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Occurrence and Properties of Lactic Dehydrogenases of Fermentative Mycoplasmas

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Eight fermentative mycoplasmas differing in genome size, deoxyribonucleic acid (DNA) base composition, or sterol dependence were examined for lactic dehydrogenase composition by spectrophotometric assay and polyacrylamide gel electrophoresis. Three completely different patterns of lactic dehydrogenase composition were found. (i) A nicotinamide adenine dinucleotide (NAD)-dependent L(+)-lactic dehydrogenase was found in *Mycoplasma pneumoniae*, *M. gallisepticum*, *M. mycoides* var. *mycoides*, mycoplasma UM 30847, *M. neurolyticum*, and *Acholeplasma axanthum*. Electrophoresis of cell-free extracts of each of these mycoplasmas produced, with the exception of *M. mycoides* var. *mycoides* and UM 30847, single, different enzyme bands. *M. mycoides* var. *mycoides* and UM 30847 were similar and formed multiple bands of enzyme activity. We were unable to establish whether these multiple bands were due to lactic dehydrogenase isoenzymes or artifacts. (ii) An NAD-dependent D(−)-lactic dehydrogenase which could not be reversed to oxidize lactate was found in *M. fermentans*. (iii) *A. laidlawii* A possessed an NAD-independent D(−)-lactic dehydrogenase capable of reducing dichlorophenol-indophenol, and an NAD-dependent L(+)-lactic dehydrogenase which is specifically activated by fructose-1,6-diphosphate. Heretofore, this enzyme regulatory mechanism was known to occur only among the *Lactobacillaceae*. No yeast-type lactic dehydrogenase activity was found in any of the mycoplasmas examined. The stereoisomer of lactic acid accumulated during growth correlated perfectly with the type of NAD-dependent lactic dehydrogenase found in each mycoplasma. The types of lactic dehydrogenase activity found in these mycoplasmas were not related to genome size, DNA base composition, or sterol dependence.

Mycoplasmas are a heterogeneous group of microorganisms that differ in such fundamental properties as genome size (3, 26), deoxyribonucleic acid (DNA) base composition (29), sterol dependence, and glucose fermentation. These properties have been used to divide the mycoplasmas into six subgroups, four of which comprise organisms that ferment glucose: (i) *Mycoplasma pneumoniae;* (ii) sterol-nonrequiring mycoplasmas belonging to the genus *Acholeplasma;* (iii) high guanine plus cytosine (GC) content fermentative mycoplasmas (GC ca. 35% and less); (iv) low GC content fermentative mycoplasmas (GC ca. 23% and greater) (29).

Approximately half of the 50 species of mycoplasmas known at present ferment glucose and accumulate acid end products. All 16 fermentative organisms so far examined produce lactic acid as a major product of glucose fermentation (30, 31, 40). Several workers (30–32, 40) have noted that the energy metabolism of some of these mycoplasmas is similar to that of the lactic acid bacteria. Organisms in both groups ferment glucose by the Embden-Meyerhof pathway and accumulate lactate, lack heme enzymes, and have a flavine-terminated respiratory system. Moreover, several of the mycoplasmas have been shown to produce hydrogen peroxide (8, 22, 31, 35, 36, 39). However, not all of the glucose-fermenting mycoplasmas that have been examined lack heme enzymes (41; Kurzepa and VanDemark, *personal communication*).

To define further the biochemical properties of the fermentative mycoplasmas, we searched

for and characterized lactic dehydrogenases in six mycoplasmas and two acholeplasmas which were selected as representatives of the four glucose-fermenting subgroups. The organisms selected represent approximately one-third of the fermentative species now recognized. Lactic dehydrogenases were chosen for study because they are constitutively synthesized enzymes that catalyze an important reaction in energy metabolism. Lactic dehydrogenase activity has been observed in three fermentative mycoplasmas (7, 31, 32) and in *M. arthritidis*, a nonfermentative species (21), but in most instances the enzyme activities were not characterized. In addition to providing information about the metabolism of fermentative mycoplasmas, knowledge of these enzymes should be useful in examining evolutionary and taxonomic relations among mycoplasmas and the bacteria. A wide variety of different lactic dehydrogenases has been found among the bacteria, in contrast to the similarity of the animal lactic dehydrogenases. Bacterial lactic dehydrogenases differ in such properties as stereospecificity, cofactor and activator requirements, reversibility, sensitivity to inhibitors, stability, and molecular weight (13, 14, 18, 34, 38). This report describes the occurrence and characteristics of lactic dehydrogenases found among the fermentative mycoplasmas.

