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L-1,2-Propanediol Exits More Rapidly Than L-Lactaldehyde from *Escherichia coli*

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Catabolism of the six-carbon compound L-fucose results in formation of dihydroxyacetone phosphate (C-1-to-C-3 fragment) and L-lactaldehyde (C-4-to-C-6 fragment) as intermediates. The fate of lactaldehyde depends on the respiratory growth conditions. Aerobically, lactaldehyde is oxidized to L-lactate by an NAD-linked dehydrogenase (*ald* product). L-Lactate, in turn, is converted to pyruvate, which enters the general metabolic pool. Anaerobically, lactaldehyde is reduced to L-1,2-propanediol by an NADH-linked oxidoreductase (*fucO* product). L-1,2-Propanediol is excreted as a terminal fermentation product. In a previous study, we showed that retention of the C-4-to-C-6 fragment of fucose depended on the competition for lactaldehyde by aldehyde dehydrogenase and propanediol oxidoreductase (Y. Zhu and E. C. C. Lin, J. Bacteriol. 169:785–789, 1987). In this study, we compared the wild-type strain and isogenic mutant strains defective in *ald*, *fucO*, or both for ability to accumulate radioactivity when incubated with fucose labeled at either the C-1 or the C-6 position. The results showed that although blocking the oxidation of lactaldehyde prevented its assimilation, rapid exit of the 3-carbon unit occurred only when the compound was reduced to propanediol. Moreover, growth experiments on fucose indicated that a double *ald fucO* mutant accumulated inhibiting concentrations of lactaldehyde. The inner cell membrane therefore appears to be much more permeable to the 3-carbon alcohol than to the 3-carbon aldehyde. The almost instantaneous exit of propanediol appears to be a facilitated process.

Because of methodological difficulties, there have been few studies on the exit process of specific end products of fermentation. The dissimilatory pathway for L-fucose in *Escherichia coli* offers an unusual opportunity for comparing the exit rate of a terminal fermentation product with that of its immediate precursor (Fig. 1). The pathway branches after the aldolase cleaves the intermediate, fuculose 1-phosphate, into dihydroxyacetone phosphate and L-lactaldehyde. Under aerobic conditions, the aldehyde is oxidized irreversibly to L-lactate by an NAD-linked dehydrogenase which is encoded by *ald*. L-Lactate then enters the general metabolic pool by being converted to pyruvate. Under anaerobic conditions, the aldehyde is reduced to L-1,2-propanediol by an NADH-linked oxidoreductase which is encoded by *fucO*. The propanediol is then excreted into the medium as a terminal fermentation product. During aerobic growth, the cell minimizes the reduction of lactaldehyde by synthesizing propanediol oxidoreductase (or modifying it posttranslationally) as molecules with low enzymatic activity or as molecules mostly in a form without catalytic activity. During anaerobic growth, the oxidation of lactaldehyde is prevented by the absence of aldehyde dehydrogenase activity as well as the lack of necessary electron acceptors. The *fucO* gene at min 60 of the chromosome clusters with the rest of the genes that specify the fucose pathway. The *ald* gene maps at min 31 (3; for a review, see reference 8).

In a previous study, we monitored the exit of the methyl half of the fucose molecule by the percentage of radioactivity retained when the cells were incubated with the sugar labeled at carbon 6. Fucose input into the cell was estimated by the radioactivity retained when the cells were incubated with the sugar labeled at carbon 1, since this carbon goes to dihydroxyacetone phosphate, which should not escape from the cell before extensive metabolism. The results indicated that effective retention of the methyl half of fucose was contingent on its conversion to the anion L-lactate by aldehyde dehydrogenase and that this fixation process was undermined by the presence of propanediol oxidoreductase activity (20). In the study reported here, we obtained more direct evidence that propanediol, but not lactaldehyde, escapes with striking rapidity from the cell despite the similarities of the compounds in size and structure.

