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Metabolism of L-Fucose and L-Rhamnose in *Escherichia coli*: Aerobic-Anaerobic Regulation of L-Lactaldehyde Dissimilation

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L-Lactaldehyde is a branching point in the metabolic pathway of L-fucose and L-rhamnose utilization. Under aerobic conditions, L-lactaldehyde is oxidized to L-lactate by the enzyme lactaldehyde dehydrogenase, while under anaerobic conditions, L-lactaldehyde is reduced to L-1,2-propanediol by the enzyme propanediol oxidoreductase. Aerobic growth on either of the methyl pentoses induces a lactaldehyde dehydrogenase enzyme which is inhibited by NADH and is very stable under anaerobic conditions. In the absence of oxygen, the cell shifts from the oxidation of L-lactaldehyde to its reduction, owing to both the induction of propanediol oxidoreductase activity and the decrease in the NAD/NADH ratio. The oxidation of L-lactaldehyde to L-lactate is again restored upon a change to aerobic conditions. In this case, only the NAD/NADH ratio may be invoked as a regulatory mechanism, since both enzymes remain active after this change. Experimental evidence in the presence of rhamnose with mutants unable to produce L-lactaldehyde and mutants capable of producing but not further metabolizing it points toward L-lactaldehyde as the effector molecule in the induction of lactaldehyde dehydrogenase. Analysis of a temperature-sensitive mutation affecting the synthesis of lactaldehyde dehydrogenase permitted us to locate an apparently single regulator gene linked to the *ald* locus at 31 min and probably acting as a positive control element on the expression of the structural gene.

L-Lactaldehyde is an intermediate metabolite formed during the dissimilation of the sugars L-fucose and L-rhamnose. These two methyl pentoses are metabolized in *Escherichia coli* through inducible pathways that show a striking parallelism. Both pathways are sequentially mediated by a permease (16), an isomerase (14, 26), a kinase (17, 27), and an aldolase (11, 13). The stereochemical difference between the configurations of both sugars at carbon 2 and carbon 4 disappears with cleavage of the fuculose 1-phosphate or rhamnulose 1-phosphate by the corresponding aldolases, yielding in each case dihydroxyacetone phosphate and L-lactaldehyde (Fig. 1). The two homologous sets of inducible proteins are specific for the metabolism of their corresponding sugars and are coded for by two different gene clusters located on the *E. coli* chromosome at 60 min for fucose and 87 min for rhamnose (1).

Further metabolism of L-lactaldehyde follows two different pathways, depending on the availability of oxygen. Under aerobic conditions, the aldehyde is oxidized to L-lactate in an irreversible reaction catalyzed by the NAD-dependent lactaldehyde dehydrogenase (24) and L-lactate is further oxidized to pyruvate by a dehydrogenase of the flavoprotein class (12). Under anaerobic conditions, L-lactaldehyde is reduced to L-1,2-propanediol by propanediol oxidoreductase in a typical fermentative mechanism in which NADH is oxidized to NAD and L-1,2-propanediol is excreted as a fermentation product (12). Anaerobic growth of wild-type cells on fucose induces the active propanediol oxidoreductase enzyme, while aerobic growth induces an inactive form of the enzyme that becomes rapidly activated when the culture is shifted to anaerobiosis (3). Growth on rhamnose, in contrast, only induces the propanediol oxidoreductase enzyme (in the active form) under anaerobic conditions (3). While propanediol oxidoreductase is encoded by the gene *fucO* of the fucose system (21), mutations affecting lactaldehyde dehydrogenase activity map well apart from the fucose and rhamnose genes in the *ald* locus at 31 min (10).

Alternatively, L-lactaldehyde may be formed in mutant cells (strain ECL3) that utilize L-1,2-propanediol (25). The diol, used as a sole carbon and energy source in the presence of oxygen, enters the cell through a facilitator (15) and is subsequently oxidized to L-lactaldehyde (25) in a reaction corresponding to the reversa of the propanediol oxidoreductase-catalyzed reduction of L-lactaldehyde. In these mutant cells, under aerobic conditions (no propanediol is metabolized anaerobically), L-lactaldehyde is metabolized to L-lactate and pyruvate and is introduced into the general metabolism as indicated above.

