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Coenzyme B_{12}-Dependent Diol Dehydratase: Regulation of Apoenzyme Synthesis in *Klebsiella pneumoniae* (*Aerobacter aerogenes*) ATCC 8724†

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Immunochemical studies demonstrated that *Klebsiella pneumoniae* (*Aerobacter aerogenes*) ATCC 8724 produces only a single diol dehydratase whether grown on glycerol or on 1,2-propanediol. The enzyme was subject to induction by 1,2-diols and to catabolite repression reversed by cyclic AMP.

Diol dehydratase (DL-1,2-propanediol hydrolyase, EC 4.2.1.28) of *Klebsiella pneumoniae* ATCC 8724 (formerly known as *Aerobacter aerogenes*) is a coenzyme B_{12} (adenosylcobalamin or Coα-[α-(5,6-dimethylbenzimidazolyl)]-Coβ-adenosylcobamide)-requiring enzyme which catalyzes the conversion of 1,2-propanediol, 1,2-ethanediol, and glycerol to propionaldehyde, acetaldehyde, and β-hydroxypropionaldehyde, respectively (7, 15). Although the mechanism of action of the enzyme has been extensively studied (1, 2), little attention has been paid to its regulation and physiological role in microorganisms. The presence of coenzyme B_{12}-dependent glycerol dehydratase, which also catalyzes the dehydrations of glycerol, 1,2-propanediol, and 1,2-ethanediol, was demonstrated in vitro with *Lactobacillus* (13) and with *K. pneumoniae* ATCC 25955 (formerly known as *A. aerogenes* PZH 572, Warsaw) (10, 12). Recently, we have reported that *K. pneumoniae* ATCC 25955 produces diol dehydratase and glycerol dehydratase in a 1,2-propanediol medium and in a glycerol medium, respectively, and that these enzymes are distinguishable in antigenic properties, in monovalent cation selectivity pattern, and in substrate specificity (14). Thus, diol dehydratase and glycerol dehydratase may be considered as isozymes in that organism. The present communication provides immunochemical and kinetic evidence that *K. pneumoniae* ATCC 8724 produces a single diol dehydratase, whether grown on 1,2-diols or on glycerol, and describes factors influencing the enzyme's synthesis, with special emphasis on its regulation by catabolite repression and cyclic AMP.

K. pneumoniae ATCC 8724 was grown at 37°C either anaerobically under He or aerobically on a rotary shaker (200 rpm) in complex media containing 5.4 g of KH_2PO_4, 1.2 g of $(NH_4)_2SO_4$, 0.4 g of $MgSO_4 \cdot 7H_2O$, 2.0 g of yeast extract, 2.0 g of tryptone, and appropriate growth substrate(s) in 1 liter of tap water. The medium was adjusted to pH 7.1 with KOH. The diol dehydratase activity was assayed with 1,2-propanediol by either the 2,4-dinitrophenylhydrazine method of Lee and Abeles (7) or the 3-methyl-2-benzothiazolinone hydrazone method of Toraya et al. (16). One unit is defined as the amount of enzyme activity catalyzing the formation of 1 μmol of propionaldehyde per min under the standard assay conditions. A homogeneous preparation of apodiol dehydratase was obtained from the cells grown on glycerol plus 1,2-propanediol (manuscript in preparation). Immunochemical titration and Ouchterlony double diffusion analysis were performed using rabbit antiserum against diol dehydratase of *K. pneumoniae* ATCC 8724, as described before (14).

Table 1 summarizes effects of growth conditions on the level of diol dehydratase activity in *K. pneumoniae* ATCC 8724. Cells grown in medium containing 1,2-propanediol (or 1,2-ethanediol) showed the highest activity. The glycerol-grown cells also exhibited activity, but to a much lesser degree. When the organism was cultivated in a glucose plus 1,2-propanediol medium, the level of enzyme activity remained very low until the glucose in the medium had been consumed. These results indicate that glucose is preferentially utilized by the bacterium, and that the diol dehydratase synthesis is regulated by glucose effects.

With all active extracts, glycerol dehydration and concomitant inactivation of the enzyme re-

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TABLE 1. *Effects of growth conditions on the level of diol dehydratase activity*

Cultivation condition	Growth substrate[a]	Cultivation time (h)	Growth (g of dry cells per liter)	Diol dehydratase activity[b]	G/P ratio[c]
Anaerobic	None	17.5	0.07	0.00	
	1,2-Propanediol	20	0.19	1.88	0.5–0.8
	1,2-Ethanediol	20	0.14	2.06	
	Glycerol	4.5	0.15	0.39	0.5–0.8
	Glycerol	8	0.44	0.21	0.5–0.8
	Citrate	17.5	0.47	0.00	
	Glucose	8.5	0.55	0.00	
	Glycerol + 1,2-propanediol[d]	20	0.47	1.17	0.5–0.8
	Citrate + 1,2-propanediol[d]	16.5	0.36	0.48	0.5–0.8
	Glucose + 1,2-propanediol[d]	14	0.47	<0.01	
	Glycerol + 1,2-ethanediol[d]	20	0.40	0.16	
Anaerobic (+KNO_3[e])	Glycerol + 1,2-propanediol[d]	23	0.63	0.79	
Aerobic	1,2-Propanediol	17	0.68	0.14	
	Glycerol + 1,2-propanediol[d]	7	1.01	0.46	
	Glycerol + 1,2-propanediol[d]	18	2.01	0.71	
	Glucose (3%) + 1,2-propanediol[d]	5	0.76[f]	<0.01	
	Glucose (3%) + 1,2-propanediol[d]	14	2.14[f]	0.22	

