Arch. Microbiol. 105, 303–312 (1975) – © by Springer-Verlag 1975

Purification and Properties of a Fructose-1,6-Diphosphate Activated L-Lactate Dehydrogenase from *Staphylococcus epidermidis*

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Received July 16, 1975

Abstract. L-(+)-lactate dehydrogenase (LDH) from *Staphylococcus epidermidis* ATCC 14990 was purified by affinity chromatography. The purified enzyme was specifically activated by fructose-1,6-diphosphate (FDP). The concentration of FDP required for 50% maximal activity was about 0.15 mM. The enzyme activity was inhibited by adenosine diphosphate (ADP) and oxamate. The inhibition by ADP appeared to be competitive with respect to reduced nicotinamide adenine dinucleotide (NADH). The catalytic activity of the LDH for pyruvate reduction exhibited an optimum at pH 5.6. The enzyme is composed of four, probably identical, subunits. Sephadex gel filtration and sedimentation velocity at pH 5.6 yielded molecular weights of about 130000 and 126000, respectively. The molecular weight at pH 6.5 and 7.0 was found to be only about 68000. Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate and sedimentation velocity at pH 2.0 or 8.5 revealed monomeric subunits with an approximate molecular weight of 36000. The thermostability of the heat labile enzyme was increased in the presence of FDP, NADH and pyruvate. The purified LDH exhibited an anomalous type of kinetic behavior. Plots of initial velocity vs. different concentrations of pyruvate, NADH or FDP led to saturation curves with intermediary plateau regions. As a consequence of these plateau regions the Hill coefficient alternated between lower and higher *n*-values. Some distinguishing properties of the *S. epidermidis* LDH and other LDHs activated by FDP are discussed.

Key words: *Staphylococcus epidermidis* – FDP-activated L-lactate dehydrogenase – Unusual kinetic behavior.

Staphylococci can grow as facultative anaerobes with the main product of glucose fermentation under anaerobic conditions being lactic acid. Previous studies by Schleifer and Kocur (1973) have demonstrated that the configuration of lactic acid is a valuable criterion for the classification of staphylococci. Stereospecific nicotinamide adenine dinucleotide (NAD) dependent lactate dehydrogenases (LDH, EC 1.1.1.27) catalyze the reduction of pyruvate to lactate. Preliminary investigations (Schleifer and Kocur, 1973) have revealed that the L-lactate dehydrogenase of *Staphylococcus epidermidis sensu stricto* is specifically activated by fructose-1,6-diphosphate (FDP).

In the present communication the purification of the FDP-activated L-LDH of *S. epidermidis* ATCC 14990 will be described. In addition, the catalytic and regulatory properties of this enzyme will be reported and compared with those of FDP-dependent LDHs from other bacteria.

Materials and Methods

Bacterial Strain. The strain used in this study was *Staphylococcus epidermidis* ATCC 14990.

Culture Conditions. The strain was grown under microaerophilic conditions in 10-l flasks of yeast extract-glucose-sodium chloride-broth (Schleifer and Kocur, 1973). The pH of the medium was adjusted to 7.5. 500 ml of a 7-hr old culture were used for inoculation. Cells were harvested at the end of the exponential phase of growth after a 7–8-hr incubation at 37° C. After centrifugation the cells were washed twice with 10 mM sodium phosphate buffer (pH 6.2) and stored at – 20° C as a wet cell paste.

Preparation of Cell-Free Extract. Cell-free extract was prepared from the thawed cell paste which was suspended in 0.1 M phosphate buffer (pH 6.2), containing 2 mM dithiothreitol (DTT). The cell suspension was mixed with glass beads and disintegrated in a water-cooled cell mill (Vibrogen-Zellmühle, Bühler, Tübingen).

Preparation of Affinity Chromatography Resin. Sepharose 4 B was first activated by treatment with cyanogen bromide and then substituted with hexamethylendiamine according to Cuatrecasas (1970). The condensation of the terminal amino group with potassium oxalate, to produce the insolubilized oxamate derivative, was carried out as described by O'Carra and Barry (1972).

Enzyme Assay. Lactate dehydrogenase activity was normally measured by following the decrease in absorption of reduced nicotinamide adenine dinucleotide (NADH) at 366 nm with an Eppendorf spectrophotometer equipped with a compensation recorder. The temperature was held constant using a thermostat. The standard assay system contained in a total volume of 1.17 ml phosphate buffer, pH 5.6 (0.1 M); sodium pyruvate (2.30 mM); NADH (0.58 mM); and FDP (0.40 mM). The reaction was initiated by addition of 0.020 ml enzyme solution. In certain stated experiments other buffers and pH values were used. All reactions were carried out at 25° C unless otherwise noted. One unit is the amount of enzyme to

catalyze the oxidation of 1 µmole of NADH per minute under the above assay conditions. Specific activity is expressed as units per milligram of protein.

Protein Determination. Protein was determined either by the A_{280}:A_{260} ratio spectrophotometric method of Warburg and Christian (1942) or by a modification of the procedure of Lowry (Hartree, 1972).

