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1,3-Propanediol:NAD$^+$ Oxidoreductases of *Lactobacillus brevis* and *Lactobacillus buchneri*

MARIA VEIGA-DA-CUNHA†* AND MICHAEL A. FOSTER

Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom

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In the cofermentation of glycerol with a sugar by *Lactobacillus brevis* and *Lactobacillus buchneri*, a 1,3-propanediol:NAD$^+$ oxidoreductase provides an additional method of NADH disposal. The enzyme has been purified from both *L. brevis* B22 and *L. buchneri* B190 and found to have properties very similar to those reported for the enzyme from *Klebsiella pneumoniae*. The enzymes require Mn^{2+} and are probably octamers with a molecular mass of 350 kDa. Although not absolutely specific for 1,3-propanediol when tested as dehydrogenases, the enzymes have less than 10% activity with glycerol, ethanol, and 1,2-propanediol. These properties contrast sharply with those of a protein isolated from another *Lactobacillus* species (*L. reuteri*) that ferments glycerol with glucose and previously designated a 1,3-propanediol dehydrogenase.

Some strains of *Lactobacillus brevis*, *Lactobacillus buchneri*, and *Lactobacillus reuteri* and *Lactobacillus* sp. strain 208-A can grow on glycerol by cofermenting it with glucose or fructose (17, 20, 21). The first two organisms (at least) can also coferment glycerol with ribose or lactate (24). All these bacteria have a coenzyme B_{12}-dependent dehydratase that converts glycerol to 3-hydroxypropionaldehyde (3-HPA), which is then reduced to 1,3-propanediol (1,3-PDL) (18, 19, 22).

The anaerobic metabolism of glycerol has been more extensively studied in *Klebsiella pneumoniae*. This organism has two parallel coenzyme B_{12}-dependent dehydratases (1, 14, 23) that produce 3-HPA, which is then reduced to 1,3-PDL by a 1,3-propanediol:NAD$^+$ oxidoreductase (1,3-PDL dehydrogenase; EC 1.1.1.202) (9). One of the dehydratases and the dehydrogenase are expressed together (7) as part of the *dha* regulon (8), being induced by dihydroxyacetone and suppressed by aerobiosis and by catabolite repression. These enzymes provide a characteristic and sole means for NADH disposal during glycerol fermentation.

In *L. brevis* and *L. buchneri*, the dehydratase and the dehydrogenase are also expressed together but without the need for induction by added glycerol and are not subject to simple catabolite repression (24). For the lactobacilli, glycerol alone does not support growth, and the enzymes provide a means of NADH disposal besides or in addition to those used during the fermentation of the single carbohydrates. However, it is not clear how the organisms allocate NADH to yield the patterns of end products found. We have examined the dehydrogenases isolated from these lactobacilli to discover whether they are obviously different from that of *K. pneumoniae*. Recently, Talarico et al. (21) isolated from *Lactobacillus reuteri* a protein able to reduce 3-HPA to 1,3-PDL but with physical properties very different from those of the *Klebsiella* oxidoreductase.

* Corresponding author.
† Present address: Centro de Tecnologia Química e Biológica, Rua da Quinta Grande, 6, Apartado 127, 2780 Oeiras, Portugal.

MATERIALS AND METHODS

Materials. MOPS (3-[*N*-Morpholino]propanesulfonic acid) buffer and protamine sulfate were from Sigma Chemical Co., Ltd. Enzyme-grade ammonium sulfate, 1,3-PDL, and EDTA were from BDH Chemicals, Ltd. Sephadex G-200, Sephadex G-25, and Blue Sepharose CL-6B were from Pharmacia Chemicals. Diethylaminoethyl cellulose (DE-52) and carboxymethylethyl cellulose (CM-52) were from Whatman Biosystems Ltd.