Several mechanisms may be invoked to explain the change from the oxidative pathway that leads to lactate under aerobic conditions to the reductive pathway that forms propanediol under anaerobic conditions. However, very little information is available to explain the adaptation to aerobic conditions by cells grown under anaerobic conditions. In this paper we present data that allow for the establishment of the factors involved in the aerobic-anaerobic regulation of lactaldehyde utilization.

MATERIALS AND METHODS

Chemicals. DL-1,2-Propanediol was obtained from E. Merck AG, Darmstadt, Federal Republic of Germany, and purified by distillation. L-Fucose, L-rhamnose, NAD, NADH, glycolaldehyde, glycerol, glucose, glutamate, and agarose were from Sigma Chemical Co., St. Louis, Mo. The other chemicals were of the purest grade available from commercial sources.

Bacterial strains. The genotypes and sources of the *E. coli* K-12 strains used are shown in Table 1. Strain JA-104, derived from strain ECL3, is a temperature-sensitive, non-L-1,2-propanediol-utilizing mutant that lacks lactaldehyde dehydrogenase activity at the restrictive temperature (6). Strain JA-105 is a Tn*5* insertion mutant derived from strain ECL3 that has lost the ability to utilize L-1,2-propanediol.

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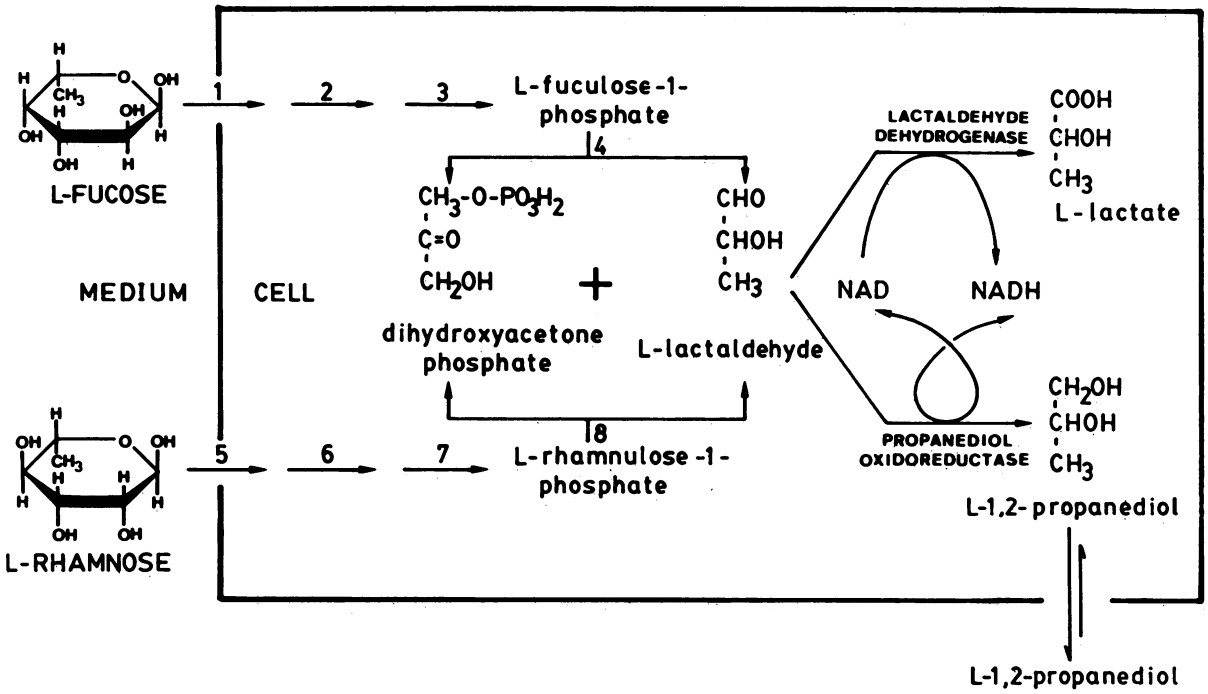

FIG. 1. Pathways of L-fucose and L-rhamnose dissimilation. The arrows indicate the corresponding permeases (1 and 5), isomerases (2 and 6), kinases (3 and 7), and aldolases (4 and 8).