Sephadex Gel Filtration. Molecular weight determinations using Sephadex G 200 were carried out according to Andrews (1965). Gel filtration was performed with a bed dimension of 2.6 by 65 cm, and a flow rate of 3.6 ml/hr. The elutant was a 20 mM phosphate buffer (pH 5.6) containing 2 mM DTT, 2 mM FDP, and 0.1 M KCl. Before the LDH was eluted, the column was equilibrated with following markers: horse heart cytochrome *c* (MW 13500), ovalbumin (MW 45000), bovine serum albumin (MW 67000), rabbit muscle aldolase (MW 147000), and blue dextran (MW 400000). The collected marker proteins were detected by light absorption at 280 nm. After elution of the markers a dialyzed cell-free extract together with cytochrome *c* and blue dextran was applied to the column. The elution buffer now contained in addition 0.1 mM NADH to stabilize the LDH. 3 ml fractions were collected and assayed for LDH-activity. The position of cytochrome *c* and blue dextran was determined by light absorption at 546 and 578 nm.

Sucrose Gradient Technique. The determination of the molecular weight with a sucrose gradient was carried out according to Martin and Ames (1961). The sucrose gradients (5–20% weight per volume) contained 0.2 mM FDP in 0.1 M HCl/KCl buffer (pH 2.0), 0.02 M phosphate buffer (pH 5.6, 6.5 or 7.0) or 0.1 M Tris/HCl buffer (pH 8.5). Ovalbumin was used as standard protein. Samples of 0.1 ml were applied to the gradients giving a total volume of 4.1 ml. Centrifugation was carried out at 48000 rpm and 20° C (for 12 hrs) in an ultracentrifuge (L-50, Beckman) with a titanium rotor (56 Ti).

SDS-Electrophoresis. Molecular weight of polypeptide chains of the *S. epidermidis* LDH was also determined by comparing its mobility in sodium dodecyl sulfate gel electrophoresis with marker proteins of known molecular weights. Electrophoresis was performed in vertical slab gel plates. The preparation of the linear gradient of 5–20% acrylamide gel and the composition of the sample buffer are described by Laemmli (1970). The only modification was that SDS was omitted from the gel solution (Stoklosa and Latz, 1974). The marker proteins used were bovine catalase (MW 60000), ovalbumin (MW 43000), α-chymotrypsinogen (MW 25700), and horse heart cytochrome *c* (MW 11700). The protein solutions were incubated at 70° C for 20 min. Electrophoresis was performed with a constant current of 6 mA for approximately 12 hrs. The gel was charged with 1 µg protein. Staining, destaining, and the determination of the molecular weight of the LDH were carried out as described by Weber and Osborn (1969).

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in a vertical slab gel was used as a means of evaluating the purity of the enzyme. A 7.5% gel-system as described by Maurer (1971; gel system No. 6) was used. The electrophoresis was performed at 15 mA for about 2 hrs. Protein staining was carried out using the method of Weber and Osborn (1969).

For localisation of LDH activity, electrophoresis was performed in glass tubes with the same gel system as that described above. Enzyme activity was detected in parallel runs; one gel being tested for pyruvate to L-(+)-lactate reaction and the other for the reverse reaction as described by Stetter (1973). The enzyme position for the reverse reaction is visible as a violet band, whereas the negative staining after the reaction from pyruvate to L-(+)-lactate leads to an uncolored LDH-band.

Chemicals. All chemicals for culture conditions, cyanogen bromide, tris (hydroxymethyl) aminomethane (Tris), and 1,6-hexanediamine were products of Merck AG, Darmstadt. The pyridine nucleotides, fructose-1,6-diphosphate, aldolase, bovine serum albumin, α-chymotrypsinogen, bovine heart catalase, and the nucleotide phosphates were obtained from Boehringer, Mannheim. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide was purchased from Sigma Chemical Co., St. Louis. Sodium 2,4,6-trinitrobenzene sulfonate, blue dextran, ovalbumin, bis-acrylamide, ammonium persulfate, N,N,N′,-N′-tetramethylethylenediamine (TEMED), and sodium dodecylsulfate were products of Serva, Heidelberg; Sepharose 4B is a product of Pharmacia, Uppsala (Sweden).

Results

Isolation and Purification of the *Staphylococcus epidermidis* LDH

6 ml of crude extract, with a protein content of 12 mg/ml, was placed on a column (2 × 7 cm) filled with 5 ml of packed oxamate substituted sepharose. The column was first washed with 100 ml 50 mM phosphate buffer containing 0.18 M ammonium chloride, 0.1 mM NADH, 2 mM FDP, and 2 mM dithiothreitol (DTT). The effluent showed a high protein content but no LDH-activity. In a second elution LDH was recovered by omitting NADH from the eluting buffer.

For regeneration the column was washed with 0.6 M NaCl solution and the purification step was repeated with the LDH-fraction previously obtained. The second purification step leads to a more purified LDH but has the disadvantage of a lower yield. The binding of the LDH to the aminohexyl linked oxamate is weak, since a small increase in the salt concentration of the elution-buffer leads to a dissociation of LDH in the presence of NADH. A summary of the purification procedure is given in Table 1. The purity of the enzyme was demonstrated by polyacrylamide gel electrophoresis (Fig. 1).

Activation of the LDH by FDP

The activity of *S. epidermidis* LDH in a cooled (4° C) cell-free extract could be increased 3 to 5-fold by addition of FDP. If the cell-free extract was kept at room temperature, the activation with FDP was even more pronounced. After dialysis of the cell-free extract against a 10 mM phosphate buffer (pH 6.5), containing 2 mM DTT, the LDH shows no activity without FDP. However, the activity of the enzyme can be restored by adding FDP. These results indicate that some FDP is bound to the enzyme. It can be partially removed under room temperature conditions or totally by dialysis.