MATERIALS AND METHODS

Organisms. The following organisms were examined: *M. pneumoniae* FH, *M. gallisepticum* X-95, *M. fermentans* PG18, *M. mycoides* var. *mycoides* KH_4J, mycoplasma UM 30847, *M. neurolyticum* KSA, *Acholeplasma laidlawii* type A, and *A. axanthum* S-743. *Leuconostoc mesenteroides* NCDO 523, a gift of Ellen I. Garvie (N.I.R.D., Reading, England), was used as a standard.

Cultivation. The media and growth conditions for the mycoplasmas were essentially as previously described (28) except that 15% unheated horse serum was used for *M. pneumoniae* and *M. fermentans* and 1% Difco PPLO serum fraction for the other strains. The final glucose concentration was 0.25 or 0.5%. Penicillin (50 units/ml) was added in some instances. *L. mesenteroides* was grown in MRS medium (10).

Preparation of cell-free extracts. Cells were harvested, washed once in 0.145 M sodium chloride–0.02 M sodium phosphate buffer (*p*H 7.5) and resuspended in the same buffer (cells were concentrated ca. 200-fold). Mycoplasma cell suspensions in an ice bath were disrupted with a Branson S-75 sonifier at a frequency of 20 kc/sec and the power output at step 3 for four 90-sec periods alternated with cooling for 3 min. *L. mesenteroides* was sonically treated for a total of 15 min, the suspension being held in an ethanol-dry ice bath at approximately −20 C. Cell debris was removed by centrifugation at 80,801 × *g* for 30 min at 4 C. The supernatant fluid was used for enzyme assays and gel electrophoresis or stored at −28 C if not examined immediately.

Enzyme assays. Nicotinamide adenine dinucleotide (NAD)-dependent lactic dehydrogenase activity was determined spectrophotometrically by measuring the rate of reduction of NAD at 340 nm by the method of Neilands (27), except that the reaction was carried out in 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 8.3) with either 0.5 M sodium DL-lactate, or 0.25 M L(+)- or D(−)-lithium lactate (Calbiochem, Los Angeles, Calif.). A unit of activity is defined as the amount of enzyme which will reduce 1 μmole of NAD per min at 22 to 24 C. Extracts were also examined for the reverse reaction, reduction of pyruvate to lactate with reduced NAD (NADH) (20).

NAD-independent D(−)-lactic dehydrogenase was determined at *p*H 6.5 by following the reduction of 2,6-dichlorophenol-indophenol (DCPIP) at 620 nm (18). Yeast-type lactic dehydrogenase was assayed as described by Dixon (11), using the supernatant fluid from an autolyzed aqueous suspension of dried yeast as a control.

Analytical methods. Total lactic acid in culture supernatant fluids was determined by the procedure of Barker and Sumerson (5). The amount of L(+)-lactic acid was determined with a commercial enzyme kit (Sigma Chemical Co., St. Louis, Mo.); D(−)-lactic acid was determined by a modification of the method of Gawehn and Bergmeyer (16) using the D(−)-specific NAD-dependent lactic dehydrogenase (5 mg/ml) of *Lactobacillus leichmannii* (Boehringer GmbH, Mannheim, Germany). Standards containing 60, 120, and 240 μg of D(−)-lithium lactate and L(+)-lithium lactate were used initially. Under our conditions the procedure was capable of detecting as little as 60 μg of D(−)-lactic acid per ml of culture medium. Formation of L(+)-lactic acid from pyruvate was detected by the yeast lactic dehydrogenase method (Worthington Biochemical Corp., Freehold, N.J.) based on the findings of Appleby and Morton (2). Samples were deproteinized by heating in a boiling water bath for 5 min.

Protein content of extracts was determined by the method of Lowry et al. (23) with crystalline bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis and enzyme staining. Enzyme extracts were further characterized by polyacrylamide gel electrophoresis (9). A 5% (w/v) gel solution was prepared by mixing one volume of water, two volumes of acrylamide solution (20.0 g of acrylamide and 0.8 *N,N'*-methylenebisacrylamide in distilled water to make 100 ml), and four volumes of freshly prepared ammonium persulfate solution (1 mg/ml), deaerating, and adding one volume of "TEMED" solution (36.3 g of Tris, 48.0 ml of 1 N HCl, 0.46 ml of *N,N,N',N'*-tetramethylethylenediamine). To compare migration in the presence of urea, amounts of urea giving final concentrations of 1, 2, or 4 M were dissolved in the gel mixture before deaeration.

