



Lactate Dehydrogenase from *Sus scrofa cristatus*

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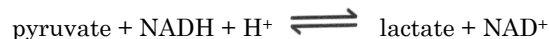
Abstract: Lactate dehydrogenase, LDH, (*EC*. 1.1.1.27), an anaerobic glycolysis enzyme present in all vertebrates, was isolated from the skeletal and heart muscle tissues of domestic pig, *Sus scrofa cristatus*. The process of purifying LDH from porcine tissue samples entailed the use of techniques like centrifugation, ammonium sulfate precipitation, dialysis, and gel filtration chromatography. At each stage of purification, enzyme and protein concentration assays were applied to each fraction collected in order to determine the degree of purification. The enzyme activity was determined by monitoring the rate of conversion of NAD⁺ to NADH as lactate is converted to pyruvate, while protein concentration was determined using the Bradford Assay. The values of the Michaelis-Menten constant (K_m), the catalytic constant (K_{cat}), and catalytic efficiency (K_{eff}) were determined using the Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots. These kinetic parameters were used to evaluate LDH in terms of binding and catalysis with respect to the coenzyme NAD⁺ in presence of the other substrate used in the assay, which is lactate. Results show that the K_m values of skeletal muscle LDH was lower as compared to that of the heart isozyme implying a higher affinity for NAD⁺. On the other hand the heart LDH isozyme showed a higher K_{cat} value while the skeletal muscle LDH isozyme has a higher K_{eff} . The isolates were also characterized using sodium dodecyl polyacrylamide gel electrophoresis (SDS PAGE). The subunits of this tetrameric enzyme were seen as distinct bands around 36,000 Daltons. This study is intended to establish an alternative source of lactate dehydrogenase for both teaching and research laboratories.

Key Words: lactate dehydrogenase; gel filtration; SDS-PAGE; isozymes; K_m ; K_{cat} ; K_{eff}

1. Introduction

Lactate dehydrogenase or LDH (*EC* 1.1.1.27), is the major catalyst at work during anaerobic (oxygen-independent) glycolysis. It

functions in the conversion of pyruvate to L(+)-lactate, and vice versa:



The reaction, sometimes denoted as the eleventh step of glycolysis, allows certain tissues to generate energy even under harsh conditions of oxygen deficiency, which happen in times of strenuous physical activity.

Lactate dehydrogenase has five isozymes depending on the tetrameric association of the two types of subunits present, namely, **M** chain and **H** chain. All LDH isozymes contain four polypeptide chains, each having a molecular weight of 33500 to 36500 Daltons (Garret and Grisham, 1988). The five isozymes are: M_4 , M_3H , M_2H_2 , MH_3 , and H_4 . The predominant form of lactate dehydrogenase in the skeletal muscles is the M_4 which favors the formation of lactate in the reversible reaction. On the other hand, the H_4 isozyme is the predominant form in the cardiac cells which favors the formation of pyruvate which proceeds to aerobic respiration. The coenzyme is in the form of oxidized nicotinamide adenine dinucleotide (NAD^+) / reduced nicotinamide adenine dinucleotide (NADH).

In this study lactate dehydrogenase from the heart and skeletal muscle tissues of domestic pig *Sus scrofa cristatus* was isolated using salting-out fractionation (Scopes, 1977) followed by dialysis. Gel filtration or size exclusion chromatography was used to purify lactate dehydrogenase (Farrel and Choo, 1989). The activity of the enzyme was measured by monitoring the formation of NADH from NAD^+ using UV Vis spectrophotometry (Farrel and Choo, 1989).

The kinetic properties of lactate dehydrogenase namely, the Michaelis-Menten constant (K_m), the Catalytic Constant or Turnover Number (K_{cat}), and Catalytic Efficiency (K_{eff}) from the two tissues were compared using the fraction obtained after gel filtration chromatography.

This study is intended to establish an alternative source of lactate dehydrogenase for use as a reagent in biochemical and biomedical research.

2. METHODOLOGY

Lactate dehydrogenase was isolated from fresh samples of skeletal and heart muscles from pig using the method of Farell, et. al 2000. Homogenized tissues suspended in phosphate buffer, pH = 7.0 were centrifuged at 20,000g at 4°C for 15 minute. The supernate was collected and subjected to ammonium sulfate precipitation at 40% and them at 65% saturation. The pellet is collected and dialyzed overnight with phosphate buffer at pH =7.0. To purify the enzyme, gel filtration chromatography using Sephadex G-100 resin was applied to the dialyzate. At each stage of isolation and purification, fractions were obtained to test for enzyme activity and protein concentration using lactate dehydrogenase assay and Bradford assay, respectively. Further, selected fractions collected in gel filtration chromatography were analyzed using SDS PAGE.

The rate of reaction was determined by monitoring the formation from NAD^+ from NADH, which absorbs radiation strongly at 340 nm. (The NAD^+ has zero absorbance at this corresponding wavelength.), and measuring how fast NADH is formed per unit time ($\Delta Abs/\Delta min$). From here, the units of LDH activity were computed in nmole/min.

The Michaelis-Menten curve was constructed by plotting the reaction velocity, represented by the units of activity against NAD^+ concentration. Also constructed were the following linear plots: Lineweaver-Burke, Eadie-Hofstee, and Hanes-Woolf. The K_m and V_{max} (maximum velocity) were determined from these plots.

Using the results generated in the Bradford assay, the catalytic constant or turnover number (K_{cat}) was computed from V_{max} using 146,000 daltons (or g/mole) as the molecular weight of the enzyme. Finally, the catalytic efficiency (K_{eff}) was solved using the empirical values obtained for K_m and K_{cat} .