Table 1. Summary of the purification of LDH from *Staphylococcus epidermidis*

Step	Volume (ml)	Total protein (mg)	Total enzyme units	Recovery (%)	Specific activity	Degree of purification
Crude extract	6	72.00	1200	100	17	1
1. Affinity chromatography	6	0.90	1020	85	1133	67
2. Affinity chromatography	5	0.25	655	55	2220	130

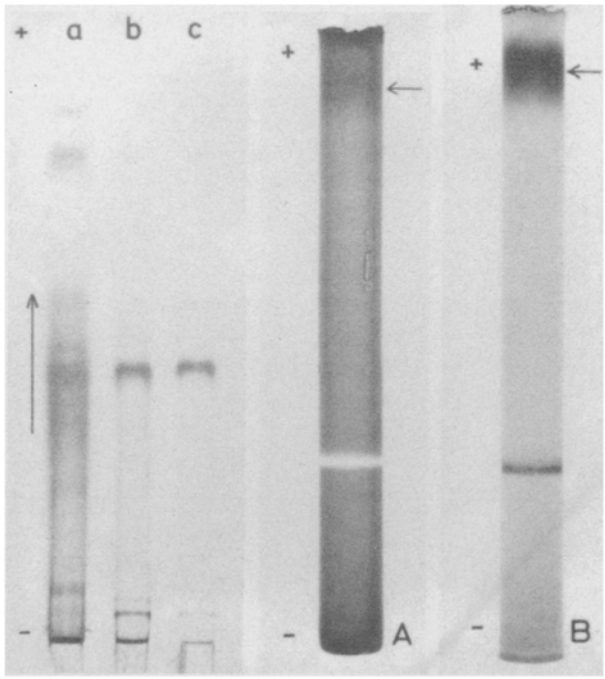


Fig. 1a–c. Control of the purification steps of LDH from *Staphylococcus epidermidis* by vertical slab gel electrophoresis. 10-µl samples were applied to slots in the stacking gel. The protein content per ml of the samples is given in Table 1. For protein staining see "Materials and Methods". The various samples are: (a) crude extract; (b) 1st step of affinity chromatography; (c) 2nd step of affinity chromatography. Arrow indicates direction of migration

Fig. 2A and B. Polyacrylamide gel electrophoresis of *S. epidermidis* LDH. Enzyme activity was detected for the reduction of pyruvate (A) and for the reverse reaction (B). The arrows indicate the position of tracking dye

FDP seems to be a very specific activator since other compounds such as glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, 6-phosphogluconate, 3-phosphoglycerate, and acetylphosphate at a final concentration of 8 mM were ineffective as activators. Neimark and Lemcke (1972) working on the FDP-activated L-(+)-LDH from *Acholeplasma laidlawii* type A found that the pyruvate concentration and the pH were able to influence the extent of activation by FDP. In contrast to these results an increase in the concentration of pyruvate to 10 mM or a variation of the pH in the test system had no influence on the activation of *S. epidermidis* LDH by FDP.

Coenzyme and Substrate Specificity

The purified enzyme only uses NADH or NAD as coenzyme, never NADPH or NADP. The enzyme is strictly specific for the L-(+) stereoisomer of lactate. It catalyzes the oxidation of L-(+)-lactate, but not of D-(−)-lactate. The oxidation of lactate to pyruvate is rather poor. Thus, the physiological function of FDP-activated LDH from *S. epidermidis* is that of a "pyruvate reductase". Polyacrylamide gel electrophoresis was used as means of detecting the activity of the LDH. Enzyme activity was determined both for the reduction of pyruvate and also for the reverse reaction (Fig. 2).

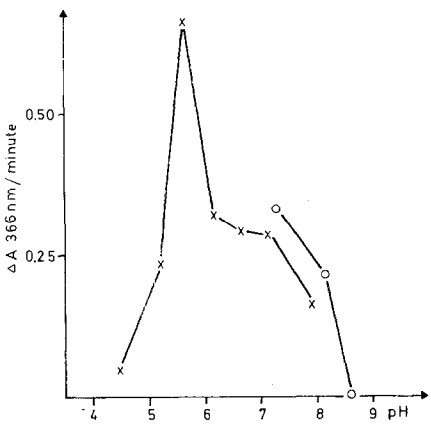
Molecular Weight Determinations of Active and Dissociated Enzyme

The molecular weight of the *S. epidermidis* LDH was determined by Sephadex gel filtration and sedimentation velocity on a sucrose gradient. The elution pattern on Sephadex G-200 of a dialysed crude extract from *S. epidermidis* LDH suggested a molecular weight of about 130000.

The sedimentation velocity of the purified *S. epidermidis* LDH was pH dependent. The molecular weight at pH 6.5 and 7.0 was found to be only 68000 ± 2000, whereas at pH 5.6 it was calculated to be 126000 ± 5000. At pH 8.5 and 2.0 the LDH is dissociated into an inactive molecular weight species of 36000. SDS gel electrophoresis of the purified LDH also leads to a single molecular weight species of 36000. These results indicate that the native enzyme is a tetramer which dissociates under certain pH conditions into active dimers or inactive monomers.