Samples were mixed with equal volumes of 40% (w/v) sucrose, 10 to 50 μliters was layered over the gels, and electrophoresis buffer (1.2 g of Tris, 5.7 g of glycine in water to make 1,000 ml, *p*H 8.3) was layered over the samples. Bromophenol blue, which served as a tracking dye, was added to the upper reservoir. Electrophoresis was performed at a constant current of 2.5 ma per tube for 60 to 90 min at 4 C after an initial 10-min period at 1.0 ma per tube.

After electrophoresis, NAD-dependent enzymes were located by staining gels in a solution containing (per ml) 1 mg of NAD, 0.4 mg of nitro blue tetrazolium, 0.14 mg of phenazine methosulfate, 100 μmoles of sodium DL-lactate [or 50 μmoles of L(+)- or D(−)-lithium lactate], 10 μmoles of NaCl, and 0.5 μmole of $MgCl_2$ in 0.1 M Tris-hydrochloride buffer (*p*H 8.3). Gels were stained normally for 5 to 20 min at 22 C in the dark; the reaction was stopped by transferring the gels to 7% acetic acid.

For locating NAD-independent lactic dehydrogenases after electrophoresis, gels were allowed to stand for 10 to 15 min in 0.1 M Tris-maleate buffer, *p*H 6.3. They were then stained for 1 to 2 hr in a solution containing (per ml) 0.052 mg of DCPIP and 100 μmoles of sodium DL-lactate [or 50 μmoles of L(+)- or D(−)-lithium lactate] in 0.1 M Tris-maleate buffer, *p*H 6.3.

RESULTS

The occurrence and types of lactic dehydrogenases of the eight mycoplasmas are shown in Table 1. With the exception of *M. fermentans* and *A. laidlawii* type A, each organism contained only a NAD-dependent L(+)-lactic dehydrogenase. *M. fermentans* possessed an NAD-dependent D(−)-lactic dehydrogenase; this enzyme was capable of reducing pyruvate to lactate but could not be reversed to oxidize lactate even at high *p*H in the presence of hydrazine. *A. laidlawii* type A had an NAD-independent D(−)-lactic dehydrogenase as well as an NAD-dependent L(+)-lactic dehydrogenase which differed from the L(+)-lactic dehydrogenases of the other species.

Stereoisomer of lactic acid produced during growth. The optical isomer of lactic acid accumulated during growth correlated perfectly with the type of NAD-dependent lactic dehydrogenase found in cell extracts of each mycoplasma (Table 1). The amounts of L(+)-lactic acid accumulated during growth ranged from approximately 9 to 13 μmoles per ml; *M. fermentans* accumulated approximately 7 μmoles of D(−)-lactic acid per ml.

Fructose-1,6-diphosphate activation of A. laidlawii A NAD-dependent L(+)-lactic dehydrogenase. We were unable to demonstrate NAD-dependent lactate oxidation in extracts from either of two strains of *A. laidlawii* A, grown with and without yeast extract. However, a low rate of NADH-dependent reduction of pyruvate to lactate was found. Addition of 10^{-3} M fructose-1,6-diphosphate (FDP) to the assay system greatly increased the reaction rate (Fig. 1). The activation by FDP is highly specific but can be masked completely by raising the pyruvate concentration from 10^{-3} to 10^{-2} M (Neimark and Tung, *manuscript in preparation*). It was proved that the activity being measured was actually that of a lactic dehydrogenase by demonstrating the formation of lactic acid (5) from the oxida-

TABLE 1. *Characteristics and lactic dehydrogenases of selected fermentative mycoplasmas*

Species	Guanine plus cytosine content[a]	Arginine deiminase[b]	Isomer of lactic acid produced[c]	Lactic dehydrogenases[d]			
				NAD-dependent		NAD-independent	
				L(+)	D(−)	L(+)	D(−)
Mycoplasma							
pneumoniae, FH	40.8	0	L(+)	+	0	0	0
gallisepticum	35.2	0	L(+)	+	0	0	0
mycoides var. *mycoides*	26.5	0	L(+)	+	0	0	0
UM 30847	26.5	0	L(+)	+	0	0	0
neurolyticum	22.8	0	L(+)	+	0	0	0
fermentans	27.6	+	D(−)	0	+	0	0
Acholeplasma							
laidlawii A	35.7	0	L(+)	+[e]	0	0	+
axanthum, S-743	31.3	0	L(+)	+	0	0	0

[a] Data taken from Neimark (29).