Growth of bacteria. *L. buchneri* B190 and *L. brevis* B22 were each grown unshaken in four 3-liter flasks filled with complex MRS medium (4) plus glycerol (100 mM). The cultures were inoculated and incubated for 40 and 30 h, respectively, and the cells were washed and broken by ultrasonic treatment as described previously (24) with 100 mM (pH 7.2) buffer containing MOPS-KOH and 1 mM $MnCl_2$. The extracts were cleared by centrifugation at 43,500 × *g* for 1 h at 4°C and diluted with buffer to yield 18 mg of protein per ml.

Assays. Protein concentrations were determined by use of the Coomassie protein assay reagent supplied commercially by Pierce & Warrier (UK) Ltd., but for column chromatography fractions, the A_{280} was taken as an approximate index of protein concentration (10).

1,3-PDL dehydrogenase activity was determined spectrophotometrically (A_{340}) by use of the initial rate of substrate-dependent NADH formation at 37°C with 1 mM NAD and 155 mM 1,3-PDL contained in 1 ml of 100 mM Tris-HCl buffer (pH 9.0). Before the assay, enzyme samples were first treated for 10 min at 37°C with 10 mM dithiothreitol (DTT). In many cases, enzyme fractions showed greatly reduced activity when the DTT treatment was omitted.

One unit of enzyme activity was the amount catalyzing the formation of 1 μmol of product per min under the specified conditions.

Purification of both 1,3-PDL dehydrogenases. All purification steps were carried out at 4°C, and the buffer used was 100 mM MOPS (pH 7.2) containing 1 mM $MnCl_2$ unless otherwise stated.

(i) Protamine sulfate fractionation. Much nucleic acid and unwanted protein was removed from the initial crude extract (E_0) by adding protamine solution (30 mg/ml of buffer) to the stirred extract gradually, so as to yield, for *L. brevis*, 0.24 mg

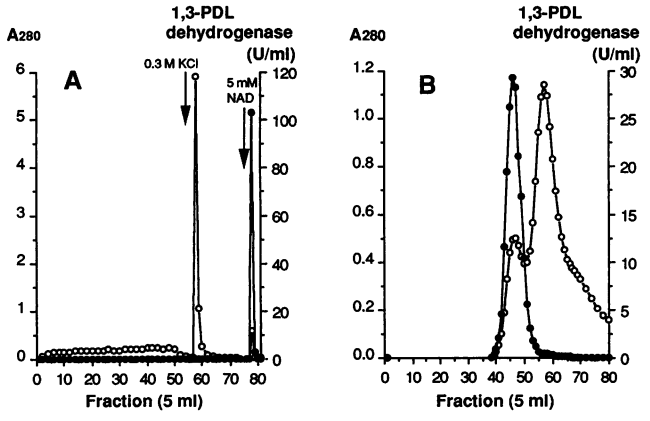

FIG. 1. Isolation of 1,3-PDL dehydrogenase from *L. buchneri* by elution with NAD from a Blue Sepharose CL-6B chromatography column (A) and from *L. brevis* by gel filtration chromatography on a Sephadex G-200 column (B). Symbols: ○, A_{280}; ●, 1,3-PDL dehydrogenase.

of protamine sulfate per mg of extract protein or, for *L. buchneri*, 0.10 mg. After 1 h, the precipitate was removed by centrifugation and discarded. The use of more protamine sulfate in either case decreased the 1,3-PDL dehydrogenase specific activity of the supernatant fluid.

(ii) Heat treatment. The protamine sulfate-treated extract (E_1) from *L. brevis* (3.8 mg · ml^{-1}) was divided into approximately 100-ml portions in 250-ml conical flasks, and the well-stirred extracts were heated quickly to 47°C in a 50°C water bath. After 5 min, with only occasional stirring while the temperature reached 50°C, the samples were cooled rapidly in ice-water. The precipitated protein was removed by centrifugation and discarded, leaving the supernatant fluid (E_2).

The dehydrogenase activity from *L. buchneri* was found to be too thermolabile for successful selective heat treatment.