Effect of pH and Temperature

The LDH activity of the purified enzyme has a sharp pH optimum at pH 5.6 (Fig. 3). A marked decrease



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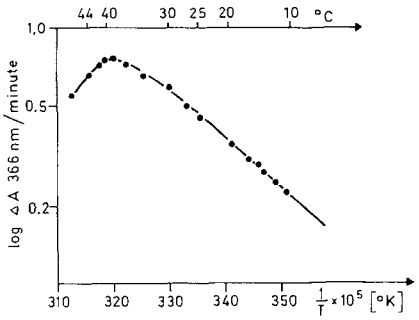

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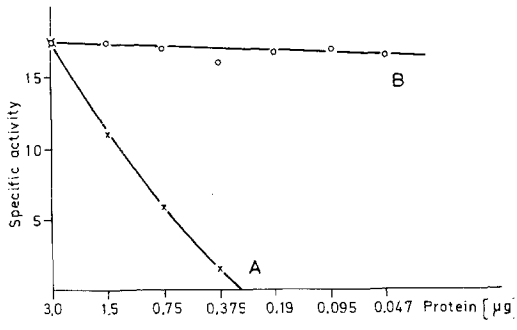

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Fig. 3. Effect of pH on *S. epidermidis* lactate dehydrogenase activity. The standard NADH-pyruvate assay was employed. Each reaction was initiated with 0.5 µg protein of purified enzyme (Table 1, step 2). × 0.1 M phosphate buffer; ○ 0.1 M Tris-HCl buffer

Fig. 4. Arrhenius plot of *S. epidermidis* lactate dehydrogenase activity. The standard assays were prewarmed to the temperature indicated and started by the addition of dialyzed cell-free extract (0.02 mg protein)

Fig. 5. Effect of dilution on the *S. epidermidis* lactate dehydrogenase activity. The standard NADH-pyruvate assay was employed. Each reaction was initiated by the addition of a dialysed cell-free extract with varying protein content as indicated. 0.1 M phosphate buffer, *A* with no added compounds, and *B* containing 0.5 mM FDP and 0.1 mM NADH, were used for dilution

Table 2. Influence of the ligands on the thermal inactivation of *S. epidermidis* LDH

Incubation time at 50°C (min)	Decrease in LDH activity (%)[a]						
	–	FDP[b]	FDP	FDP	–	–	–
	–	–	NADH	NADH	NADH	NADH	–
	–	–	–	Pyruvate	Pyruvate	–	Pyruvate
2	100	100	89	6	100	100	100
4			100	43			
8				71			
12				100			

[a] Enzyme activity of the sample before heat-treatment was taken as 100% of the maximum rate.
[b] Each ligand or combination was incubated at a final concentration of 25 mM with purified LDH and exposed to a 50°C water bath. 0.020-ml samples were removed at the times indicated above and immediately assayed for LDH activity as described in "Materials and Methods".

in activity occurs above this pH up to the range of pH 6.0–7.0 where a plateau is reached. At higher pH values the activity falls again and above pH 8.5 the enzyme is completely inactive (Fig. 3).

The temperature optimum of the enzyme reaction was determined by means of the Arrhenius plot (Fig. 4) and was found to be at 39°C.

Enzyme Stability

The enzyme is very sensitive to dilution and heat treatment. Dilution of the enzyme with buffer causes an inactivation of 50 or more percent (Fig. 5). To determine the pH-optimum for enzyme stability, the cell free extract was diluted with 0.1 M phosphate buffer of varying pH and kept at 4°C for 3 hrs. It was found that the loss of catalytic activity of the LDH reached a minimum at pH 6.5. Following chromatography on Sephadex G-200, activity was only found when FDP and NADH were present in the elution buffer. These two ligands together with pyruvate also protected the rather heat-labile enzyme from thermal inactivation (Table 2).

Unusual Kinetic Behavior

The saturation curves of the enzyme for substrate (pyruvate), coenzyme (NADH), and activator (FDP) show an unusual kinetic behavior. The kinetic pat-

