retained, the subunit can form no multimer greater than a dimer and cooperativity with dihydrolipoamide is lost. However, we would not expect maintenance of enzymatic activity when immunological activity is destroyed. A clear answer could be obtained through genetic means.

aceF10 and *aceF19* are two of the *aceF* mutants in our collection which map furthest apart in that gene[20]. If the hypothetical translocated transacetylase gene had suffered a small deletion, the deleted segment certainly should not cover both the mutant sites mentioned. Therefore, strains *aceF10* and *aceF19* possessing the transacetylase X locus were used as donors in phage P1-mediated transduction with strains *aceF10* and *aceF19* lacking transacetylase X as recipients. Recombination to *ace*$^+$ is to be expected if the transacetylase X locus is a translocated *aceF* gene. Table III shows that such recombination does not occur, and thus it is clear that transacetylase X is entirely distinct from the transacetylase component of the pyruvate dehydrogenase complex.

TABLE III

RECOMBINATIONAL ANALYSIS OF THE TRANSACETYLASE X LOCUS

In each experiment $2 \cdot 10^9$ cells were infected with $5 \cdot 10^9$ phages. The frequencies of recombinants are expressed as *ace*$^+$ colonies per phage. The symbols X$^+$ and X$^-$ are used for presence and absence of transacetylase X, respectively.

Donor (P1 lysate)	*Recipient*	*ace$^+$ recombinants*
aceF10 X$^+$	*aceF10* X$^-$	$< 4 \cdot 10^{-9}$
aceF10 X$^+$	*aceF19* X$^-$	$5 \cdot 10^{-7}$
aceF19 X$^+$	*aceF19* X$^-$	$< 4 \cdot 10^{-9}$
aceF19 X$^+$	*aceF10* X$^-$	$3 \cdot 10^{-6}$

The high specific activity of transacetylase X in KL 16-99 is probably the consequence of a mutation to constitutivity of the expression of that gene. What may its normal function be? Lipoate auxotrophy can be satisfied with acetate plus succinate supplementation, *i.e.* no other defect than that of pyruvate and α-ketoglutarate oxidation becomes phenotypically apparent. It seems, therefore, that transacetylase X is not required for growth on carbon sources such as those listed in Table I. Oxidative decarboxylation of the type in Reaction 1 also occurs in the degradation of valine (α-keto isovalerate → isobutyryl-CoA), leucine (α-keto isocaproate → isovaleryl-CoA), and isoleucine (α-keto-β-methylvalerate → α-methyl butyryl-CoA). Although *E. coli* cannot grow on these amino acids, we have asked whether or not extracts containing transacetylase X are able to oxidatively decarboxylate α-keto isovalerate, α-keto isocaproate, or α-keto valerate (α-keto-β-methyl valerate is not available commercially) because the specificity of the corresponding transacetylases is not known. With none of these substrates was any reaction observed and it is thus very unlikely that transacetylase X is a component of any of these enzymes. It appears that *E. coli* still retains some secret corners in its metabolism.

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