MATERIALS AND METHODS

Chemicals. L-Lactaldehyde, synthesized by the reaction of ninhydrin with D-threonine (18), was provided by Y.-M. Chen. DL-1,2-Propanediol and L-fucose were purchased from Sigma Chemical Co., St. Louis, Mo. L-[6-^{3}H]fucose (specific activity, 27 Ci/mmol) was purchased from ICN Pharmaceuticals Inc., Irvine, Calif. L-[1-^{14}C]fucose (specific activity, 59.2 mCi/mmol) was obtained from Dupont, NEN Research Products, Boston, Mass. Vitamin-free casein acid hydrolysate was obtained from ICN Nutritional Biochemicals, Cleveland, Ohio. All other reagents used were commercial products of the highest grade available.

Bacterial strains. Bacterial strains are listed in Table 1. To construct isogenic strains with different combinations of the *ald* and *fucO* alleles, a bacteriophage P1 lysate of strain ECL471 (*ald-1 zdb-1*::Tn*10*) with the transposon 60% linked to the *ald* mutation was used as the donor to transduce strain ECL116 (*fucO*$^+$) or ECL326 (Φ[*fucO-lac*]). Tc^r transductants were screened on minimal agar supplemented with either 0.04% L-fucose or 0.2% L-rhamnose. (complete aerobic utilization of rhamnose also requires aldehyde dehydrogenase [3]). On both sugars, the *ald*$^+$ transductants (e.g., strains ECL732 and ECL733, derived transductionally from

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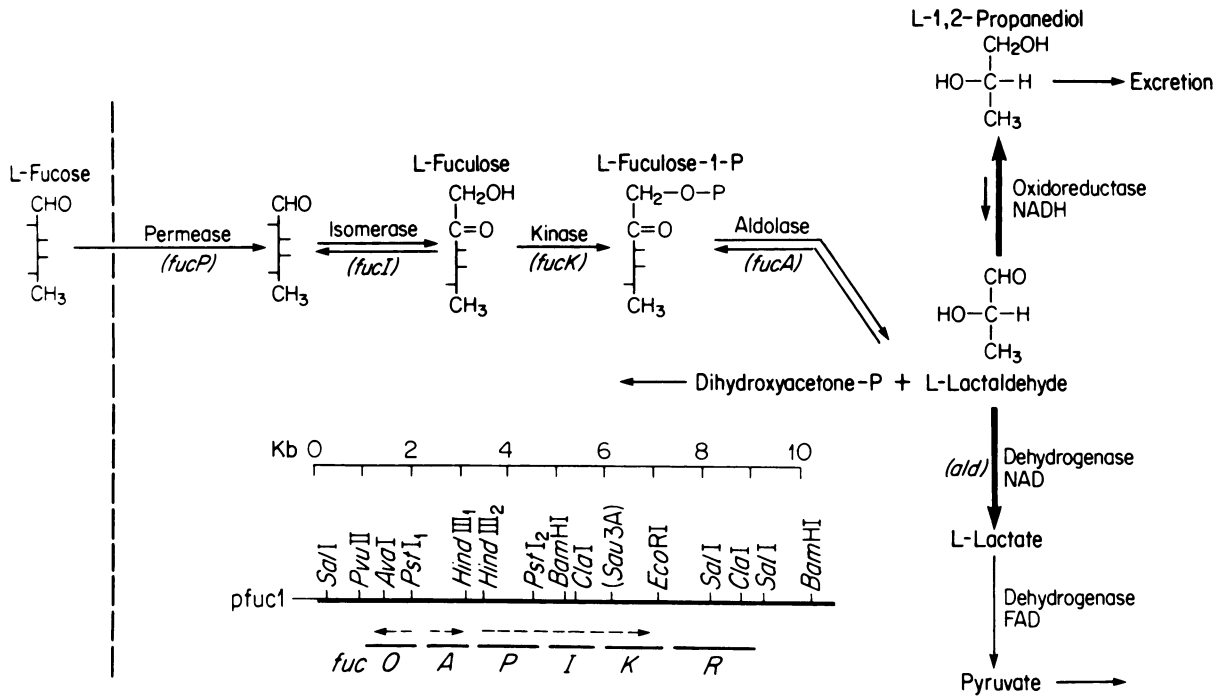

FIG. 1. Catabolic pathway of L-fucose and the genetic map of the *fuc* regulon in *E. coli*. kb, Kilobases; FAD, flavin adenine, dinucleotide.

strain ECL116, and strains ECL734 and ECL735, derived from strain ECL326) gave colonies of normal size, whereas *ald* transductants (e.g., strains ECL736 and ECL737, derived from strain ECL116, and ECL738 and ECL739, derived from strain ECL326) gave colonies with diminished size.