(iii) Anion-exchange chromatography. Most of the dehydrogenase activity was not retained by DEAE-cellulose equilibrated with buffer. Therefore, extract E_2 of *L. brevis* (1.2 g of protein) or E_1 of *L. buchneri* (2.2 g of protein) was passed through columns (80 ml) of DE-52. For *L. brevis*, 85% of the enzyme activity was recovered in the pooled fractions (E_3), but for *L. buchneri*, only 61% was recovered in E_3. However, for the latter, some of the unaccounted activity was retained by the column and was eluted as a sharp peak ($E_{3.1}$) after loading of the column with buffer containing 100 mM KCl.

(iv) Cation-exchange chromatography. Excess protamine sulfate and further unwanted protein were removed by filtration of the E_3 samples (*L. brevis*, 1.2 mg of protein ml^{-1}; *L. buchneri*, 0.54 mg of protein ml^{-1}) through columns of buffered CM-52 (30 ml for *L. brevis*; 18.5 ml for *L. buchneri*) to yield pooled fractions (E_4).

(v) Affinity chromatography. Very little (15%) of the dehydrogenase activity of E_4 from *L. brevis* (1.04 mg of protein ml^{-1}) was bound to a buffered Blue Sepharose CL-6B matrix, but all that of E_4 from *L. buchneri* (0.38 mg of protein ml^{-1}) was retained by a column (8 ml) of this affinity gel together with about half of the E_4 protein. Washing of this latter column with buffer with added 0.3 M KCl removed most of the bound protein before 85% of the *L. buchneri* enzyme was selectively eluted with buffered NAD (5 mM) as fraction E_5 (5 ml) (Fig. 1A). To remove NAD from the enzyme, we filtered 1-ml aliquots of E_5 through a 25-ml column of buffered Sephadex G-25 without significant activity loss.

(vi) Ammonium sulfate concentration. The bulk of E_4 from *L. brevis* (390 ml) was mixed gently with an equal volume of saturated ammonium sulfate solution to precipitate inactive protein, which was removed after 1 h by centrifugation. More ammonium sulfate solution was added to the supernatant fluid to yield 70% saturation, and the enzyme precipitate (collected by centrifugation 1 h later) was dissolved in buffer as sample E_6 (3.5 ml). This concentrated solution (33.0 mg of protein ml^{-1}) was taken for gel filtration.

(vii) Gel filtration. Sample E_6 was passed down a column (500 ml) of Sephadex G-200 in buffer, and the enzyme activity appeared close to the void volume of the column, coinciding with a minor protein peak (Fig. 1B). The active fractions (5 ml) were of constant specific activity and were pooled to form sample E_7, which was used for the characterization of the *L. brevis* dehydrogenase.

PAGE. For polyacrylamide gel electrophoresis (PAGE), nondenaturing polyacrylamide gels were made as described by Chrambach et al. (3) and used in two discontinuous buffer systems. The first was the common Tris-glycine system used by Forage and Foster (6) but without 1,2-propanediol and in which the pH of the resolving gel is about 9.6 at 4°C. In the second, the buffers were *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and N-(2-acetamido)-2-aminoethanesulfonic acid (ACES) used as described by Forage (5); in this system the pH of the resolving gel is reduced to 8.4 at 4°C. Electrophoresis was done at 4°C, and the samples used contained up to 0.1 U of enzyme activity. 1,3-PDL dehydrogenase activity was located by formazan formation on incubation of the gels for 10 min in Tris-HCl buffer (125 mM, pH 9.0) containing 1,3-PDL (155 mM), NAD (1 mM), phenazine methosulfate (25 μg/ml), and nitroblue tetrazolium (1 μg/ml). Before loading of the gels, the samples were treated at 37°C for 10 min first with EDTA (1.5 mM) and then with $MnCl_2$ (2 mM). This routine treatment of the samples prevented the loss of all enzyme activity during electrophoresis and was necessary to allow the visualization of any active bands. Protein bands were located by positive staining with Coomassie blue G-250 (15). Mobilities (R_b) are reported relative to that of the tracking dye.