[b] Data taken from Barile, Schimke, and Riggs (4).

[c] Produced in deep nonaerated cultures (20 ml in 25 by 150 mm test tubes); determined enzymatically; see Materials and Methods.

[d] Determined spectrophotometrically; see Materials and Methods.

[e] Fructose-1,6-diphosphate-activated lactic dehydrogenase (see Fig. 2).

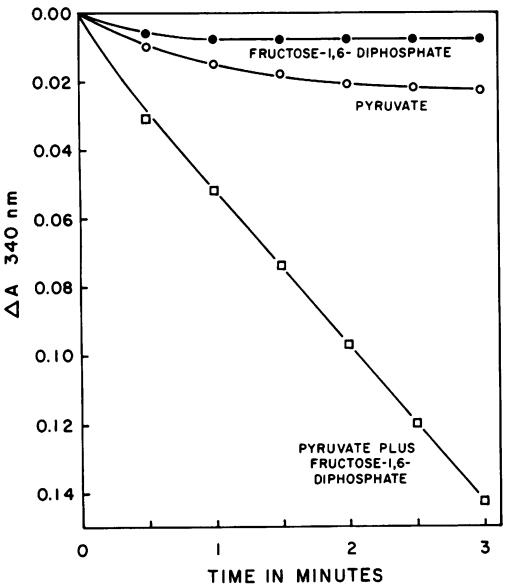

FIG. 1. *Activation of Acholeplasma laidlawii type A NAD-dependent lactic dehydrogenase by fructose-1,6-diphosphate (FDP). Cuvettes (1-cm light path) contained Tris-hydrochloride buffer (pH 7.2), 100 μmoles; NADH, 0.1 μmole; water to a total volume of 1.0 ml and the following substrates, as indicated: FDP, 1 μmole (●); sodium pyruvate, 1 μmole, (○); sodium pyruvate plus FDP, 1 μmole each (□). Approximately 30 μg of crude extract protein was added to start the reaction. Omitting both sodium pyruvate and FDP resulted in a reaction essentially identical to that obtained with FDP alone.*

tion of approximately 1 μmole of NADH in a system composed of crude extract, NADH, FDP, and sodium pyruvate in Tris-hydrochloride buffer. No significant amount of lactic acid was detected in controls from which pyruvate or enzyme extract was omitted.

Electrophoretic properties of NAD-dependent lactic dehydrogenase enzymes. Cell extracts were subjected to polyacrylamide gel electrophoresis, and the positions of the enzymes in the gel columns were located with enzyme stain. The appearance of the enzyme bands of each species was characteristic and reproducible. Extracts of *M. pneumoniae*, *M. gallisepticum*, *M. neurolyticum*, and *A. axanthum* each produced a single moderately narrow enzyme band, but extracts of *M. mycoides* var. *mycoides* and mycoplasma UM 30847 each produced a broad major band, which appeared first, and two to four additional diffuse minor bands. In addition to the bands, some material with enzyme activity did not penetrate into the gel, but remained at the origin. Migration (R_F) values for the major bands are listed in Table 2. The migration rate of the single band of *A. axanthum* was similar to that of the major bands of *M. mycoides* var. *mycoides* and mycoplasma UM 30847. The R_F values of the *M. gallisepticum* and *M. neurolyticum* enzymes were also quite close. No other similarities in electrophoretic mobility were observed. None of the lactic dehydrogenases of the mycoplasmas migrated as fast as the D(−) enzyme of *Leuconostoc mesenteroides* which produced an intense, sharp band immediately behind the tracking dye.

Similarities in mobility were not paralleled by similarities in capacity to reduce tetrazolium slowly in the absence of substrate or NAD. In the complete system, most enzyme bands appeared in 1 to 5 min. In the absence of lactate or NAD, bands did not form with extracts of most species during the course of normal incubation periods. Extracts of *A. axanthum*, upon prolonged incubation (90 min), were able to reduce tetrazolium in the absence of NAD or lactate and produce a faint band at the lactic dehydrogenase position.

