GROWTH OF ESCHERICHIA COLI ON FATTY ACIDS: REQUIREMENT FOR COENZYME A TRANSFERASE ACTIVITY

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The ability of an Escherichia coli strain (E-26) to grow on butyrate or valerate as the sole source of carbon has previously been correlated with the glyoxylate-short chained fatty acid acyl-CoA ester condensing activities (1).

In attempting to confirm and expand these results, several strains of Escherichia coli K12, of previously defined genotype and physiology (2) were employed. From one of them, constitutive for the enzymes of the glyoxylate shunt, a new mutant, V10, has been selected for its ability to grow on butyrate or valerate as the sole source of carbon. The growth of this strain on these fatty acids is rapid and is not preceded by any significant lag when transfered from a glucose medium. This physiological behavior is related to the presence of a constitutive level of an acyl-CoA: acetate transferase activity. This enzymatic reaction, not previously reported in Escherichia coli, is measurable by spectrophotometric assays and a radioisotopic microassay. A second mutant, derived from V10, is simultaneously deficient in the transferase activity and in the ability to grow on butyrate or valerate.

The data presented suggest that the required transferase functions as an activating mechanism which permits the organism to utilize these fatty acids as a carbon source for growth.

Experimental: Escherichia coli strain $D_5H_3G_7$ (aceD, his) is a glu^+ recombinant obtained by transduction of strain D_5H_3 (glu-1, ace D, his) with a phage 363 lysate from a glucose positive strain, W678 (2,3). Growth conditions and preparation of cell-free extracts have previously been described (1), except that the extraction buffer was 0.1M phosphate, pH 6.8, containing 2×10^{-4}M dithiothreitol.

Enzyme Assays: Coenzyme A transferases were measured spectrophotometrically by coupling the production of acetyl-CoA with 1) arsenolysis in the presence of phosphotransacetylase (4); 2) malic dehydrogenase and citrate synthase (5); 3) malate synthase under the same conditions used for the malate synthase assay (6) except that butyryl-CoA was used in place of acetyl-CoA and the rate of decrease in absorbancy at 232 mμ in the presence of glyoxylate determined following the addition of potassium acetate (30 μm in 2.5 ml final volume). Coenzyme A transferases were also assayed using a radioisotopic microassay based on the determination of the acid-nonvolatile radioactive product formed upon incubation of a mixture containing 1-C^{14} acetate, butyryl-CoA (or valeryl-CoA) and extract. The details and efficacy of this technique will be published elsewhere (7). The β-oxidation enzymes: crotonase (E.C. 4.2.1.17), 3-hydroxy-acyl-CoA dehydrogenase (E.C. 1.1.1.35) and thiolase (E.C. 2.3.1.9) were assayed as previously described (8); butyryl-CoA and valeryl-CoA dehydrogenase (E.C. 1.3.99.2) were measured by the indophenol assay (9) using phenazine methosulfate (10).

Results: The several "normal" K12 strains tested did not grow on butyrate or valerate as the sole source of carbon even after a two week incubation. This is in accord with results previously reported by Overath et al. (8). However, using a strain ($D_5H_3G_7$) constitutive for the enzymes of the glyoxylate shunt (ace D) (2), growth was observed on valerate after a six day lag. The growth was the result of the selection of a spontaneous mutant (V10), which, following purification, was able to grow on valerate or butyrate with a doubling time of 140 min. (fig. 1). Furthermore, no significant lag was observed when washed cells of V10 were transferred from a glucose to a valerate or butyrate mineral salts medium. The V10 strain does

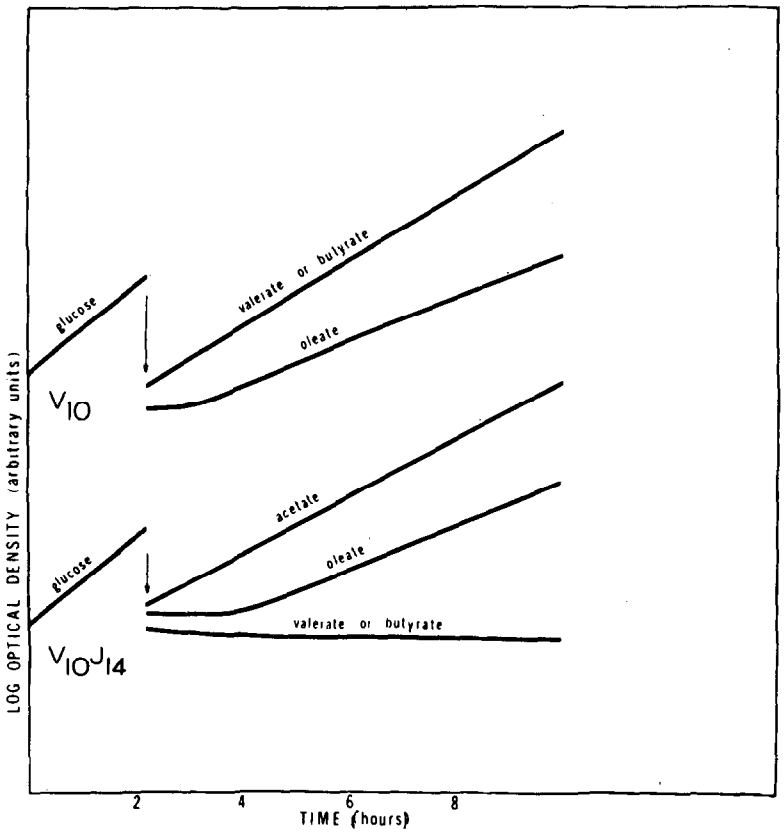

Figure 1: Growth of E. coli strains on fatty acids. The arrow indicates the transfer of washed cells from glucose to the indicated media.

not differ from its parent in the ability to grow on various other carbon sources, including long chained fatty acids such as oleate or palmitate, and has retained the parental genotype: constitutivity for the glyoxylate shunt (ace D) and histidine auxotrophy (his).