In the sodium dodecyl sulfate (SDS)-polyacrylamide denaturing gels, the resolving gel contained 10% (wt/vol) acrylamide, of which 2% was *N',N'*-bisacrylamide. The stacking gel was the same as that for nondenaturating polyacrylamide gels. These gels were used in the Tris-glycine system at room temperature, and the upper electrode buffer contained 0.05% (wt/vol) SDS. Before loading of the gels, the samples were diluted in electrode buffer containing 2% SDS and heated in boiling water for 5 min. Protein bands were located by staining with Coomassie blue R-250. For calculation of denatured protein molecular weights, a commercial low-molecular-weight calibration kit of standard proteins (Pharmacia Great Britain, Ltd.) was used (25).

Removal of metal ions. For removal of any reversibly bound metal ions, the E_5 and E_7 enzyme preparations were passed slowly (4 h total) through 10 ml of Dowex A-1 chelating resin equilibrated with 100 mM MOPS (pH 7.2). The protein-containing fraction contained 4% (*L. buchneri*) or 9% (*L. brevis*) dehydrogenase activity before treatment.

Molecular weight determinations. The molecular weights of the purified enzymes were determined by gel filtration in a buffered Pharmacia Superose 6-prepacked 10/30 column by use of a commercial computer-controlled low-pressure liquid

TABLE 1. Purification of 1,3-PDL dehydrogenase from *L. brevis*

Procedure (extract)	Total U[a]	Yield (%)	Sp act (U · mg of protein^{-1})	Purification (fold)
Crude cell (E_0)	4,600	100	1.0	1
Protamine sulfate (E_1)	6,730	146	3.9	4
Heat denaturation (E_2)	7,900	172	6.5	7
DE-52 (E_3)	6,160	140	12.8	13
CM-52 (E_4)	4,940	107	12.5	13
Ammonium sulfate (E_6)	1,320	30	13.0	13
Gel filtration (E_7)	800	17	53.0	53

[a] The activity of 1,3-PDL dehydrogenase was assayed with 1,3-PDL as the substrate and after DTT treatment at 37°C (10 mM, 10 min) as described in Materials and Methods.

chromatography system (Gilson) with apoferritin, β-amylase, and bovine serum albumin as standards. They were also determined by sedimentation in a linear sucrose density gradient (5 to 20% sucrose [wt/vol]) by the method of Martin and Ames (12) with, as standards, beef liver catalase (240 kDa) and yeast alcohol dehydrogenase (150 kDa). Centrifugation was done for 5 h at 4°C and 370,000 × *g*.

RESULTS

Isolation of the dehydrogenases. Even though 1,3-PDL is the final product of the metabolism of glycerol and is excreted into the growth medium (7, 8, 21, 24), there was no evidence for the 1,3-PDL dehydrogenase from either of the organisms being attached to a membrane, since it was found exclusively in the supernatant fluid of the broken-cell suspension. Summaries of the results of the purification procedures are given in Table 1 for *L. brevis* and in Table 2 for *L. buchneri*. The enzyme from *L. buchneri* was purified almost 1,000-fold and in 42% yield. In the case of *L. brevis*, purification was notably less successful (only 53-fold and in 17% yield); this result was especially disappointing, since the enzyme in E_0 appeared to be at least 40% inhibited, the total enzyme activity rising markedly during the first two purification steps (protamine sulfate precipitation and heat treatment). However, E_0 from *L. brevis* was fivefold richer in dehydrogenase than that from the other organism. Both the E_5 and E_7 final preparations were judged to be at least 95% pure on the basis of protein staining after electrophoresis in either buffer system or with SDS denaturation.