[a] Glycerol-grown cells were washed twice with 0.9% KCl and inoculated into fresh media at an initial concentration of about 0.002 g of dry cells per liter. Unless otherwise indicated, the growth substrate was added to a concentration of 0.3 g-atom of carbon per liter of medium.

[b] Specific activity: micromoles of propionaldehyde formed per minute per milligram of protein.

[c] The ratio of glycerol-dehydrating activity to 1,2-propanediol-dehydrating activity measured by the 1-min assay (14).

[d] The concentration of 1,2-propanediol and of 1,2-ethanediol was 0.075 M.

[e] The concentration of KNO_3 was 10 g/liter.

[f] The concentration of glucose remaining was 1.66% after 5 h and 0% after 14 h when determined as described by Horikoshi (6).

sulted in almost complete cessation of the dehydration reaction within about 3 min, as observed with purified diol dehydratase (15). On the other hand, the rate of 1,2-propanediol dehydration with the extracts was linear for at least 20 min. The glycerol-dehydrating activity/1,2-propanediol-dehydrating activity ratio measured by the 1-min assay was in the range of 0.5 to 0.8 with all the active extracts tested, which is close to that obtained with homogeneous diol dehydratase (14). Figure 1 shows Ouchterlony double-diffusion patterns of anti-diol dehydratase antiserum with crude extracts of cells grown on 1,2-propanediol, on glycerol, and on glycerol plus 1,2-propanediol together with a purified enzyme preparation. Antiserum in the center well reacted with all of the extracts, forming the continuous and fused precipitin band with the band between antiserum and the purified enzyme. This result indicates not only that the enzyme preparation employed as antigen was homogeneous, but also that the antigen identical with diol dehydratase is present in all the extracts tested. Glucose-grown and citrate-grown cell extracts did not form any precipitin line with antiserum (data not shown). Furthermore, upon immunochemical titration both the 1,2-propanediol-dehydrating activity and the glycerol-dehydrating activity were completely precipitated by antiserum, and with all three extracts the amount of antiserum required to precipitate 1 unit of activity was in the range of 10 to 12 μl, which is in good agreement with the equivalence point with purified diol dehydratase (14). Thus, it seems clear that 1,2-propanediol and glycerol induce the same enzyme, diol dehydratase, although the latter is a much poorer inducer.

The addition of 1,2-propanediol to the growing culture (glycerol medium) brought about a marked increase in the enzyme activity, and the increase was completely inhibited by chloramphenicol (100 μg/ml of medium). This result, together with the lack of any cross-reactive proteins in the inactive extracts, indicates that the increase in diol dehydratase activity is not attributable to conversion of an inactive form of enzyme into an active form but rather to a de novo protein synthesis. Table 2 shows that the diol dehydratase synthesis was strongly repressed by glucose. Galactose and fructose also partially suppressed the enzyme induction by 1,2-propanediol. These data imply that the synthesis of diol dehydratase is subject to catabolite

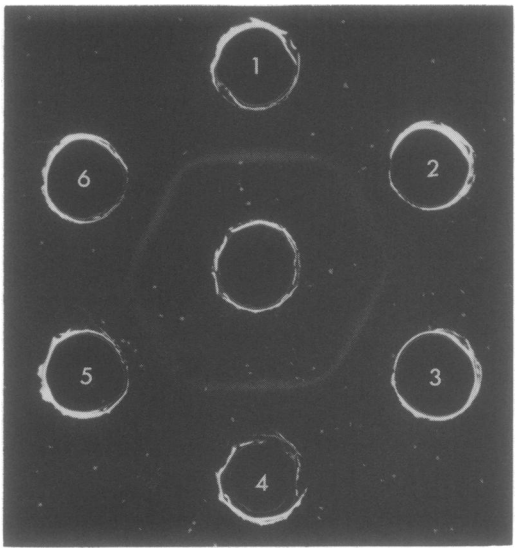

FIG. 1. *Ouchterlony double-diffusion pattern of anti-diol dehydratase antiserum with crude extracts of the cells grown on various growth substrates. Each well was filled with 44 μl of a protein solution containing 0.05 M potassium phosphate buffer (pH 8.0) and 10% glycerol. Center well, 22 μl of rabbit antiserum against diol dehydratase: well 1, 0.60 U of purified diol dehydratase; wells 2 and 5, crude extracts (0.39 mg of protein and 0.14 U of activity) of cells grown on glycerol (middle exponential phase); wells 3 and 6, crude extracts (0.19 mg of protein and 0.35 U of activity) of cells grown on 1,2-propanediol; well 4, crude extracts (0.46 mg of protein and 0.43 U of activity) of cells grown on glycerol plus 1,2-propanediol.*