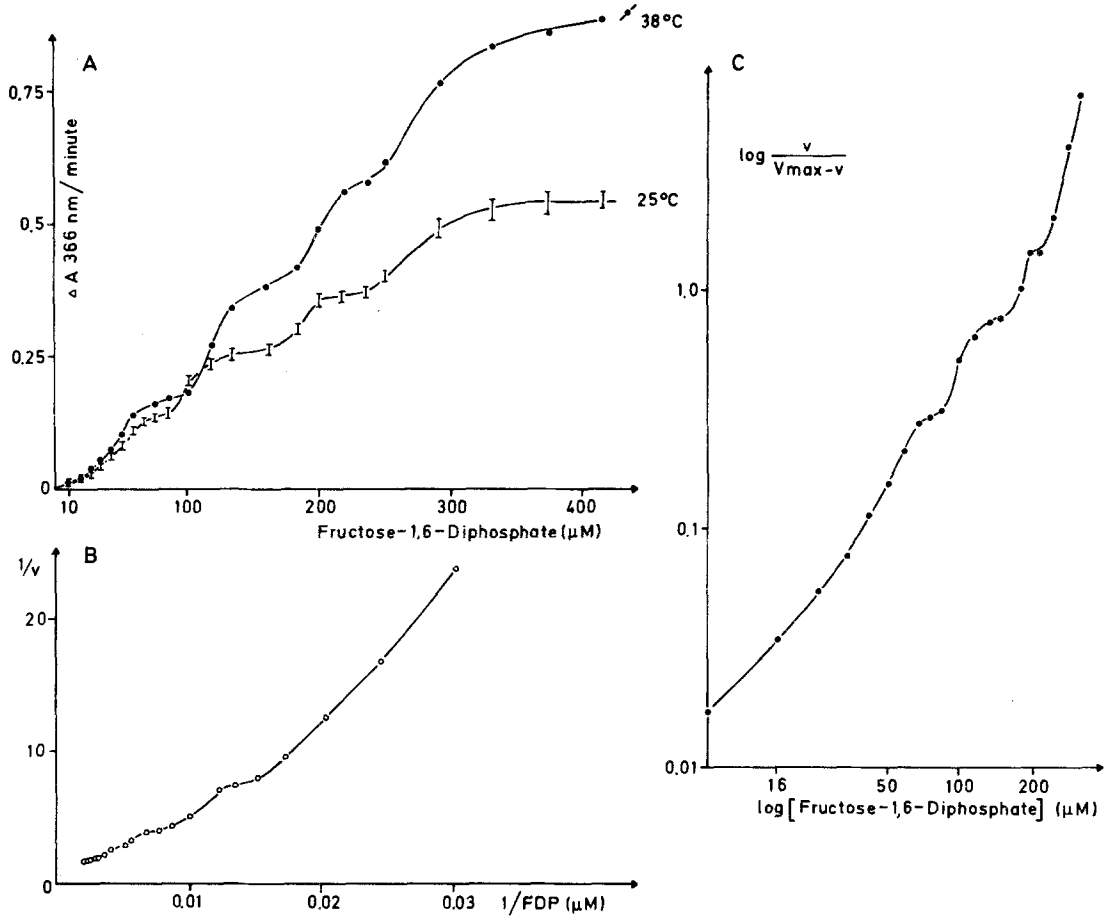

Fig. 6. (A) Dependence of the LDH from *S. epidermidis* ATCC 14990 on FDP for catalytic activity at various temperatures. The standard LDH assay was employed (see "Materials and Methods") except that the concentration of FDP was varied as shown. Each reaction was initiated with 0.5 μg protein of purified enzyme solution (Table 1, step 2). The maximal experimental deviation was determined by three values which are represented by the vertical lines on the 25° C curve. (B) Lineweaver-Burk plot of data from Fig. 6A at 25° C. (C) Hill plot of data from Fig. 6A at 25° C

terns of the saturation curves have a step-like character due to several intermediary plateaux. Fig. 6A shows the saturation curve for FDP at two different temperatures. The $(M)_{0.5v}$ value (Atkinson, 1966) is about 0.15 mM under the assay conditions. Similar intermediary plateaux are present in the Lineweaver-Burk plot (Fig. 6B) and also in the Hill plot (Fig. 6C). The slopes between the plateaux in the Hill plot (Atkinson, 1966) have *n*-values of about 3.0, while the plateaux themselves show an *n*-value of about 0.5 (Fig. 6C). This indicates that there are changes in the cooperativity.

In order to insure that these plateaux were not artefacts, enzyme kinetic studies were performed with various buffers, hydrogen ion concentrations, salt concentrations and temperatures. In each case the intermediary plateau regions of the kinetic curves were preserved.

In Fig. 7A the initial velocity vs. NADH concentration plots are shown. These saturation curves also display intermediary plateau regions. In comparison to the FDP saturation pattern, however, some differences are found. Firstly, the intermediary plateau regions are not as distinct with NADH as with FDP. Secondly, the initial part of the saturation curve shows a hyperbolic character whereas this part is sigmoidal in the FDP saturation curve. A raising of the temperature to 38° C leads to an increase in enzyme activity without significantly changing the undulating character of the saturation curve.

The $(M)_{0.5v}$ value for NADH is about 0.05 mM. The overall nature of the Lineweaver-Burk plot for NADH in Fig. 7B shows a slight downward concave curve. The Hill plot in Fig. 7C exhibits that at lower substrate concentrations the *n*-value is about 0.8 and at higher NADH concentrations this increased to 2. This again indicates that there is a change in cooperativity.

The initial velocity vs. pyruvate concentration plots at various temperatures also exhibited inter-