A requirement for a divalent metal ion was reported for the 1,3-PDL dehydrogenase isolated from *K. pneumoniae* (9), and the enzyme from either lactobacillus was found to be very unstable during fractionation unless Mn^{2+} was freshly included in the solutions. This instability may have been connected with the large loss (up to 70%) of the activity of either enzyme during ammonium sulfate treatment. However, the latter effect was not due to the salt simply blocking the binding of Mn^{2+}, because not more than one-third of the lost activity could be recovered by removal of the ammonium sulfate by dialysis, by incubation with DTT, or by the treatment with EDTA and Mn^{2+} found to be required before gel electrophoresis. A successful alternative means of concentrating the *L. brevis* enzyme was not found.

There was also a further loss (40%) of the *L. brevis* enzyme activity during the gentle procedure of gel filtration (with Mn^{2+}), and no treatment to recover this activity was found. Nor was there any evidence for reversible dissociation into less active subunits (2), tested by recombining fractions that had been reconcentrated by ultrafiltration. The addition of 1,3-PDL (250 mM), NAD (1 mM), or DTT (3 mM) to the gel filtration buffer had no effect.

Both of the dehydrogenases activities were also found to be lost completely during gel electrophoresis unless the enzyme samples were treated immediately beforehand with EDTA and then with an excess of $MnCl_2$. When EDTA was omitted and only Mn^{2+} was added, the loss of enzyme activity during electrophoresis was still considerable and the visualization of dehydrogenase as an activity band required a longer incubation period in the staining mixture.

Evidence for a second 1,3-PDL dehydrogenase in *L. buchneri*. Part of the dehydrogenase activity from *L. buchneri* was bound to the anion-exchange cellulose and subsequently eluted to yield sample $E_{3.1}$. That this activity was of a distinct second dehydrogenase able to act on 1,3-PDL was strongly suggested by PAGE analysis. In contrast to more highly purified sample E_5, sample $E_{3.1}$ gave a detectable band of enzyme activity (R_f = 0.08) only in the HEPES-ACES-buffered gels and not in the more alkaline Tris-glycine system. The activity of sample $E_{3.1}$ was found to be completely lost after incubation at pH 9.5 in 100 mM Tris-HCl buffer for only 3 min at 4°C, a treatment that had no effect on the activity of sample E_5.

That neither of the two dehydrogenases was formed readily from the other during the column separation was confirmed by subjecting either E_3 or $E_{3.1}$ to a second round of DEAE-cellulose chromatography. In each case, the other enzyme was not produced. Furthermore, PAGE analysis of E_0 in HEPES-ACES revealed two enzymatically active bands, the less mobile (R_f = 0.04) of these corresponding to the single band produced by purified preparation E_5. The occurrence of two different 1,3-PDL dehydrogenases in E_0 was also suggested during a kinetic analysis by use of biphasic plots (11) that corresponded to apparent K_m values of about 7 and 30 mM for 1,3-PDL.

Physical properties of the dehydrogenases. The molecular masses of the purified 1,3-PDL dehydrogenase from either organism (in E_5 or E_7) were found to be 355 ± 5 kDa when determined by gel filtration and 350 ± 20 kDa when determined by sucrose density gradient centrifugation.

SDS-PAGE of the activated 1,3-PDL dehydrogenases (i.e., after the sample had been treated with EDTA and Mn^{2+}) resulted in two stained bands of protein (42 and 46 kDa) (Fig. 2). However, if Mn^{2+} was not added after incubation with EDTA, so that the enzyme remained inactive, or if the enzyme was subsequently incubated with 10 mM DTT (whether Mn^{2+} was present or not), the stained gels contained only the upper 42-kDa band.

Activation by divalent metal ions. The 1,3-PDL dehydrogenase activities of all the preparations were completely lost after incubation with EDTA (1.5 mM for 10 min at 37°C). When the two purified enzymes (E_5 and E_7) were so treated

TABLE 2. Purification of 1,3-PDL dehydrogenase from *L. buchneri*

Procedure (extract)	Total U[a]	Yield (%)	Sp act (U · mg of protein^{-1})	Purification (fold)
Crude cell (E_0)	1,880	100	0.2	1
Protamine sulfate (E_1)	1,755	93	0.8	4
DE-52 (E_3)	1,080	57	5.13	29
CM-52 (E_4)	930	49	6.8	38
Blue Sepharose CL-6B (E_5)	790	42	178.0	990

[a] The activity of 1,3-PDL dehydrogenase was assayed with 1,3-PDL as the substrate and after DTT treatment at 37°C (10 mM, 10 min) as described in Materials and Methods.