This strain was obtained by a modification of the procedure described by Bruijn and Lupski (4). A Luria broth culture of strain ECL3 (10^9 cells per ml) was infected at 37°C for 30 min with λ467 (*b*221 *c*Its857 *rex*::Tn*5* *O*am29 *P*am80) at a multiplicity of infection of 0.5. Selection of the propanediol-negative mutants was made by plating 200 to 400 cells on MacConkey plates containing 40 mM 1,2-propanediol and 25 μg of kanamycin per ml. White colonies were picked, grown aerobically on rhamnose, and processed to assay for propanediol oxidoreductase and lactaldehyde dehydrogenase. In this way, a propanediol oxidoreductase-deficient clone (strain JA-105) was selected. Transduction with phage P1 was carried out by the method of Miller (23).

Growth media and preparation of cell extracts. Cells were grown and harvested as described previously (2). The carbon sources L-fucose, L-rhamnose, and glucose were added to a basal inorganic medium at a 10 mM concentration for aerobic growth and at a 20 mM concentration for anaerobic growth. For aerobic conditions glycerol was added at a 20 mM concentration and DL-1,2-propanediol was added at a 40 mM concentration; for anaerobic conditions glycerol was added at a 40 mM concentration and supplemented with 10 mM fumarate. Strain ECL289 (*argA*::Tn*10* *eno*) was grown on arginine-supplemented (20 μg/ml) mineral medium containing glycerol and succinate as combined carbon and energy sources (7). To counterselect routinely for the excision mutants, we cultured strain JA-105 in the presence of 50 μg of kanamycin per ml and strain ECL289 in the presence of 12.5 μg of tetracycline per ml. Extracts were prepared as described previously (3), except that the buffer used was 10 mM sodium phosphate (pH 7.3) containing 10 mM β-mercaptoethanol and 1 mM EDTA.

Enzyme assays. Lactaldehyde dehydrogenase was measured by using glycolaldehyde instead of lactaldehyde as the substrate (6). Spectrophotometric assays were performed at 25°C by measuring the A_{340} (NADH formation) in a mixture (1 ml) that consisted of 1 mM glycolaldehyde, 2.5 mM NAD, and 100 mM sodium glycine buffer at pH 9.5. Propanediol oxidoreductase activity, measured during the selection of mutant strain JA-105, was determined as described by Boronat and Aguilar (3). One unit of enzyme activity was defined as the amount of enzyme that transformed 1 μmol of substrate per min. The concentration of protein in cell extracts and purified preparations was determined by the method of Lowry et al. (22).

TABLE 1. *E. coli* K-12 strains used

Strain	Genotype[a]	Source or reference
ECL1	HfrC *phoA8 relA1 tonA22* $T2^r$ (lambda)	E. C. C. Lin (20)
ECL3	HfrC *fucO*(Con) *fucA*(Con) *fucPIK* (Non) *phoA8 relA1 tonA22* $T2^r$ (lambda)	E. C. C. Lin (25)
ECL289	HfrC *argA*::Tn*10 eno phoA8 relA1 tonA22* $T2^r$ (lambda)	E. C. C. Lin (7)
ECL493	HfrC *ald-1 trg-2*::Tn*10 fucO*(Con) *fucA*(Con) *fucPIK*(Non) *phoA8 relA1 tonA22* $T2^r$ (lambda)	E. C. C. Lin (10)
JA-104	HfrC *ald*(Ts) *fucO*(Con) *fucA*(Con) *fucPIK*(Non) *ppc phoA8 relA1 tonA22* $T2^r$ (lambda)	6
JA-105	HfrC *fucO*::Tn*5* *fucA*(Con) *fucPIK* (Non) *phoA8 relA1 tonA22* $T2^r$ (lambda)	This study
ET-6016	*araD139* Δ(*argF-lac*)*205 flbBS301 ptsF25 relA1 rpsL150* Δ(*glnG-glnL*)*228 rha-10 deoC1*	CGSC[b]

[a] Symbols: *ald*, gene for aldehyde dehydrogenase; *fucO*, gene for L-1,2-propanediol oxidoreductase; *fucA*, gene for L-fuculose 1-phosphate aldolase; *fucPIK*, operon encoding L-fucose permease, L-fucose isomerase, and L-fuculose kinase; Con, constitutivity; Non, noninducibility; and Ts, temperature sensitivity.

[b] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

Purification procedure. Lactaldehyde dehydrogenase was purified to homogeneity by a modification of the procedure described by Caballero et al. (6): hydroxylapatite column chromatography was substituted for Ultrogel AcA 34 gel filtration in the last step (1a).