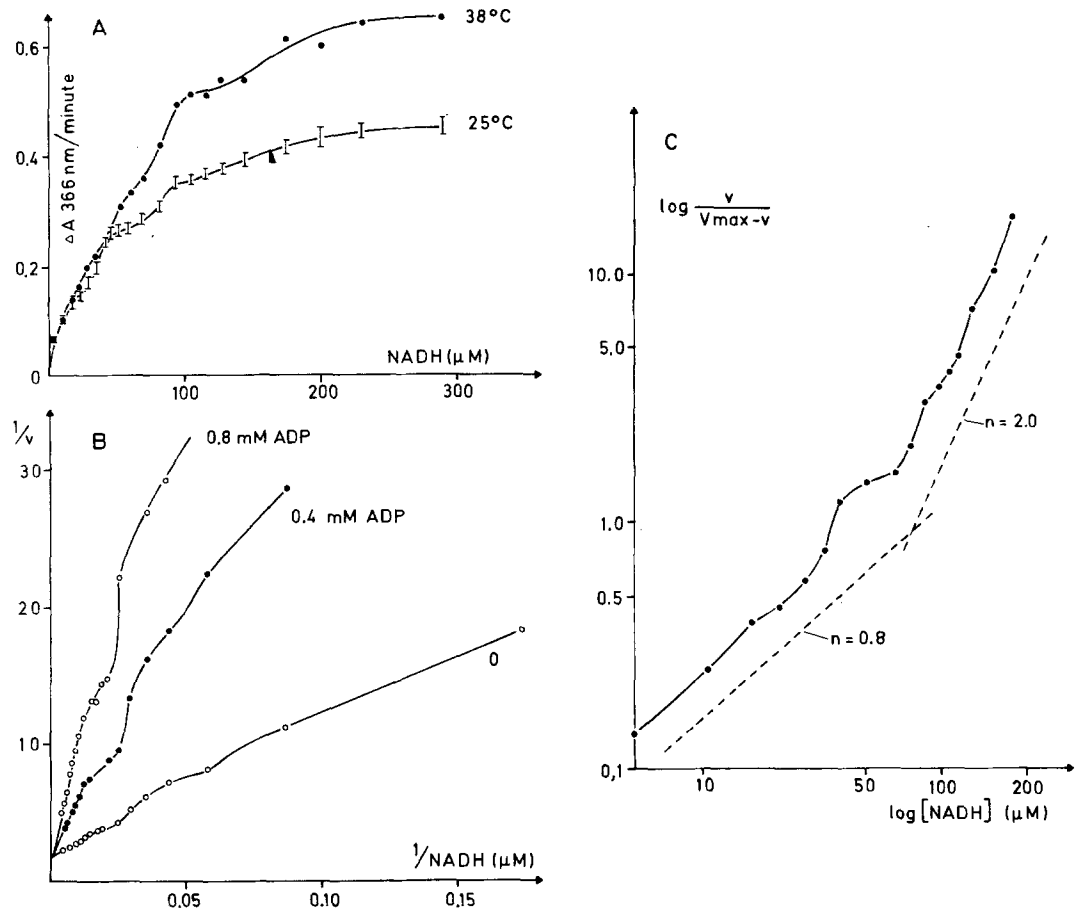

Fig. 7. (A) Coenzyme saturation curves for the LDH from *S. epidermidis* ATCC 14990 at various temperatures. The standard LDH assay was employed except that the concentration of NADH was varied as indicated. Each reaction was initiated with 0.5 μg protein of purified enzyme solution (Table 1, step 2). The maximal experimental deviation was determined from three values and is represented by the vertical lines on the 25° C curve. (B) Lineweaver-Burk plots at 25° C for NADH with 0.8 mM and 0.4 mM ADP and without ADP. (C) Hill plot of data from 7A at 25° C

Table 3. Nucleotide specificity for inhibition

Nucleotide phosphates tested	Final concentration (mM)	Inhibition of initial reaction rate (%)
ADP	4	100
ATP	4	36
AMP	4	31
GTP	4	10
CTP	4	5
UTP	4	4

LDH-activity was tested as in the previously described enzyme assay. Several nucleotide phosphates were added singly to the reaction mixture to a final concentration of 4 mM. Each reaction was initiated by addition of 0.5 μg purified enzyme solution (Table 1, step 2).

mediary plateau regions (Fig. 8A). The Lineweaver-Burk plot (Fig. 8B) shows an undulating slope but in contrast to the same plots for FDP and NADH it has no tendency to exhibit a concave upward or concave downward bent curve to the 1/v-axis. The $(S)_{0.5\,v}$ value for pyruvate was about 1 mM. From Fig. 8B it is also evident that oxamate is a competitive inhibitor of pyruvate. The Hill plot (Fig. 8C) shows different *n*-values for the plateaux and regions between the plateaux; for the former the *n*-value is 0.5 and for the latter 1.6.

Inhibition by Nucleotide Phosphates

A variety of nucleotide phosphates have an inhibitory effect upon the activity of the *S. epidermidis* LDH (Table 3). ADP was found to be the most potent inhibitor and could completely inhibit the LDH-activity. The ADP-inhibition appeared to be competitive with respect to NADH, since the Lineweaver-Burk plot (Fig. 7B) yielded nonlineary slopes intercepting on the 1/v-coordinate.