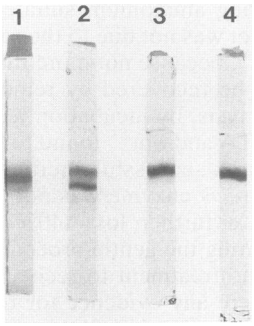

FIG. 2. SDS-PAGE analysis of 1,3-PDL dehydrogenase isolated from *L. brevis*. Samples (160 mU; 3 μg of protein) of purified enzyme (E_7) were applied to the top of each gel rod. They were untreated (gel 1) or had been treated with EDTA and Mn^{2+} (gel 2) and then DTT (gel 3) or treated with EDTA alone (gel 4) as detailed in the text. After electrophoresis, the gels were stained with Commassie blue R-250. Virtually identical results were obtained with sample E_5 from *L. buchneri*.

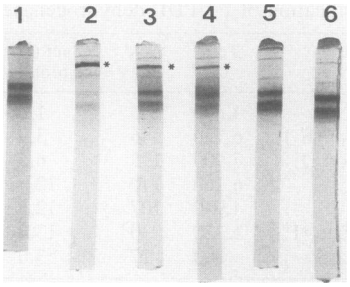

FIG. 3. Effect of metal ion treatment on PAGE of 1,3-PDL dehydrogenase isolated from *L. brevis*. PAGE was done with the Tris-glycine system. The samples (250 mU of E_7) were applied to the gel rods after treatment with EDTA (gel 1) and then with excess Mn^{2+} (gel 2), Fe^{2+} (gel 3), Ca^{2+} (gel 4), Mg^{2+} (gel 5), or Zn^{2+} (gel 6) as detailed in the text. After electrophoresis, the gels were stained to visualise the active 1,3-PDL dehydrogenase band (*). Subsequently, Commassie blue G-250 stain was applied.

and any chelated metal had been removed by gel filtration through Sephadex G-25 equilibrated with buffer containing 0.5 mM EDTA instead of $MnCl_2$, they could be reactivated by the addition of excess (1 mM) Mn^{2+} or Fe^{2+} (Table 3). Surprisingly, Ca^{2+} was almost as effective as Fe^{2+}, and even Mg^{2+} resulted in slight but significant reactivation, but Zn^{2+} did not.

PAGE analysis with the Tris-glycine system of these samples from *L. brevis* showed (Fig. 3) that the different ions supported the appearance of a sharp protein band at R_f = 0.09 at the expense of the broad bands (R_f = 0.16 and R_f = 0.22) found after EDTA inactivation. The sharp band coincided precisely in all cases with the sharp band of enzymatic activity that was detected before protein staining; also, the extent of formazan production was similar to the degree of apparent enzyme reactivation measured before electrophoresis. The minor protein bands at R_f = 0.06 and R_f = 0.14 were unchanged by metal ions and presumably represented protein impurities. In the case of *L. buchneri*, whose dehydrogenase was more easily inactivated during electrophoresis, an active band was found only after treatment with Mn^{2+}.

Since Mn^{2+} seemed especially effective at stabilizing the enzymes, the apparent degree of binding of this ion to the two purified preparations was measured kinetically after the samples had been passed through a chelating resin. The apparent K_m values for Mn^{2+} were 1.7 μM (*L. brevis*) and 3.1 μM (*L. buchneri*).