The multiple bands of enzyme activity observed with *M. mycoides* var. *mycoides* and mycoplasma UM 30847 could have been due to lactic dehydrogenase isoenzymes, or they could have been artifacts. Multiple bands can result, for example, from an enzyme complexing with other proteins and migrating with them. However, such effects can be prevented by reducing agents or including urea in the gels. A number of experiments were done to determine the cause of the multiple bands. Electrophoresis in a 7.5% gel with a discontinuous *p*H stacking gel (9) produced essentially identical multiple bands, with some sharpening of the minor bands. Treatment of extracts with reducing agents (0.1 to 1.0% 2-mercaptoethanol or dithiothreitol) did not affect the pattern, but with either agent an additional fast-moving band appeared ahead of the main band; this fast band was an artifact due to the

TABLE 2. *Electrophoretic mobilities of mycoplasma NAD-dependent* L (+)*-lactic dehydrogenases*

Species	R_F[a]
Mycoplasma pneumoniae FH	0.09
M. gallisepticum X-95	0.24
M. neurolyticum KSA	0.26
M. mycoides var. *mycoides* KH_3J	0.53
Mycoplasma UM 30847	0.55
Acholeplasma axanthum S-743	0.57

[a] Distance from origin to center of lactic dehydrogenase activity band divided by distance migrated by tracking dye.

reducing agent itself, since it also occurred in gels from which the enzyme sample was omitted. It is known that the oxidizing effects of ammonium persulfate used as a catalyst for gel polymerization can generate multiple band artifacts (6, 25), but electrophoresis of reduced samples in gels prerun with buffer to remove ammonium persulfate (25) did not decrease the number of bands. Extracts of *M. mycoides* var. *mycoides* and mycoplasma UM 30847 run in gels containing 2 M urea produced only a single band which migrated slightly faster than the major band did in gels without urea. Electrophoresis in 4 M urea gels diminished the intensity of the band (Fig. 2). The single enzyme band of *A. axanthum* behaved similarly, in that the migration rate was slightly increased in 2 and 4 M urea. The activity of the extracts of *M. pneumoniae*, *M. gallisepticum*, and *M. neurolyticum* was completely lost in 2 M urea.

NAD-independent D(−)-lactic dehydrogenase. An NAD-independent lactic dehydrogenase specific for D(−)-lactic acid was found only in *A. laidlawii* (Fig. 3). The activity was low relative to the FDP-activated NAD-dependent L(+)-lactic dehydrogenase. The nonspecific decrease in absorbance in the first 30 sec was caused by the large amount of cell extract required to demonstrate activity. Polyacrylamide gel electrophoresis at *p*H 8.3 followed by enzyme staining at *p*H 6.3 revealed a 4-mm wide band of reduction with DL- or D(−)-lactate but not with L(+)-lactate as substrate. The R_F was approximately 0.57 (Fig. 4). The enzyme migrated very little at *p*H 6.3 or 7.0. No NAD-independent lactic dehydrogenase was found in any of the other mycoplasmas

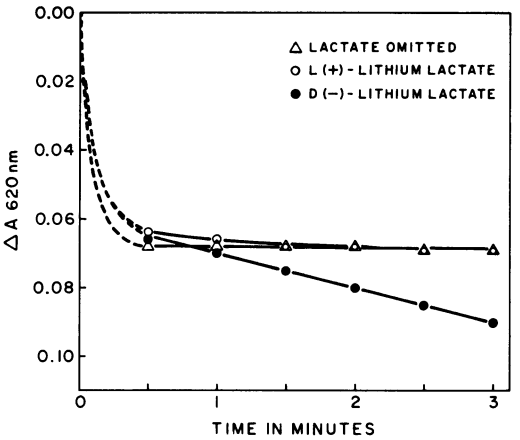

FIG. 3. *Acholeplasma laidlawii type A NAD-independent D(−)-lactic dehydrogenase. Cuvettes (1-cm light path) contained sodium phosphate buffer (pH 6.5), 100 μmoles; 2,6-dichlorophenol-indolphenol, 0.1 μmole; D(−)- or L(+)-lithium lactate, 20 μmoles; and distilled water to give a volume of 0.9 ml. The reaction was started by adding 0.1 ml of crude extract containing approximately 300 μg of protein.*