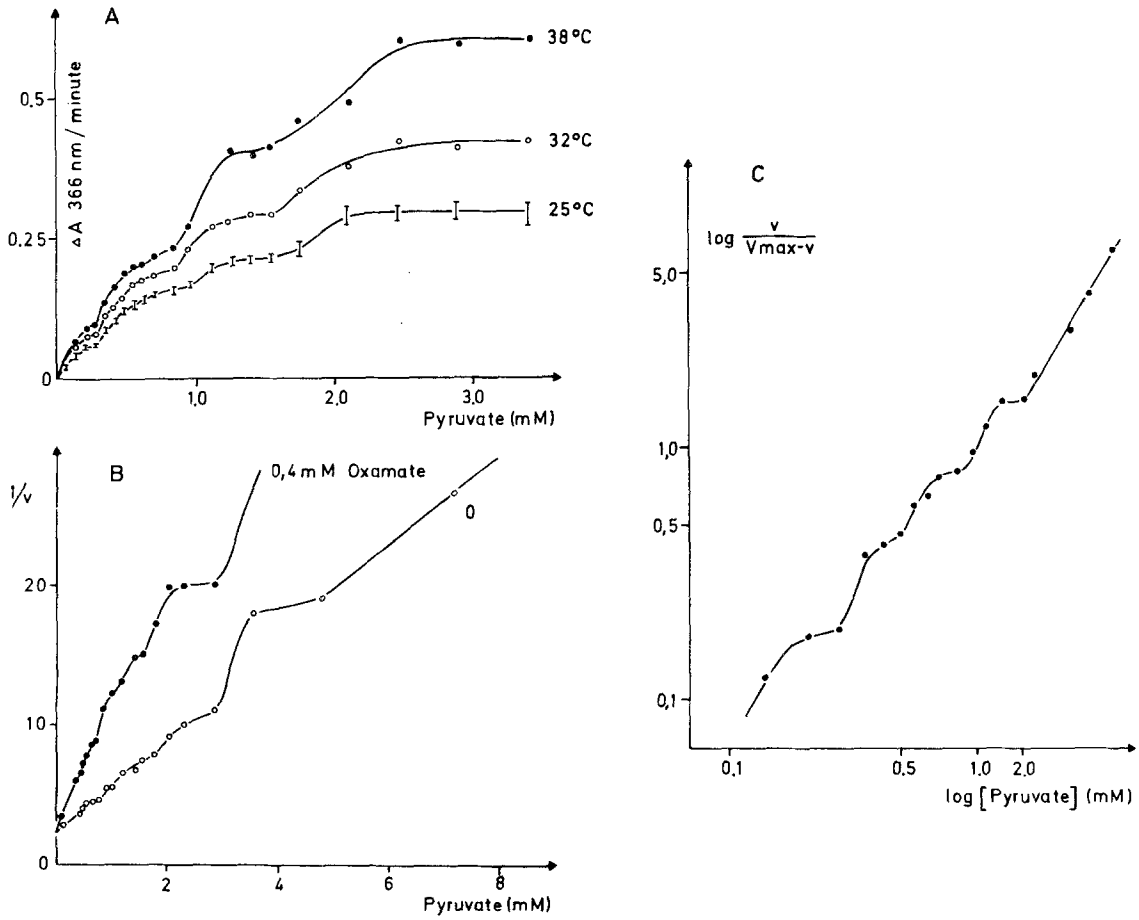

Fig. 8. (A) Pyruvate saturation curve for the LDH from *S. epidermidis* ATCC 14990 at various temperatures. The standard LDH assay was employed except that the concentration of pyruvate was varied as indicated. Each reaction was initiated with 0.5 µg protein of enzyme solution from step 2 (Table 1). The maximal experimental deviation was determined from three values and is represented by the vertical lines on the 25° C curve. (B) Lineweaver-Burk plots at 25° C for pyruvate with and without 0.4 mM oxamate. (C) Hill plot of data from 8A at 25° C

Discussion

Fructose-1,6-diphosphate activated LDHs have now been found in various bacteria such as streptococci (Wolin, 1964; Wittenberger and Angelo, 1970; Brown and Wittenberger, 1972; Jago *et al.*, 1971; Jonas *et al.*, 1972; Mou *et al.*, 1972), bifidobacteria (de Vries and Stouthamer, 1968; Lauer, 1974), *Acholeplasma laidlawii* (Neimark and Tung, 1973); and *Lactobacillus casei* (de Vries *et al.*, 1970; Stetter, 1973; Holland and Pritchard, 1975). The kinetic and physical properties of the FDP-dependent L-LDHs from different bacteria are summarized in Table 4 and compared with the properties of the L-LDH isolated from *Staphylococcus epidermidis* ATCC 14990. The NAD-dependent L-LDH from *S. epidermidis* exhibits an absolute requirement of FDP for its catalytic activity, whereas some LDHs which are listed in Table 4 show a catalytic activity even without FDP at certain pyruvate concentrations or within a certain pH range. The L-LDHs of *Streptococcus cremoris* and *S. lactis* are active without FDP at pH of 8.0 (Jago *et al.*, 1971; Mou *et al.*, 1972). For the enzymes of *Acholeplasma laidlawii* type A (Neimark and Tung, 1973), *Bifidobacterium animalis* and *B. adolescentis* (Lauer, 1974) a low activity is demonstrable in the absence of FDP at a pH near 6.5. Addition of FDP, however, increased the reaction rates of the enzymes. Like the LDHs of most streptococci and of *L. casei* the enzyme from *S. epidermidis* ATCC 14990 exhibited a pH optimum at about 5.6. In contrast to the LDHs of group N streptococci (Jago *et al.*, 1971; Mou *et al.*, 1972; Jonas *et al.*, 1972) which are still active at pH 8.0, the enzyme of *S. epidermidis* underwent irreversible denaturation above pH 7.5.

The concentration of FDP required by the enzymes for one-half maximum reaction velocity ($M_{0.5v}$ value) is quite different for the various bacteria ranging from 0.2 µM (*B. pseudolongum*) to 10 mM (*S. cremoris*, *S. lactis*). The LDH from *S. epidermidis* has an $(M)_{0.5v}$ value for FDP of 0.15 mM. This rather high concen-