Substrate specificity and kinetic properties. Although the in vivo substrate for 1,3-PDL dehydrogenase is believed to be 3-HPA, this unstable aldehyde is not available commercially or readily accessible in the laboratory. Therefore, the enzyme is more conveniently examined as a dehydrogenase rather than as a reductase (Table 4). Further alcohols that did not act as substrates (or inhibitors of equimolar 1,3-PDL) were methanol, ethanediol, propan-2-ol, and *R*(−)-*S*(+) mixtures of *sec*-butyl alcohol, 1,2-butanediol, and 2,3-butanediol. Both enzymes were most active with diols containing two primary alcohol groups separated by one or two carbon atoms, i.e.; 1,3-PDL resulted in the fastest reaction rate.

When tested as reductases by following the removal of NADH (0.16 mM) in 100 mM 2-(*N*-morpholino)ethanesulfonic acid buffer at pH 6.6 and 25°C with 100 mM aldehyde, the enzymes from both strains were active with DL-glyceraldehyde, propionaldehyde, butyraldehyde, and acetaldehyde (in that order of decreasing relative activity); the enzymes were not active with dihydroxyacetone. Neither enzyme had any detectable activity with NADP (1 mM) when tested with 1,3-PDL.

Both isolated enzymes displayed classical Michaelis-Menten kinetics, with apparent K_m values for 1,3-PDL of 2 mM (*L. brevis*) and 14 mM (*L. buchneri*) and for glycerol of

TABLE 3. Divalent cations and the activity of the purified 1,3-PDL dehydrogenases from *L. brevis* and *L. buchneri*

Cation added[a]	1,3-PDL dehydrogenase activity ($U \cdot ml^{-1}$)[b] of:	
	L. brevis	*L. buchneri*
None	0.0	0.0
Mn^{2+}	10.0	40.0
Fe^{2+}	5.0	8.0
Ca^{2+}	4.0	3.5
Mg^{2+}	0.5	1.1
Zn^{2+}	0.0	0.0

[a] Added as solutions of chloride salts.
[b] Measured after inactivation with EDTA and reactivation as described in the text.

TABLE 4. Alcohol specificity of 1,3-PDL dehydrogenases from *L. brevis* and *L. buchneri*

Substrate	1,3-PDL dehydrogenase activity (% of that with 1,3-PDL)[a] of:	
	L. brevis	*L. buchneri*
CH_2OH-CH_2-CH_2OH (1,3-propanediol)	100	100
CH_3-$CHOH$-CH_2OH (1,2-propanediol)	0	0
CH_2OH-$CHOH$-CH_2OH (glycerol)	7	1
CH_3-CH_2OH (ethanol)	3	0
CH_3-CH_2-CH_2-CH_2OH (butan-1-ol)	13	5
CH_2OH-CH_2-CH_2-CH_2OH (1,4-butanediol)	26	10
CH_3-CH_2-CH_2OH (propan-1-ol)	10	3

[a] Activity with the various substrates (155 mM) was measured at 25°C as described in Materials and Methods.

300 and 700 mM, respectively. These very high values for glycerol are in accordance with the ability of these organisms to continue producing 1,3-PDL even when the cells are growing in 100 mM glycerol (25).

The apparent K_m values for NAD (with 1,3-PDL) were 76 μM (*L. brevis*) and 370 μM (*L. buchneri*) and, as expected, NADH acted as a competitive inhibitor. The apparent K_m values for NADH (with DL-glyceraldehyde) were much lower at 13 μM for both enzymes, and NAD acted as a competitive inhibitor.

DISCUSSION

The 1,3-PDL dehydrogenases isolated from *L. brevis* and *L. buchneri* are very similar to each other and are probably octameric proteins. The number of subunit types is not completely clear, for although a single protein band was found after SDS-PAGE, two protein bands (of not very dissimilar sizes) were found after treatment with EDTA and then Mn^{2+}. If these represent two forms of a single type of subunit, the nature of the difference between them is obscure. Similar unexpected observations have been made in our laboratory with purified 1,3-PDL dehydrogenase from *K. pneumoniae* (16) and remain unexplained. The prima facie proposition that the more mobile subunit form has been induced to bind extra Mn^{2+} seems unlikely; such binding would have to be remarkably strong to survive heating in alkaline SDS and subsequent electrophoresis yet be abolished by treatment with either EDTA or DTT. The ability of Mn^{2+} treatment to protect the lactobacillus enzyme activity during PAGE without SDS is also unusual, because the best retention of activity required incubation with EDTA first.