Growth and preparation of cell extracts. For enzyme assays and measurements of fucose uptake, cells were grown in mineral medium supplied with 0.2% fucose and 0.5% vitamin-free casein acid hydrolysate. Aerobic or anaerobic growth was carried out at 37°C, and cell extracts were prepared as previously described (20).

Transport and enzyme assays. Assays of retention of radioactivity by cells incubated with L-fucose labeled at either carbon 1 (1-^{14}C) or carbon 6 (6-^{3}H) and of the activity levels of fucose isomerase, propanediol oxidoreductase, and aldehyde dehydrogenase were performed according to published methods (19, 20). Protein concentration was determined with bovine serum albumin as the standard (9).

Assay of propanediol and lactaldehyde in culture media. Growth of cells in mineral medium supplied with 5 mM fucose was monitored by measuring the increase in Klett units (no. 42 filter). The cells were removed by centrifugation, and the supernatant fraction was passed through a membrane filter (pore diameter, 0.45 μm; Millipore Corp., Bedford, Mass.).

For gas chromatographic analysis of 1,2-propanediol, 0.9 ml of the filtrate was acidified with 0.1 ml of 6 N HCl, and 5 μl of the acidified mixture was injected into a 5809A gas chromatography unit (Hewlett-Packard Co., Palo Alto, Calif.) with a Hewlett-Packard silar 10C column operated from 140 to 190°C, with the temperature raised at 10°C/min. The injector port was at 250°C, and the detector was at 280°C. A Hewlett-Packard 3392A integrator was connected for data processing.

For chemical analysis of 1,2-propanediol by sodium bisulfite (7), 0.1 ml of the medium was mixed with 0.5 ml of concentrated H_2SO_4 at 70°C for 10 min and cooled to room temperature. A 0.02-ml volume of 5% $NaHSO_4$ in 3% ninhydrin was added to the reaction mixture, and incubation continued for 1 h. Samples were then diluted with 1.25 ml of concentration H_2SO_4, and the optical density at 595 nm was read after 5 min.

TABLE 1. Bacterial strains

Strain	Derivation	Genotype or phenotype	Source or reference
ECL116	YMC9	F⁻ Δ*lacU169 thi endA hsdR*	B. Magasanik
ECL326	ECL116	Δ[*fucO-lac*::λ*p1*(*209*)]	2
ECL471	ECL40	*ald-1 zdb-1*::Tn*10*	3
ECL732	ECL116	*ald*⁺ *zdb-1*::Tn*10*	This study
ECL733	ECL116	*ald*⁺ *zdb-1*::Tn*10*	This study
ECL734	ECL326	*ald*⁺ *zdb-1*::Tn*10* Φ(*fucO-lac*)	This study
ECL735	ECL326	*ald*⁺ *zdb-1*::Tn*10* Φ(*fucO-lac*)	This study
ECL736	ECL116	*ald-1 zdb-1*::Tn*10*	This study
ECL737	ECL116	*ald-1 zdb-1*::Tn*10*	This study
ECL738	ECL326	*ald-1 zdb-1*::Tn*10* Φ(*fucO-lac*)	This study
ECL739	ECL326	*ald-1 zdb-1*::Tn*10* Φ(*fucO-lac*)	This study

RESULTS

Construction and characterization of isogenic *ald* and *fucO* mutations. The genotypes of the strains constructed for the study of growth on fucose and metabolite excretion were verified by the enzymatic patterns of the strains (Table 2). As expected, aldehyde dehydrogenase activity in the wild-type strains increased with oxygenation of the growth medium,

TABLE 2. Specific enzyme activities of strains bearing different alleles of *ald* and *fucO* grown under aerobic or anaerobic conditions[a]