Immunological techniques. Antisera against lactaldehyde dehydrogenase were raised in New Zealand White rabbits with purified enzyme from strain ECL3 as the antigen. Purified lactaldehyde dehydrogenase (250 μg) in 300 μl of 10 mM sodium phosphate (pH 7.3) containing 150 mM NaCl was emulsified in an equal volume of Freund complete adjuvant, and the mixture was injected subcutaneously into each rabbit. Booster injections of 400 μg were given subcutaneously 2 and 4 weeks later. The rabbits were bled 1 week after the last booster injection. The globulin fraction was partially purified by 35% ammonium sulfate precipitation. Quantitative immunoelectrophoresis was performed as described by Laurell (19). The agarose gel contained 0.4% specific globulins, and 50 μg of protein in 10 μl of cell extract was applied to each of the wells.

RESULTS

Induction of lactaldehyde dehydrogenase. Wild-type *E. coli* K-12 induced the synthesis of lactaldehyde dehydrogenase when grown on L-fucose or L-rhamnose under aerobic conditions (Table 2). This induction was absolutely dependent on the presence of oxygen, as indicated by the absence of both activity and enzymatic protein when growth was carried out under anaerobic conditions. No inactive enzyme was detected under any conditions, as indicated by the constant ratio of specific activity to the height of the immunoprecipitate (rocket), except for the glucose culture, in which, owing to the low concentration of the enzyme in the extracts, the height of the rocket deviated from linearity. Control cultures on glucose or glycerol corroborated the absolute dependence of enzyme synthesis on oxygen, and they showed some catabolic repression effect by glucose and a rather high basal level of enzyme synthesis with a noninducing carbon source such as glycerol.

Role of L-lactaldehyde as an inducer molecule. Attempts to identify the inducer molecule were carried out by adding the aldehyde to cells growing aerobically on a noninducing carbon source. No induction was observed under these conditions, but we would like to stress the toxicity problems, reflected by an immediate cessation of growth, that appeared upon the addition of the aldehyde to a growing culture of cells. To bypass these problems, we used mutant cells unable to produce L-lactaldehyde but able to produce precursor intermediate metabolites during the dissimilation of rhamnose as well as mutant cells able to produce L-lactaldehyde when incubated anaerobically in the presence of the sugar but unable to further metabolize the aldehyde under these conditions, resulting in an increase in its intracellular concentration.

TABLE 2. Lactaldehyde dehydrogenase activity in crude extracts of wild-type *E. coli* (ECL1) grown under different conditions

Carbon source	Aerobic conditions		Anaerobic conditions	
	Enzyme sp act (mU/mg of protein)	Immunoprecipitate height (mm)	Enzyme sp act (mU/mg of protein)	Immunoprecipitate height (mm)
Glucose	40	5	<10	ND[a]
Glycerol	80	7	<10	ND
Rhamnose	340	27	20	ND
Fucose	360	28	25	ND

[a] ND, Not detectable.

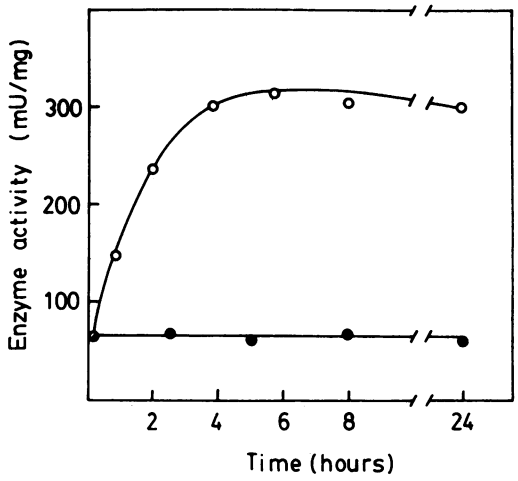

FIG. 2. Effect of the addition of rhamnose on the aerobic induction of lactaldehyde dehydrogenase in strain ECL1 and strain ET-6016. Cells of strain ECL1 (○) and strain ET-6016 (●) were grown aerobically on glycerol as described in the text. At zero time, rhamnose was added, incubation was continued, and samples were removed for enzyme activity analysis at the indicated times.