Table 4. Comparison of some of the kinetic and physical properties

Organism	Requirement of FDP for catalytic activity	pH-optimum (with FDP)	Saturation curve for FDP	FDP(M)$_{0.5v}$ value or K_m(mM)	Effect of FDP on thermal stability	
					minus FDP	plus FDP
1. *Streptococcus mutans* NCTC 10449	absolute	5.5	sigmoidal	5.0	labile	stable
2. *S. faecalis*	absolute	5.8	sigmoidal	0.045	stable	labile
3. *S. cremoris* US3	not absolute[b]	5.0–7.0	–	10.0	labile	stable
4. *S. bovis*	absolute	–	–	0.050	–	–
5. *S. lactis* N760	not absolute[b]	5.0–7.0	–	10.0	labile	stable
6. *Acholeplasma laidlawii* A	not absolute[e]	7.0	hyperbolic	0.0024	labile	stable
7. *Bifidobacterium animalis* R 101-8	–	7.0	sigmoidal	0.028	–	–
8. *B. pseudolongum* PNC-296	absolute	5.7	hyperbolic	0.0002	–	–
9. *B. adolescentis* ATCC 15703	–	6.6	sigmoidal	0.005	–	–
10. *Lactobacillus casei* var. *rhamnosus* ATCC 7469	–	5.5	–	0.002	–	–
11. *Staphylococcus epidermidis* ATCC 14990	absolute	5.6	sigmoidal[h]	0.15	labile	stable[i]

[a] Oxamate is a competitive inhibitor with respect to pyruvate.
[b] Catalytic activity was also found without FDP at pH 8.
[c] ATP is a competitive inhibitor with respect to NADH.
[d] Inorganic phosphate inhibits the FDP-activation and increases the stability of the enzyme.
[e] The activation by FDP can be masked completely by raising the pyruvate concentration.

tration of FDP required for the activation of the enzyme may be of physiological significance. A low intracellular pool of FDP under aerobic growth conditions should prevent the formation of lactate and favor the further degradation of pyruvate. Under anaerobic conditions, however, the intracellular pool of FDP may be high enough to activate the LDH and stimulate the glycolytic pathway. Like the enzyme from *S. mutans*, the LDH from *S. epidermidis* was quite heat-labile and only addition of NADH, pyruvate and FDP protected the enzyme from thermal inactivation. This may indicate that all three ligands are necessary to stabilize the quaternary structure of the enzyme. Most of the other FDP-dependent L-LDHs only need FDP for protection against heat inactivation. An exception is the enzyme of *S. faecalis* which is rendered more heat-labile in the presence of FDP. The protecting effect of pyridine nucleotides on various dehydrogenases has been shown before by several authors (Chilson *et al.*, 1965a, b; Jaenicke *et al.*, 1971; Lovell and Winzor, 1974).

The molecular weight of the LDH of *S. epidermidis* is about 130000 daltons at the pH optimum of the enzyme (pH 5.6). This is in good agreement with the molecular weights of other FDP-dependent L-LDHs (Table 4). A shift in the pH leads to dissociation of the enzyme. Between pH 6.5 and 7.0 a dimeric state of the enzyme (68000 daltons) is found which is still active. At pH 2.0 or above 7.5 the activity of the enzyme was completely destroyed and an inactive monomeric subunit of the enzyme (36000 daltons) could be demonstrated.

In contrast to the FDP-dependent L-LDHs studied so far, the LDH of *S. epidermidis* exhibits significant deviations from the usual kinetics of enzyme-catalyzed reactions. The saturation curves are non-linear and exhibit a step-like form. Koshland and coworkers (Conway and Koshland, 1968; Teipel and Koshland, 1969; Cornish-Bowden and Koshland, 1970; Cook and Koshland, 1970) have described similar "bumpy saturation curves" for several enzymes. A nonlinearity for the double reciprocal plot of the reaction velocities at varying concentrations of pyridine nucleotides was also found for the human heart LDH (Nisselbaum and Bodansky, 1961). The reason for the abnormal Michaelis-Menten behavior of the LDH from *S. epidermidis* is not yet known. A possible explanation may be the lability of the quaternary structure of this enzyme. The occurrence of the step-like saturation curves for FDP, NADH and pyruvate may be due to the fact that all three ligands have to be present to render the enzyme stable. The cooperative interaction of the three ligands may influence the transition between the more active tetrameric and the less active dimeric state of the enzyme. This alteration of the quarternary structure of the enzyme would change the reaction rate and may explain the non-linear saturation curves. To prove this hypothesis, however, binding studies of the ligands combined with the determination of the quaternary structure of the enzyme would be necessary.

of FDP-dependent lactate dehydrogenases from several bacteria

Pyruvate$(S)_{0.5v}$ value or K_m(mM)	Saturation curve for pyruvate	Molecular weight (daltons)	Inhibitor	Reference
5.0	sigmoidal	135000	oxamate, ATP and ADP	Brown and Wittenberger (1972)
1.0	hyperbolic	135000	oxamate[a]	Wittenberger and Angelo (1970)
–	–	140000	ATP[c] inorganic phosphate[d]	Jago *et al.* (1971); Jonas *et al.* (1972)
–	–	–		Wolin (1964)
–	–	–		Mou *et al.* (1972)
0.058	hyperbolic	–	ATP, high phosphate and NADH concentrations	Neimark and Lemcke (1972) Neimark and Tung (1973)
1.7	sigmoidal	138000	inorganic phosphate[f]	Lauer (1974)
0.062	sigmoidal	–		
3.2	sigmoidal	140000		
–	–	–	inorganic phosphate[g], citrate and tris maleate[g]	Holland and Pritchard (1975)
1.0	hyperbolic[h]	130000	oxamate[a], ADP[j] and ATP	This study

[f] Inorganic phosphate competitively inhibits the binding of FDP.
[g] Inhibition caused by affecting the binding of FDP to the enzyme.
[h] The saturation curve shows plateau regions.
[i] Addition of NADH and pyruvate was necessary to render the enzyme more heat-stable.
[j] ADP is a competitive inhibitor with respect to NADH.

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