Strain	Genotype	Sp act[b]					
		$+O_2$			$-O_2$		
		ISO	ALD	POR	ISO	ALD	POR
ECL732	*ald*$^+$ *fucO*$^+$	2,700	55	150	4,200	0	500
ECL733	*ald*$^+$ *fucO*$^+$	3,100	53	100	4,000	0	490
ECL734	*ald*$^+$ *fucO*	2,100	44	9	5,900	3	0
ECL735	*ald*$^+$ *fucO*	2,200	66	0	5,000	1	0
ECL736	*ald fucO*$^+$	2,500	0	75	4,200	0	500
ECL737	*ald fucO*$^+$	2,700	0	100	3,400	0	470
ECL738	*ald fucO*	2,800	2	0	4,900	0	0
ECL739	*ald fucO*	2,700	0	0	5,200	0	2

[a] All cells were grown on fucose plus vitamin-free casein acid hydrolysate. Abbreviations: ISO, fucose isomerase; ALD, aldehyde dehydrogenase; POR, propanediol oxidoreductase.

[b] Expressed as nanomoles per minute per milligram of protein at 25°C.

TABLE 3. Effects of *ald* and *fucO* mutations on retention of the methyl fragment of fucose[a]

Strain	Genotype	Radioactivity retained[b] after incubation with:		Avg 6-^{3}H/1-^{14}C of paired strains
		[6-^{3}H]fucose	[1-^{14}C]fucose[c]	
ECL732	*ald*$^+$ *fucO*$^+$	40	60	
ECL733	*ald*$^+$ *fucO*$^+$	34	67	0.59
ECL734	*ald*$^+$ *fucO*	77	58	
ECL735	*ald*$^+$ *fucO*	74	57	1.30
ECL736	*ald fucO*$^+$	8	65	
ECL737	*ald fucO*$^+$	7	73	0.11
ECL738	*ald fucO*	57	81	
ECL739	*ald fucO*	62	84	0.72

[a] Cells grown aerobically on focuse were incubated with 10 μM labeled substrate at 32°C. Radioactivity accumulated by the cells was calculated by subtracting the zero-time value from the value obtained at 1 min.

[b] Expressed as nanomoles of L-fucose equivalent retained per milligram (dry weight) of cells.

[c] Retention average of all strains was 68 ± 10 (standard deviation). Theoretically, values for the retention of carbon-1-labeled fucose should be the same. Factors contributing to the variations are obscure.

whereas the opposite change occurred in propanediol oxidoreductase activity.

It should be noted, however, that under the aerobic conditions used, appreciable levels of propanediol oxidoreductase activity in the cells (about one-fourth the levels found in anaerobically grown cells) were present. Although we suspected that the higher than expected basal activity of propanediol oxidoreductase resulted from insufficiency of the oxygen tension in the culture medium shortly before harvest, a test showed that this was not true. When wild-type cells were grown in 200-ml cultures agitated in 2-liter flasks with triple baffles on a rotatory platform operated at 250 rpm and harvested at about 90 Klett units, extracts of duplicate experiments gave specific activities of propanediol oxidoreductase of close to 150 U.

In contrast, mutants defective in *fucO* showed no significant propanediol oxidoreductase activity regardless of whether the mutants were grown aerobically or anaerobically, although aldehyde dehydrogenase and fucose isomerase activity levels were normal. Likewise, there was no aldehyde dehydrogenase activity in mutants defective in *ald*, but the activity patterns of the two other enzymes were normal. In mutants defective in both *ald* and *fucO*, only the isomerase activity remained.

Effects of *ald* and *fucO* mutations on retention of the methyl fragment of fucose. Using the isogenic strains described above, we measured retention of the C-4-to-C-6 fragment of fucose by determining the accumulation of radioactivity when the cells were incubated with the compound labeled at carbon 6. We used the C-1-labeled compound as an indicator for fucose uptake, since mutations in *ald* or *fucO* are not expected to affect significantly the metabolism of the derived dehydroxyacetone phosphate. The C-6/C-1 retention ratio should therefore indicate the portion of the methyl fragment kept by the cells.