repression. As expected, the glucose repression of the enzyme was overcome by cyclic AMP. The fact that both an inducer (i.e., 1,2-propanediol) and cyclic AMP are necessary for the diol dehydratase synthesis can be accounted for by the current model for the role of cyclic AMP in mediation of catabolite effects on enzyme induction (3, 4, 8, 9, 17). Furthermore, glycerol proved to be a much less effective inducer than 1,2-propanediol even when tested in the presence of cyclic AMP added exogenously (Table 2). Therefore, it is unlikely that the poorer effectiveness of glycerol as an inducer is due to its effect on cyclic AMP levels.

As depicted in Fig. 2, the transfer of the induced cells to a fresh glucose medium did not result in any significant change in the total enzyme activity in the culture, as compared with the control culture without glucose, indicating that glucose does not affect the rate of degradation of the already existing enzyme. Thus, it is unlikely that diol dehydratase undergoes catabolite inactivation (5).

From the data presented in this paper, it is evident that diol dehydratase is inducible by 1,2-propanediol (or 1,2-ethanediol), and that its synthesis is regulated by catabolite repression reversed by cyclic AMP. Glycerol is also effective as an inducer for the enzyme, but to a much lesser extent. It seems reasonable to assume that in *K. pneumoniae* ATCC 8724 diol dehydratase is implicated in the fermentation of 1,2-propanediol, 1,2-ethanediol, and glycerol, and that glucose and some other sugars are used preferentially to those substrates by the bacterium.

TABLE 2. *Effects of various sugars and cyclic AMP on diol dehydratase induction by 1,2-propanediol or glycerol*

Run no.	Medium[a]	Sp act (U/mg)					
		Zero time[b] (control)	After 1 h		After 3 h		
			Without inducer	With propanediol[b]	Without inducer	With propanediol[b]	With glycerol[b]
1	Glycerol	0.17	0.14	0.95			
	Glucose	0.00	0.00	0.03			
	Galactose	0.00	0.00	0.27			
	Fructose	0.00	0.00	0.64			
	Mannose	0.00	0.00	0.84			
2	Glucose	0.00	0.00	0.00			
	Glucose (+cAMP)[c]		0.00	0.50			
3	Citrate	0.00	0.00		0.00		
	Citrate (+cAMP)[c]		0.00		0.00	0.47 (22.3)[d]	0.15 (6.9)[d]

[a] Each carbon source, 0.05 M. Glycerol-grown cells were washed twice with 0.9% KCl and inoculated into fresh media at an initial concentration of about 0.002 g of dry cells per liter.

[b] An inducer (0.1 M 1,2-propanediol or 0.1 M glycerol) was added after 4.5 h of growth in runs no. 1 and 2 and after 10.5 h of growth in run no. 3 (zero time).

[c] Cyclic AMP (2 mM) was added together with an inducer.

[d] Total activity (units per 500-ml culture) in parentheses.

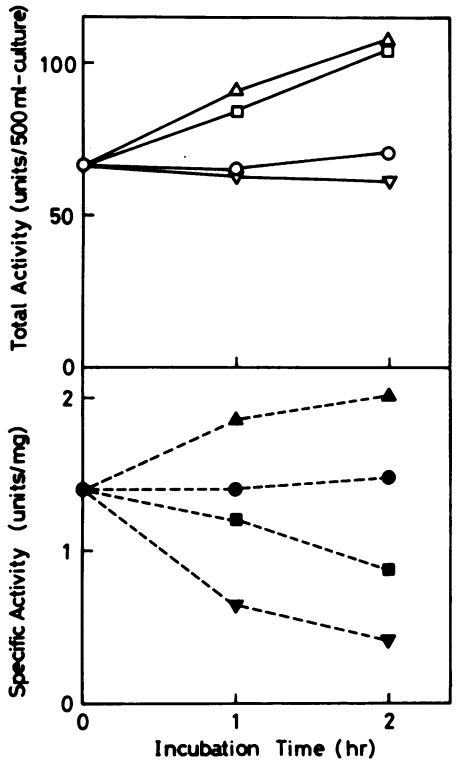

FIG. 2. *Effects of various compounds on changes in the apoenzyme level of the induced cells. The induced cells, grown on 0.1 M glycerol plus 0.075 M 1,2-propanediol for 13 h, were washed twice with 0.05 M potassium phosphate buffer (pH 8.0) and then transferred to fresh medium to approximately the same concentration of cells as that obtained after 13 h of growth in a glycerol-propanediol medium. The total and specific activities of diol dehydratase were determined after 1 and 2 h. Additions to fresh media:* (○) *and* (●) *None;* (△) *and* (▲) *1,2-propanediol (0.1 M);* (□) *and* (■) *glycerol (0.1 M);* (▽) *and* (▼) *glucose (0.05 M).*

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