Overall, the properties of the lactobacillus enzymes are very similar to those reported for the *K. pneumoniae* enzyme (Table 5): i.e., a molecular mass of 330 kDa (subunit size, 45 ± 3 kDa) and a requirement of either Mn^{2+} or Fe^{2+} for activity (9). However, one difference is that the *K. pneumoniae* protein is inactive as glycerol dehydrogenase, but the lactobacillus enzymes can dehydrogenate glycerol at a significant rate when the glycerol concentration is very high.

In contrast, the other lactobacillus enzyme able to dehydrogenate 1,3-PDL, obtained from *L. reuteri* (21), is quite different (Table 5). It is smaller (180 kDa), requires only a monovalent cation (K^+), is much less active as a dehydrogenase with 1,3-PDL than with glycerol, and is as active as a reductase with dihydroxyacetone and acetol as with 3-HPA. It is surprising that the 1,3-PDL dehydrogenase isolated from *L. reuteri* is a protein different from the one found in *L. brevis* and *L. buchneri*, since all three strains of lactobacilli ferment glucose and produce 1,3-PDL from glycerol in a similar way (17, 21, 24). This *L. reuteri* enzyme resembles more closely the *K. pneumoniae* glycerol dehydrogenase described by McGregor et al. (13). It is therefore possible that the *L. reuteri* enzyme described acts in vivo, during growth with glycerol, as a glycerol dehydrogenase and that a 1,3-PDL dehydrogenase actually responsible for 3-HPA reduction in this organism has so far escaped detection because of inactivation similar to that observed for *L. buchneri* during PAGE analysis.

TABLE 5. Comparison of 1,3-PDL dehydrogenases from *L. brevis*, *L. buchneri*, *L. reuteri*, and *K. pneumoniae*

Organism	K_m for:						Effect of cations on activity of the following enzyme:		pH of activity assay under the following substrate conditions:		Subunits		Molecular mass (kDa) as determined by gel filtration
	1,3-PDL (mM)	Glycerol (mM)	3-HPA (mM)	Dihydroxyacetone (mM)	DL-Glyceraldehyde (mM)	NAD with the following substrate (μM): 1,3-PDL	NAD with the following substrate (μM): Glycerol	NADH with the following substrate (μM): Glyceraldehyde	NADH with the following substrate (μM): Dihydroxyacetone	Active	Inactive	Oxidation	Reduction	No.	Molecular mass (kDa)	
L. brevis	2	300	ND[a]	—[b]	26	76	ND	13	ND	$Mn^{2+} > Fe^{2+} > Zn^{2+}$		9.0	6.6	8	42 or 46	355 ± 5
L. buchneri	14	700	ND	—	29	370	ND	13	ND	Mg^{2+} $Mn^{2+} > Fe^{2+} > Zn^{2+}$		9.0	6.6	8	42 or 46	355 ± 5
L. reuteri[c]	ND[d]	12	7.8	7.2	ND[d]	ND	100	30	30	Mg^{2+} $K^+ > Na^+ > Li^+$		7.3	7.3	4	41	180
K. pneumoniae[e]	18	—	ND	—	ND	310	ND	ND	ND	Mn^{2+}, Fe^{2+}	Co^{2+}, Zn^{2+}	9.0	7.4	8 or 6	45 ± 3	328 ± 15

[a] ND, not determined.
[b] —, no reaction.
[c] Data are from reference 21.
[d] The K_m values with these two substrates were not determined, but the activities measured were 5% of the 100% relative activities determined with 3-HPA, acetol and dihydroxyacetone, or 1,2-propanediol and glycerol.
[e] Data are from reference 9.

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