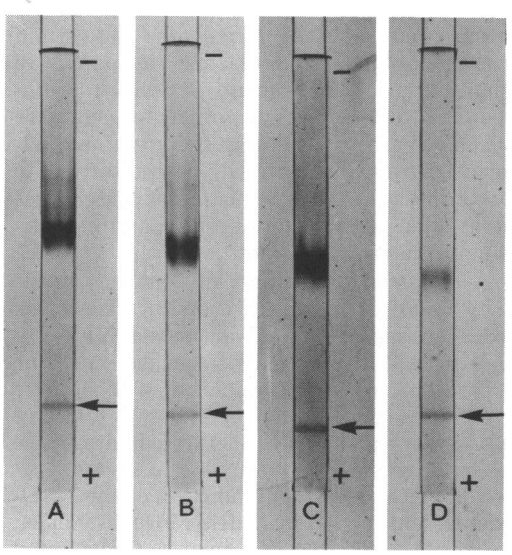

FIG. 2. *Effect of urea on migration of NAD-dependent L(+)-lactic dehydrogenase of Mycoplasma mycoides* var *mycoides* (*KH*$_3$*J*) *in polyacrylamide gels stained for enzyme activity. A, no urea; B, 1 M urea; C, 2 M urea; D, 4 M urea. Arrows indicate position of tracking dye.*

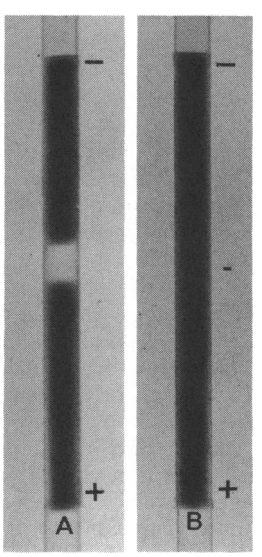

FIG. 4. *Polyacrylamide gel electrophoresis of NAD-independent D(−)-lactic dehydrogenase of Acholeplasma laidlawii, type A. A, Band of reduction with D(−)-lithium lactate as substrate indicates position of enzyme; B, no reduction with L(+)-lithium lactate.*

either spectrophotometrically or by gel electrophoresis. Cell extracts of *M. pneumoniae* and mycoplasma UM 30847 slowly reduced DCPIP at the same rate in the presence or absence of substrate; this activity was destroyed by boiling the extracts for 5 min.

Yeast-type lactic dehydrogenase. The only strain which completely decolorized methylene blue in the presence of DL-lactate, but not in its absence, was mycoplasma UM 30847. However, the extract of UM 30847 (6.25 mg of protein per ml) took 30 min for complete reduction, compared with less than 2 min for the yeast autolysate (10.75 mg of protein per ml).

DISCUSSION

Three different patterns of lactic dehydrogenase enzyme composition were observed among the mycoplasmas selected for examination: (i) possession of a single NAD-dependent L(+)-lactic dehydrogenase; (ii) possession of a single NAD-dependent D(−)-lactic dehydrogenase; (iii) possession of a FDP-activated NAD-dependent L(+)-lactic dehydrogenase as well as a NAD-independent D(−)-lactic dehydrogenase. A result of the enzyme compositions is that these mycoplasmas have the characteristic property of producing a specific isomer of lactic acid. This property could prove generally useful for characterizing fermentative mycoplasmas.

The possession of only an NAD-dependent L(+)-lactic dehydrogenase and production of L(+)-lactic acid during growth was found in both large and small genome mycoplasmas and in organisms with widely different DNA base compositions. The L(+)-lactic dehydrogenase of each species could be readily distinguished by electrophoretic mobility. The similarity in electrophoretic mobility and enzymatic properties between the lactic dehydrogenases of *M. mycoides* var. *mycoides* and mycoplasma UM 30847 parallels the similarity in GC content and growth properties of these organisms and supports the inclusion of mycoplasma UM 30847 in the biotype composed of *M. mycoides* var. *mycoides* and its relatives (29). It remains to be determined whether the lactic dehydrogenases of different strains of other species of mycoplasmas will also have common electrophoretic mobilities. Intraspecific uniformity of electrophoretic mobility of lactic dehydrogenases does occur among lactobacilli (14, 15) and also leuconostocs where electrophoretic uniformity can extend to different species (13), showing that certain lactic dehydrogenases have remained stable during evolution.