Cells of mutant strain ET-6016 (rhamnose negative, owing to the lack of rhamnulose-1-phosphate aldolase activity), able to produce the intermediate metabolites up to rhamnulose 1-phosphate, and cells of strain ECL1 were grown aerobically on glycerol in parallel. At the late logarithmic phase, 10 mM rhamnose was added to each culture, and incubation was continued at 37°C (Fig. 2). At various times, aliquots were removed for enzyme activity assays. No induction was present in the mutant cells, even after 24 h of incubation, while wild-type cells induced lactaldehyde dehydrogenase under the same conditions. The entry and metabolism of rhamnose in this experiment with mutant strain ET-6016 were assessed by the cessation of growth upon the addition of the sugar to a growing culture of cells on glycerol, presumably owing to the toxicity of sugar phosphate accumulation (data not shown). In another experiment (anaerobic conditions), cells of strain JA-105, which contains a Tn*5* insertion hindering propanediol oxidoreductase expression, and cells of strains ET-6016 and ECL1 as reference cultures were also grown on glycerol. At the end of the logarithmic phase, 20 mM rhamnose was added, and incubation was continued. Analysis of the aliquots removed at various times (Fig. 3) revealed a four- to fivefold induction of lactaldehyde dehydrogenase, although specific activities were seven times lower than those obtained under aerobic conditions. Reference cultures (Fig. 3) of strain ET-6016, which is unable to produce L-lactaldehyde, showed no induction, and those of strain ECL1, which produces and simultaneously metabolizes L-lactaldehyde, showed a very slight induction.

Location of the regulatory gene for the expression of lactaldehyde dehydrogenase. Strain JA-104 was shown to be a temperature-sensitive mutant unable to utilize L-1,2-propanediol, owing to the absence of lactaldehyde dehydrogenase activity when grown at the restrictive temperature of 42°C in the presence of any of the known inducers, namely, rhamnose, propanediol, and glutamate (1a). Analysis of the thermostability of the mutant enzyme revealed no differences with respect to the wild-type enzyme (data not shown). In

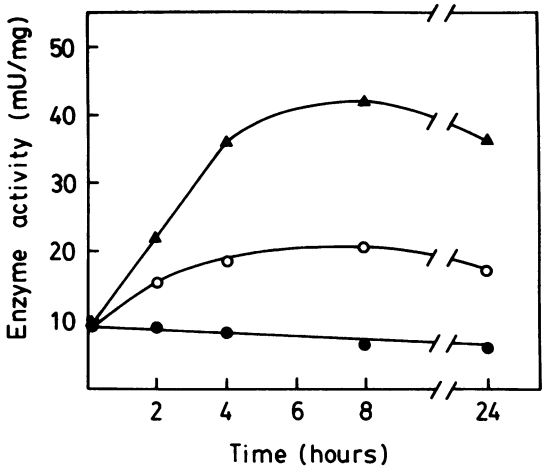

FIG. 3. Effect of the addition of rhamnose on the anaerobic induction of lactaldehyde dehydrogenase in strains JA-105, ET-6016, and ECL1. Cells of strain JA-105 (▲), strain ET-6016 (●), and strain ECL1 (○) were grown anaerobically on glycerol as described in the text. At zero time, rhamnose was added, incubation was continued, and samples were removed for enzyme activity analysis at the indicated times.

addition, an immunological analysis of lactaldehyde dehydrogenase in cell extracts of strain JA-104 grown at the restrictive temperature revealed a complete absence of the enzymatic protein, indicating that the inability to metabolize L-1,2-propanediol at this temperature was due to the absence of the enzyme rather than to its stability. The mutation was thus affecting a regulatory gene rather than a structural gene for lactaldehyde dehydrogenase.

The mapping of the genes responsible for propanediol oxidoreductase activity in the fucose locus led us to investigate the possible location in this locus of the regulatory temperature-sensitive mutation affecting lactaldehyde dehydrogenase. To this end, we used strain JA-104 [*ald*(Ts) *fucO*(Con)] as the transduction donor and strain ECL289 (*ald*$^+$), with the markers *eno* (59.6 min) and *argA* (60.5 min) flanking *fuc*$^+$ (60.2 min), as the recipient. Transductants were selected for *eno*$^+$ and *arg*$^+$ (growth on glucose without arginine supplementation) and scored for the ability to grow on propanediol at 30 and 42°C. If the *ald*(Ts) mutation was within the segment of the genome selected, the transductants should have been propanediol negative at 42°C, although growing on propanediol at 30°C. All (98 of 98) of the transductants scored were propanediol positive at 42°C, indicating that *ald*(Ts) is not between the markers *eno* and *argA*.