3. RESULTS AND DISCUSSION

Table 1 and 2 presents the purification progress (Concepcion, et. al 1989; Farrel, et. al 2000) for the isolation and purification of LDH from skeletal and heart tissues. Results show an increase in the specific activity after each step with a dramatic increase in the specific activity and purification factor when the samples were purified using gel filtration chromatography (GFC).

The GFC eluates were characterized by SDS-PAGE. Figure 1 show results of SDS-PAGE. LDH is a tetrameric enzyme composed of M and H subunits, both with molecular weight of approximately 36 KDa. The two bands between 30 and 43 KDa correspond to the two subunits with the M subunit having a slightly higher molecular weight than the H subunit.

Table 1. Purification Progress (skeletal muscle LDH)

Fraction	Activity U/ml	Total Activity	Protein mg/ml	Specific Activity U/mg	Purification Factor
Crude Homogenate	0	0	0.2514	0	N/A
Supernate 1	0.000772	23.16	0.1090	7.08	1
Supernate 2	0.009646	270.10	0.0290	332.62	47
Pellet 2	0.013794	179.32	0.0259	532.60	75
Dialyzed Sample	0.014951	179.41	0.0252	593.30	84
GFC Eluate	0.019327	109.96	0.0055	3332.18	470

Table 2. Purification Progress (cardiac muscle LDH)

Fraction	Activity U/ml	Total Activity	Protein mg/ml	Specific Activity U/mg	Purification Factor
Crude Homogenate	0	0	0.0900	0	N/A
Supernate 1	0.000627	17.56	0.0517	12.13	1
Supernate 2	0.001061	28.65	0.0262	40.50	4
Pellet 2	0.001399	19.59	0.0054	259.07	21
Dialyzed Sample	0.007524	97.81	0.0053	1419.62	117
GFC Eluate	0.008247	41.24	0.0038	2170.00	170

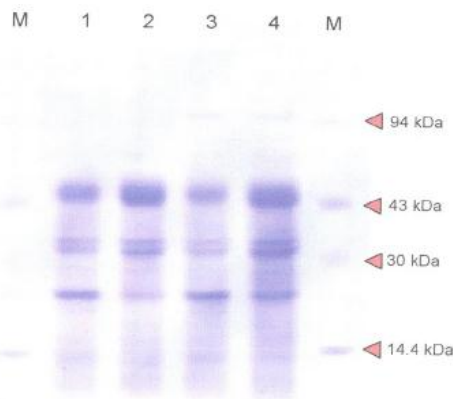


Figure 1. SDS-PAGE. Lane M (molecular weight standards); Lane 1 Heart LDH (Trial 1); Lane 2 Skeletal Muscles LDH (Trial 1); Lane 3 Heart LDH (Trial 2); Lane 4 Skeletal Muscles LDH (Trial 2);

Kinetic studies were done on the last fraction (GFC eluate). A comparison of the K_m , K_{cat} , and K_{eff} using various plots in the kinetic studies are shown in Tables 3 and 4. Results show that the K_m of the LDH in skeletal muscles cells is lower as compared to that of the heart counterpart indicating a higher affinity for NAD^+ . This implies that the LDH from skeletal muscles is able to catalyze reactions even at relatively lower concentration of NAD^+ . The catalytic constant K_{cat} , is a measure of the rate of catalysis interpreted as the number of molecules converted into per molecule of the enzyme per unit time. A higher K_{cat} was observed for the heart LDH but the difference is not that significant. The last kinetic parameter, the catalytic efficiency represents the $K_{cat} \cdot K_m$ ratio. An ideal enzyme is one with a low K_m (higher substrate affinity) and a high K_{cat} in order to convert the enzyme-substrate complex (ES) into the product efficiently. Therefore the higher is the K_{eff} , the greater is the catalytic efficiency of the enzyme. Results show the skeletal LDH has a higher catalytic efficiency as compared to the heart counterpart. Likewise, it should be noted that differences are not significant and that both isozymes are fairly efficient as catalyst.

Table 3. Values of K_m , K_{cat} , and K_{eff} (skeletal LDH)

Plot	K_m mM	K_{cat} min^{-1}	K_{eff} $\text{min}^{-1}\text{mM}^{-1}$
Michaelis-Menten	0.30	12698	42327
Eadie-Hofstee	0.45	15873	35273
Lineweaver-Burk	0.33	12698	38479
Hanes-Woolf	0.36	13757	38214
Standard deviation	0.065	1497	2894

Table 4. Values of K_m , K_{cat} , and K_{eff} (heart LDH)

Plot	K_m mM	K_{cat} min^{-1}	K_{eff} $\text{min}^{-1}\text{mM}^{-1}$
Michaelis-Menten	0.38	15385	40486
Eadie-Hofstee	0.57	20000	35086
Lineweaver-Burk	0.45	16923	37606
Hanes-Woolf	0.50	18462	36924
Standard deviation	0.080	1968	2242

4. CONCLUSIONS

The enzyme lactate dehydrogenase (LDH) was isolated and purified from the skeletal and heart muscles of Southeast Asian domestic pig. Both tissues provided significant amount of enzyme as proven by the high values of activities obtained in the enzyme assay conducted. A kinetic study was done using the eluate from both samples and three kinetic parameters pertaining to NAD^+ were reported, namely, K_m , K_{cat} , and K_{eff} . These quantities were indicators of how effectively LDH would interact with coenzyme NAD^+ in terms of binding and catalysis in the presence of the other substrate used in the assay, which is lactate. From the study, it is recommended that isolation and

purification of LDH be done using a different enzyme activity assay, for example, one which will monitor the formation or disappearance of lactate or pyruvate. Likewise, the kinetic study on LDH may be broadened so as to include kinetic LDH inhibitors.

5. ACKNOWLEDGMENT

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