When wild-type strains (*ald*$^+$ *fucO*$^+$) were incubated with fucose labeled at different positions for 1 min at 32°C, the retention ratio was 0.59 (Table 3). The partial loss of the methyl fragment probably resulted from the reduction of lactaldehyde to propanediol by the low level of oxidoreductase activity (Table 2). In the *ald*$^+$ *fucO* mutants, the retention ratio increased to 1.3. In principle, the maximal value should not exceed 1.0. A ratio in excess of unity would indicate that during the short incubation period there was actually a smaller loss of carbon 6 than of carbon 1 (either as carbon dioxide or as other end products). In *ald fucO*$^+$ mutants, the retention ratio was lowered from the wild-type value of 0.59 to 0.11. This drop apparently was caused by the failure to trap lactaldehyde as lactate, which allowed almost all of the 3-carbon fragment to escape as propanediol. In *ald fucO* double mutants, lactaldehyde should be neither oxidized nor reduced. The retention ratio was 0.72. Comparison of this value with the retention ratio of 1.3 exhibited by *ald*$^+$ *fucO* strains indicated that there was a significant rate of escape of lactaldehyde from the *ald fucO* cells. More important, comparison of the value 0.72 with the retention ratio of 0.11 exhibited by the *ald fucO*$^+$ strains indicates that the reduction of lactaldehyde to propanediol allowed a much more rapid escape rate of the 3-carbon fragment. It should be emphasized that the sixfold difference in the retention ratio between the *ald fucO*$^+$ and *ald fucO* strains is much greater than the standard deviations observed in the amount of carbon-1 retained by the various strains used (see footnote *c* of Table 3).

Aerobic growth of different strains on fucose. The effects of *ald* and *fucO* mutations on aerobic growth were examined with 5 mM fucose as the sole source of carbon and energy. Wild-type strains (*ald*$^+$ *fucO*$^+$) showed a doubling time of 1.4 h; by 12 h the carbon and energy source was exhausted, giving a growth yield of 0.19 U (Table 4). The growth properties of the *ald*$^+$ *fucO* strains were similar to those of the wild-type strains, although a higher than normal final yield would be expected on the basis of diminished excretion of propanediol. The *ald fucO*$^+$ strains grew with an increased doubling time of 2.8 h and gave half the growth yield of the wild-type strains. Since only one half of the fucose molecule could be utilized by *ald* mutants, the decreased growth yield was predictable. The lengthened doubling time would indicate that the input rate of the carbon and energy source became limiting when half of the material was wasted. The *ald fucO* double mutants were highly impaired in their growth rates, with a doubling time of about 8 h. Stationary phase was reached after 24 h, giving a full potential yield of 0.09 U (data not shown). The aggravated growth retardation was probably caused by blockage of both the oxidation and reduction of lactaldehyde, resulting in toxic accumulation. (For instance, the compound might attack proteins by forming Schiff's bases.)

Excretion of 1,2-propanediol into medium with fucose as the

TABLE 4. Propanediol accumulation in medium after aerobic growth of different strains on fucose

Strain	Genotype	Growth on fucose[a]		Propanediol accumulated in medium at 12 h (mM)
		Doubling time (h)	Yield at 12 h[b]	
ECL732	*ald*$^+$ *fucO*$^+$	1.4	0.19	1.3
ECL733	*ald*$^+$ *fucO*$^+$	1.4	0.19	1.6
ECL734	*ald*$^+$ *fucO*	1.6	0.19	0.1
ECL735	*ald*$^+$ *fucO*	1.6	0.19	0.3
ECL736	*ald fucO*$^+$	2.8	0.10	4.7
ECL737	*ald fucO*$^+$	2.8	0.10	4.8
ECL738	*ald fucO*	8.0	0.07	0.6
ECL739	*ald fucO*	8.0	0.07	0.6

[a] Cells were grown aerobically on 5 mM fucose.