The recent mapping of mutations affecting lactaldehyde dehydrogenase activity at 31 min by Chen et al. (10) gave us a second possible location for the temperature-sensitive mutation. To check this alternative, we transduced the *ald*(Ts) mutation in strain JA-104 into strain ECL493 (*ald-1 trg-2*::Tn*10*). In this cross, 169 transductants were selected by growth on propanediol at 30°C and scored for *ald*(Ts) by growth on propanediol at 42°C and for the loss of Tn*10*. All of the transductants analyzed were temperature sensitive for the utilization of propanediol, and 137 of them were tetracycline sensitive. Thus, *ald*(Ts) was 100% linked with *ald-1* and 81% linked with *trg-2*.

Lack of inactivation or degradation of lactaldehyde dehydrogenase under anaerobic conditions. To gain more information on the possible mechanisms of regulation of lactaldehyde dehydrogenase in the aerobic-anaerobic adaptation of wild-type cells, we shifted cultures from one condition to the other condition. Obviously, a shift from anaerobiosis, in which no enzyme was present, to aerobiosis triggered the induction of the enzyme and the appearance of lactaldehyde dehydrogenase activity. On the other hand, cells were grown aerobically on rhamnose until the late logarithmic phase, washed twice, suspended in different media, and incubated under anaerobic conditions. At various times, samples were processed to determine enzyme activity and the presence of enzymatic protein measured immunologically. Enzyme was not inactivated or degraded when analyzed up to 4 h after the shift to minimal-rhamnose medium (Fig. 4). The same result was obtained when minimal-fucose or minimal-glucose medium was used (data not shown). Deprivation of a nitrogen and sulfate source at the time of anaerobic incubation had no effect on the stability of the enzyme. However, deprivation of a carbon source promoted rapid inactivation, although no degradation of enzymatic protein was apparent (Fig. 4). The amount of enzymatic protein measured immunologically remained stable up to 24 h (data not shown) under any of the conditions used above.

Inhibition of lactaldehyde dehydrogenase by NADH. The activity of purified lactaldehyde dehydrogenase was analyzed at increasing concentrations of NADH. All assays were carried out with 1 mM glycolaldehyde and 2.5 mM NAD under the standard conditions described above. Fifty percent inhibition was obtained with 0.45 mM NADH, and enzyme activity was virtually zero with 1 mM NADH (Fig. 5).

DISCUSSION

Information on the regulation of propanediol oxidoreductase (3, 8, 9) together with the results presented in this report concerning lactaldehyde dehydrogenase permitted us to improve the understanding of the regulatory mechanisms involved in the metabolism of L-lactaldehyde, the branching

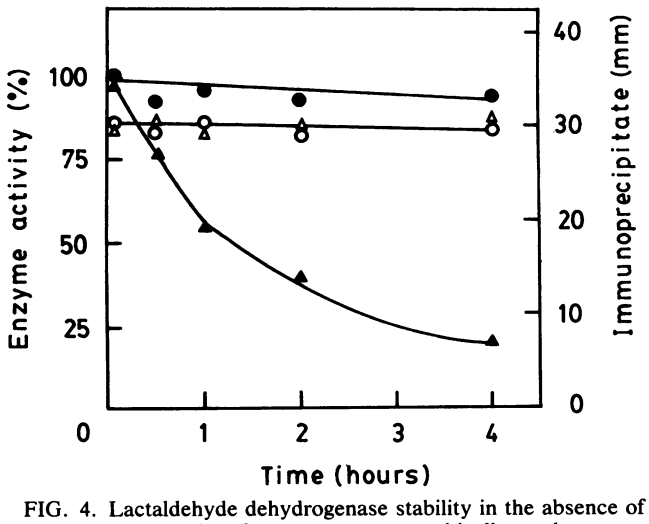

FIG. 4. Lactaldehyde dehydrogenase stability in the absence of oxygen. Cells of strain ECL1 were grown aerobically on rhamnose. At zero time, the culture was shifted to anaerobic conditions; at the indicated times, samples were removed to measure enzyme activity (closed symbols) and for the immunological quantification of enzymatic protein (open symbols). The experiment was carried out in the presence of rhamnose (circles) and in the absence of any carbon source (triangles).