To our knowledge, isoenzymes of bacterial lactic dehydrogenases have not been described (multiple types of lactic dehydrogenases, however, are known in a number of bacteria; for example see references 13–15, 19, 25, 37, 44). We were unable to establish whether the multiple bands seen with extracts of *M. mycoides* var. *mycoides* and mycoplasma UM 30847 resulted from an artifact or isoenzymes. Garvie occasionally observed two merging adjacent bands with extracts of *Leuconostoc* species on starch gel electrophoresis, and suggested that these could be conformational isoenzymes (13). Our results obtained with urea-containing gels could indicate that enzyme binding to other proteins had occurred, but it is possible that the minor bands were merely destroyed by urea. The observation that enzyme activity was present in material that could not enter the gels indicates that enzyme aggregation may have occurred; it is possible, too, that enzyme activity was bound/to larger cell components such as membrane fragments. It should be possible to resolve the question by separating the bands by preparative acrylamide gel electrophoresis and rerunning the major band on an analytical acrylamide gel to determine whether minor bands are again produced.

The NAD-dependent lactic dehydrogenase of *M. fermentans* was unique in being specific for D(−)-lactic acid. *M. fermentans* and certain recently isolated mycoplasmas belonging to avian group I (J. Fabricant, *personal communication*) differ from all other fermentative mycoplasmas by possessing the arginine dihydrolase pathway (4). It will be interesting to determine what types of lactic dehydrogenases these other glucose-fermenting, arginine-utilizing mycoplasmas possess.

A. laidlawii was the only mycoplasma in the study found to possess two distinct lactic dehydrogenases. The FDP-activated NAD-dependent L(+)-lactic dehydrogenase must be responsible for the accumulation of L(+)-lactic acid during growth. The relatively less active NAD-independent D(−)-lactic dehydrogenase is similar to enzymes occurring in several species of bacteria (34). The function of the NAD-independent D(−)-lactic dehydrogenase in *A. laidlawii* is unknown. Snoswell (33) suggested that such enzymes probably do not function in the production of lactic acid in lactobacilli. In *Escherichia coli* an NAD-independent D(−)-lactic dehydrogenase appears to function in membrane transport systems (17).

The FDP activation of the NAD-dependent

lactic dehydrogenase of *A. laidlawii* type A is particularly significant. This enzyme appears to differ markedly from those of *A. axanthum* and the other mycoplasmas examined thus far. A similar enzyme could be expected to occur in *A. laidlawii* type B and also in *A. granularum* because of its nucleic acid hybridization with *A. laidlawii* (29). Studies that will be reported in detail elsewhere have shown that FDP activation of the lactic dehydrogenase is highly specific and that a number of substrates and glycolytic intermediates fail to substitute for FDP. Our use of FDP was prompted by the discovery by Wolin (46) that the lactic dehydrogenase from certain species of streptococci was specifically activated by FDP. We concur with Wolin's suggestion (46) that FDP acts as an allosteric activator of the lactic dehydrogenase and that FDP activation could be a significant metabolic control mechanism. This highly specific activation is unusual and at present is known to occur only among the *Lactobacillaceae*, specifically in streptococci, where this property is common to lactic dehydrogenases of all species which have been examined (43, 45, 46) and in strains of *Lactobacillus bifidus* (42). Even though *L. bifidus* possesses a FDP-activated lactic dehydrogenase, its carbohydrate metabolism differs considerably from that of the streptococci and *A. laidlawii* (7) in that it lacks key enzymes of the Embden-Meyerhof and hexose monophosphate pathways and catabolizes glucose by the fructose-6-phosphate phosphoketolase route (42).

The FDP-activated lactic dehydrogenases of *A. laidlawii* and the lactic acid bacteria may have molecular similarities, since all must have binding sites for the three ligands, pyruvate, FDP, and NADH. This type of lactic dehydrogenase is particularly advantageous for studying evolutionary relations because it is essential for energy metabolism and has a regulatory function. The regulated functional site could confer additional constraint on amino acid substitution during evolution and result in relative conservation of primary structure (1, 12, 24). Because of the similarity of these enzymes, a comparative biochemical and immunochemical study is being carried out.

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