[b] Expressed as milligrams (dry weight) of cells per milliliter of culture (100 Klett units = 0.17 mg [dry weight] of cells [21]). All cultures reached stationary phase at 12 h except those of the *ald fucO* strains, which reached the full yield of 0.09 after 24 h.

carbon and energy source. The data in Table 4 on propanediol excreted into the medium by cells grown aerobically on fucose were obtained from gas chromatographic analysis. The standard 1,2-propanediol eluted as a distinct peak at 3.3 min after injection of the sample (Fig. 2A). The area under the peak was proportional to the concentration of the compound in the sample at least up to 5 mM (Fig. 2A, insert). When the medium of a fully grown culture of strain ECL736 (*ald fucO*$^+$) was tested, a peak with an elution time similar to that of the standard propanediol was observed (Fig. 2B). To confirm that the unknown peak represented propanediol, an equal-volume mixture of the culture medium and a solution of 5 mM propanediol was tested, and a single peak was observed (Fig. 2C).

The wild-type strains, in which the fully induced aldehyde dehydrogenase and the slightly induced propanediol oxidoreductase competed for lactaldehyde, excreted about 1.5 mM propanediol after full growth on 5 mM fucose (Table 4). The excretion of propanediol by strains lacking propanediol oxidoreductase (*ald*$^+$ *fucO*) was reduced to about 0.2 mM. In contrast, propanediol excretion by strains lacking aldehyde dehydrogenase but possessing propanediol oxidoreductase (*ald fucO*$^+$) approached the maximal expected concentration of 5 mM. Propanediol excretion by strains lacking both enzymes (*ald fucO*), in which propanediol should be neither oxidized nor reduced, was expected not to exceed the value of 0.2 mM observed with *ald*$^+$ *fucO* strains. The actual excretion, however, reached 0.6 mM. Perhaps when the intracellular concentration of lactaldehyde became high, its nonspecific reduction by an unknown enzyme occurred. Analysis of 1,2-propanediol by the chemical method gave qualitatively similar results, although higher base levels were obtained. For example, an average of 1.3 mM was observed with cultures of the *ald*$^+$ *fucO* strains. In contrast, an average value of 4 mM was observed with the *ald fucO* strains.

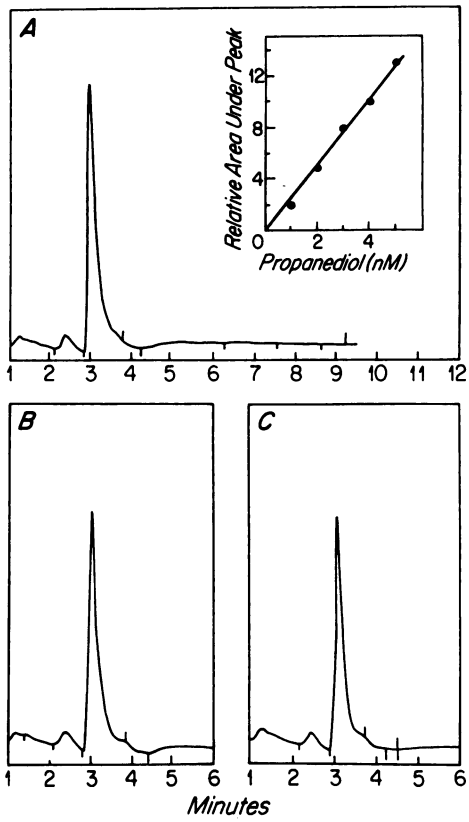

FIG. 2. Determination by gas chromatography of 1,2-propanediol excreted into the medium of fucose-grown cultures. (A) Elution time of standard 1,2-propanediol (5 mM). Insert shows standard curve based on the area under the peaks as a function of propanediol concentration. (B) Eluant from the medium after full growth of strain ECL736 on fucose. (C) Eluant from a mixture of equal volumes of the medium and a 5 mM standard.

DISCUSSION

In contrast to the extensive knowledge acquired on the numerous systems for the uptake of nutrient molecules, relatively little attention has been paid to the question of how metabolic end products exit from the cell. Since the outer cell membrane contains a large number of porins that provide diffusion channels for molecules with a cutoff size in the vicinity of 600 daltons (for review, see reference 10), the exit rates of fermentation products are almost certainly controlled by the inner cell membrane. A combination of three circumstances allowed us to compare the relative permeability of this membrane with that of two similar 3-carbon compounds which are nonionic: the fact that an intermediate in fucose catabolism, lactaldehyde, can be either oxidized or reduced; the successful construction of isogenic mutants blocked in one or both of the redox reactions; and the availability of fucose labeled at either the C-1 or the C-6 position. Our results showed that the plasma membrane is considerably more permeable to propanediol than to lactaldehyde.