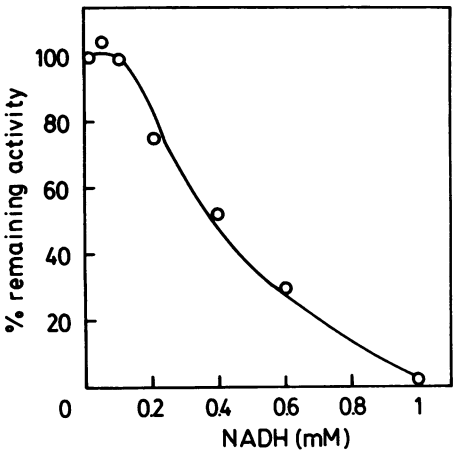

FIG. 5. Inhibition of purified lactaldehyde dehydrogenase by NADH. Enzyme activity was measured under standard conditions (see the text) in the presence of increasing concentrations of NADH in the assay mixture.

point in the aerobic-anaerobic utilization of L-fucose and L-rhamnose.

The experiments done with strain ET-6016 or strain JA-105 indirectly point toward L-lactaldehyde as the effector molecule in the induction of lactaldehyde dehydrogenase. However, in the case of strain ET-6016, the alternative explanation of the lack of induction by the toxic effects of the sugar phosphate rather than by the absence of the inducing molecule cannot be eliminated.

Under aerobic conditions, both sugars induce lactaldehyde dehydrogenase, channeling the three carbons of L-lactaldehyde to L-lactate. Under these conditions, propanediol oxidoreductase is present in an inactive form when fucose is the carbon source but is not induced at all when rhamnose is the carbon source. A change to anaerobic conditions causes the activation of propanediol oxidoreductase, by a yet-unknown mechanism, in fucose-grown cells or the induction of active enzyme in rhamnose-grown cells (3). However, no inactivation or degradation of lactaldehyde dehydrogenase occurs under these conditions. Thus, shifting from oxidation of lactaldehyde by lactaldehyde dehydrogenase to reduction of this metabolite by propanediol oxidoreductase could be explained by the conjunction of at least two factors. One of them is the higher efficiency of propanediol oxidoreductase than of lactaldehyde dehydrogenase in the utilization of L-lactaldehyde. This would account for the preferential transformation of L-lactaldehyde to L-1,2-propanediol when both enzymes are present. Actually, the catalytic power (V_{max}/K_m), as defined by Kelleti and Welch (18), has a value of 530 for propanediol oxidoreductase and a value of 190 for lactaldehyde dehydrogenase. The other factor to be considered is the decrease in the high intracellular NAD/NADH ratio when the growth conditions change to anaerobiosis (28), which favors the reduction reaction, while the transient increase in NADH (28) stops the oxidation reaction by inhibiting lactaldehyde dehydrogenase. Estimates of intracellular concentration of NADH close to 1 mM (5, 28, 29) consistently support the physiological role of this inhibition.

Shifting from anaerobic to aerobic conditions promotes an increase in the NAD/NADH ratio, which shifts the oxidation-reduction balance back to the oxidation of lactaldehyde. It has already been pointed out that growth in the presence of inducing carbon sources under aerobic conditions triggers the synthesis of lactaldehyde dehydrogenase if the enzyme is not already present. It is also known that propanediol oxidoreductase is not degraded or inactivated when a carbon source is present and is only inactivated in the absence of a carbon source (J. Aguilar, unpublished results). Hence, the shift toward the oxidation of lactaldehyde under these conditions can only be justified in terms of the NAD/NADH ratio, according to the information available at present.

Finally, in strain JA-104 the effect of the restrictive temperature on the induction of lactaldehyde dehydrogenase rather than on its activity suggests that the temperature-sensitive mutation affects a regulatory gene that has been mapped close to the *ald* locus at 31 min by P1 transduction. This regulatory gene is apparently shared by the different known inducing carbon sources since, in all cases, inductions are affected by the mutation. The fact that this mutation leads to a negative rather than to a constitutive phenotype suggests a positive control mechanism for lactaldehyde dehydrogenase.

The regulatory protein activates a unique structural gene, as indicated by an analysis of an ethylene glycol-positive mutant that displays, presumably as a consequence of an up-promoter mutation, increased enzyme production with any of the different carbon sources and a simultaneous loss of sensitivity to glucose catabolite repression (1a). The structural gene is shared by L-fucose, L-rhamnose, or other inducing carbon sources, such as glutamate or ethylene glycol (1a).

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