Assays of the glyoxylate condensing enzymes by the valeryl-CoA or butyryl-CoA dependent disappearance of glyoxylate-1-C^{14} (11) was catalyzed only by extracts of V10 cells grown either on glucose, valerate, butyrate or oleate. However, the only detectable product formed during these reactions was malate; it was subsequently shown that the activity was dependent upon the presence of acetate in the

TABLE I SPECIFIC ACTIVITIES (μm/hr/mg protein) OF THE ENZYMES OF FATTY ACID DEGRADATION IN E. COLI

strain	carbon source	MALATE SYNTHASE	BUTYRYL-CoA ACETATE TRANSFERASE	ACYL CoA DEHYDROGENASE		CROTONASE	β-HYDROXY ACYL-CoA DEHYDROGENASE	THIOLASE
				BUTYRYL-CoA	VALERYL CoA			
$D_5H_3G_1$	glucose	64	0	03	09	9	5	2
	oleate	69	0	90	1 31	282	264	15
V_{10}	glucose	51	9	1.30	37	25	47	58
	butyrate	67	12	80	50	86	89	73
	valerate	83	5	43	72	55	63	46
	oleate	60	6	50	85	110	113	45
$V_{10}J_{14}$	glucose	49	0	13	20	18	22	5
	oleate	83	0	54	90	125	107	13

reaction mixture or endogenously in the extract. That most, if not all, of this activity was due to a coenzyme A transferase (butyryl or valeryl-CoA + acetate $\rightleftharpoons$ acetyl CoA + butyrate or valerate) coupled with the malate synthase (glyoxylate + acetyl-CoA $\rightarrow$ malate + CoASH) constitutively present in the extract was assessed by measuring transferase activity directly.

Three spectrophotometric assays employed to measure transferase (see Experimental) gave essentially similar results for the butyryl-CoA: acetate transferase. The results shown in Table 1 were those obtained by coupling with the malate synthase reaction. The spectrophotometric assays with valeryl-CoA: acetate transferase gave much lower specific activities than with butyryl-CoA (approximately 1μM/hr/mg). However, using the radioisotopic microassay (7), activity with valeryl-CoA was evident, similar to the activity obtained with

butyryl-CoA, and in both cases, acetyl CoA was identified as the product of the reaction by paper chromatography (12). Further, the reversibility of these reactions was demonstrated by this technique.

The results given in Table 1 also show that the V10 strain has acquired the transferase activity constitutively and that each of the several enzymes of the β-oxidation system of fatty acid degradation examined appears at a much higher level in the glucose grown mutant than in the parent. The parent strain grown on oleate is normally induced for the β-oxidation enzymes, without any detectable transferase activity; so induced, it has not acquired the capacity to grow on butyrate or valerate.

The penicillin selection technique was employed to select a U.V. induced valerate-negative mutant from the V10 (V10J14). Strain V10J14 is unable to grow on valerate or butyrate but retains the parental behavior on other substrates, including oleate. Its only enzymatic deficiency appears to be the lack of transferase activity, and the activities of the β-oxidation enzymes are more similar (although somewhat higher) to those of the original strain of this series ($D_5H_3G_7$).

Discussion: As described several years ago, Coenzyme A transferase activity is known to occur in Clostridium Kluyverii (13), and in some mammalian tissues (14). The appearance of this activity in E. coli under the conditions described suggests the following physiological function: it provides the ability for cells to grow on various fatty acids by enabling them to activate these substrates using available and easily regenerated acetyl-CoA.

Some interesting hypothetical features concerning this reaction - besides its intriguing mechanism (15) - can be pointed out:
1) it may represent the activation component of a more elaborate

transport system for fatty acids by analogy to the PEP-phosphotransferase system for sugar transport (16). The observation that oleyl-synthetase, the activating enzyme for oleic acid in E. coli, is membrane bound (8) supports the contention that transport and activation of fatty acids are tightly coupled.

2) the fact that "derepressed" levels of the enzymes of β-oxidation appear simultaneously with transferase activity in V10 and are lower in the V10 derived transferase-less mutant V10J14 cannot be explained by assuming a common regulatory mechanism since the β-oxidation enzymes can be induced alone by growth on oleate. Rather, it implies that the CoA esters of the fatty acids could be the inducers of their own oxidation system.

A coupling of the above two hypothetical schemes would result in a process by which fatty acids might be transported through conversion to the CoA thioesters, and, in this form, would then induce the enzymes necessary for their subsequent degradation.

The mutational event resulting in the V10 phenotype and its biochemical and regulatory implications are under current investigation.

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