Effective retention of lactaldehyde is biologically important, since the compound could serve the cell either aerobically as a carbon and energy source or anaerobically as a hydrogen sink. On the other hand, speedy excretion of propanediol is probably also vital. On the basis of hydrophobicity, an aldehydic compound should be able to diffuse more readily than its alcoholic counterpart across the phospholipid membrane (for a rough comparison, it might be noted that whereas at 20°C *n*-propyl alcohol is miscible with water, the solution of propylaldehyde requires 5 volumes of water [17]). Moreover, there was no indication of the existence of a sequestered pool of lactaldehyde in the cytoplasm, since practically all of the compound was subject to instant reduction by propanediol oxidoreductase. It is therefore tempting to postulate that a special mechanism, such as

facilitated diffusion, selectively accelerated the discharge of propanediol. In the absence of this mechanism, a deleteriously elevated intracellular concentration of the compound might be required to provide an adequate concentration gradient for steady-state release of the waste product by nonspecific diffusion. For instance, propanediol was observed to inhibit fuculose kinase activity in vitro (unpublished data of T. T. Wu, quoted in reference 5).

What appeared to be facilitated diffusion of propanediol was also encountered in a study of mutants that acquired the ability to grow aerobically on the compound as the sole carbon and energy source (5, 16). Two important changes made this growth property possible. First, the *fucO* gene encoding propanediol oxidoreductase became constitutively expressed. Second, the enzyme synthesized under aerobic conditions became fully active catalytically. Thus, in the propanediol-positive mutant, an enzyme that normally catalyzed a terminal reaction for a specific fermentation pathway was genetically converted to an enzyme that initiated an aerobic dissimilatory pathway. After the propanediol oxidoreductase activity was abolished in these mutants by a further mutation, the mutants became incapable of accumulating labeled propanediol. Consequently, it was concluded that propanediol was not actively transported against a concentration gradient in the acquired dissimilatory pathway. A clue to the nature of the entry process was provided by a transport inhibition experiment. If the cells capable of growth on propanediol were appropriately induced with a substrate of the phosphoenolpyruvate:phosphotransferase system (PTS), propanediol uptake was inhibited in the presence of the inducing PTS compound. This kind of exclusion (a control mechanism evolved for the preferential utilization of carbon and energy source) usually indicates inhibition at the plasma membrane barrier (for review, see reference 12). There is only one known case in which the hindrance of utilization of the less-favored substrate occurs at the level of an intracellular enzyme. Glycerol enters the cell by facilitated diffusion (6, 14, 15), and inhibition of utilization of this compound by a PTS sugar operates at the level of glycerol kinase (4, 11, 13). In propanediol-positive mutants, however, substrate capture is not likely to be regulated at the level of the oxidoreductase, because the enzyme evolved naturally for end product formation and not for substrate scavenging. No obvious selective advantage would accrue from the kinetic control of a terminal fermentation enzyme by a PTS substrate. The inference that propanediol enters by a mediated process based on the inhibition studies therefore seems valid.

Thus, in studies of both the exit and the entry of propanediol, there is circumstantial evidence for facilitated diffusion of the compound. The exit process is a normal physiological process, whereas the entry process occurs in mutants selected in the laboratory. Whether the same mechanism is accountable for the solute movement remains to be clarified.

Our finding that *E. coli* cells lacking aldehyde dehydrogenase activity excrete half of the carbon atoms derived from fucose as propanediol even under aerobic conditions coincides with results of an independent study of the terminal metabolism of rhamnose by *Salmonella typhimurium* LT2 (ATCC 23564). This strain is a spontaneous *ald* mutant. When grown on 10 mM rhamnose under aeration, equimolar propanediol was recovered in the final growth medium (1). Although the propanediol oxidoreductase was not assayed in that study, it is highly probable that a significant level of the activity was also present despite the aerobic growth conditions. It is known that the lactaldehyde molecules derived from fucose and rhamnose are metabolized by the same set of enzymes (8).

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