Electronic Journal of Biotechnology 35 (2018) 18–24

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Recombinant production and biochemical and *in silico* characterization of lactate dehydrogenase from *Geobacillus thermodenitrificans* DSM-465

Muhammad Shahid Nadeem *, Maryam A. Al-Ghamdi, Jalaluddin Azam Khan, Saida Sadath, Abdulaziz Al-Malki

Department of Biochemistry, Faculty of Science, Building A-90, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 19 February 2018
Accepted 23 June 2018
Available online 03 July 2018

Keywords:
Docking
Escherichia coli expression
Geobacillus thermodenitrificans
Glycolytic pathway
In silico studies
Inhibitors
Lactate dehydrogenase
Recombinant enzyme
Thermostable

ABSTRACT

Background: Lactate dehydrogenase (LDH) is an enzyme of glycolytic pathway, ubiquitously found in living organisms. Increased glycolysis and LDH activity are associated with many pathologic conditions including inflammation and cancer, thereby making the enzyme a suitable drug target. Studies on conserved structural and functional domains of LDH from various species reveal novel inhibitory molecules. Our study describes *Escherichia coli* production and characterization of a moderately thermostable LDH (LDH-GT) from *Geobacillus thermodenitrificans* DSM-465. An *in silico* 3D model of recombinant enzyme and molecular docking with a set of potential inhibitors are also described.

Results: The recombinant enzyme was overexpressed in *E. coli* and purified to electrophoretic homogeneity. The molecular weight of the enzyme determined by MALDI-TOF was 34,798.96 Da. It exhibited maximum activity at 65°C and pH 7.5 with a K_M value for pyruvate as 45 μM. LDH-GT and human LDH-A have only 35.6% identity in the amino acid sequence. On the contrary, comparison by *in silico* structural alignment reveals that LDH-GT monomer has approximately 80% identity to that of truncated LDH-A. The amino acids "GEHGD" as well as His^{179} and His^{193} in the active site are conserved. Docking studies have shown the binding free energy changes of potential inhibitors with LDH-A and LDH-GT ranging from −407.11 to −127.31 kJ mol^{-1}.

Conclusions: By highlighting the conserved structural and functional domains of LDH from two entirely different species, this study has graded potential inhibitory molecules on the basis of their binding affinities so that they can be applied for *in vivo* anticancer studies.

How to cite: Nadeem MS, Al-Ghamdi MA, Khan JA, et al. Recombinant production, biochemical and *in silico* characterization of lactate dehydrogenase from *Geobacillus thermodenitrificans* DSM-465. Electron J Biotechnol 2018;35. https://doi.org/10.1016/j.ejbt.2018.06.003.

© 2018 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Lactate dehydrogenase (LDH; EC 1.1.1.27) is a glycolytic enzyme catalyzing the simultaneous interconversion of pyruvate and lactate [1]. Lactate is produced under hypoxic conditions by normal cells and under aerobic conditions by human cancer cells [2, 3]. Because of metabolic reprogramming, the lactate production is increased up to 40 times in cancer cells compared to that in normal cells [4]. Lactate is considered as an important energy fuel and a starting molecule for gluconeogenesis [5]. The increase in enzyme activity in malignant cells results in acidosis and pain. Hence, LDH inhibitors are considered as the key molecules for cancer treatment [6, 7]. LDH sensor strips have been introduced to replace the conventional plasma enzyme detection procedures [8]. Recently, LDH-based wearable biosensors have been introduced to detect lactate in the sweat to evaluate stress response and human performance [9]. Similarly, LDH-based glucose sensing cells are also applied in clinical investigations [10]. In addition to their clinical applications, such biosensors are also used for the detection of lactate in food and beverages [11]. LDH has been isolated and characterized from a wide range of organisms including animals, plants, and bacteria [12, 13]. Nucleotide sequences of DNA encoding the enzyme have been cloned and analyzed from a variety of organisms including mammals [14], bacteria [15], silkworm [16], and protozoans [17], and the characteristics of recombinant enzymes have also been studied.

In silico 3D structure determination and molecular docking techniques have been extensively applied to explore the affinities of small molecules in the binding site of targeted enzymes [18]. As an increasingly used bunch of tools and techniques in drug discovery, the binding specificity of small compounds against an enzyme can be estimated for applications *in vivo*. In case of target enzymes, the algorithms are applied to determine the inhibitory molecules with minimum binding energies [19]. During the last two decades,

* Corresponding author.
E-mail address: mhalim@kau.edu.sa (M.S. Nadeem).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2018.06.003
0717-3458/© 2018 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

approximately 60 docking software and tools were introduced under different names, for commercial and academic applications [20,21]. The present study describes *Escherichia coli* expression, purification, and properties of L-LDH from *Geobacillus thermodenitrificans* DSM-465 (LDH-GT). We have demonstrated a 3D model for the recombinant enzyme that was further subjected to molecular docking studies against substrates, coenzymes, and potential inhibitors. The conservation of structural and functional residues was also analyzed by comparison with human LDH-A.

2. Materials and methods

2.1. Materials and chemicals for DNA manipulations

All the kits for PCR, DNA restriction, and ligation as well as chemicals and materials for cloning of recombinant plasmids, DNA isolation from agarose gel, plasmid isolation and purification, and characterization of recombinant enzyme were purchased from Sigma-Aldrich and Thermo Fisher. The genomic DNA of *Geobacillus* strain DSM-465 was obtained from DSMZ Germany. Modified bacterial strain (BL21 (DE3) codon plus RIL) and T7 promoter-based expression plasmid (pET21a (+)) were generously provided by the laboratories of the School of Biological Sciences, University of the Punjab, Lahore, Pakistan.

2.2. PCR amplification of the LDH-GT gene

The PCR amplification of the LDH gene was carried out using the primer sequences 5′-catatgaaaaacggaggaggaaacagag-3′ and 5′-ggatc cttactgcgcaaaggagc-3′. The restriction sites for *Nde*I and *BamH*I were introduced in the primer sequences to obtain the sticky ends along the complete open reading frame (ORF). The PCR reaction mixture consisted of 0.75 mM dNTPs; 1× *Taq* polymerase buffer containing KCl, 2.5 mM $MgCl_2$, and 2.5 U of *Taq* polymerase; 20 pM of each primer; and 2 μL of diluted DNA template with nuclease-free water, thus making a final volume of 25 μL. The thermocycler was initially adjusted for denaturation at 94°C for 4 min, followed by another denaturation step at 94°C for 40 s, annealing at 63°C for 1 min, and extension at 72°C for 1.5 min in the second step; these steps were repeated as 35 cycles. The final extension at 72°C for 25 min was carried out for the addition of poly-A sequence at the 3′-end of the amplified fragment.

2.3. Gene cloning and expression

The purified PCR product was ligated to the pTZ57R/T plasmid, and *E. coli* DH5α cells were transformed. Colonies successfully transformed with recombinant plasmids were selected initially by blue–white screening and further confirmed by the restriction analysis of isolated plasmids. The gene was cleaved from the pTZ57R/T plasmid using the restriction enzymes *Nde*I and *BamH*I and ligated to the pET21a(+) plasmid cleaved by the same pair of enzymes. The ligation mixture was used for the transformation of BL21 (DE3) RIL Codon plus cells according to the procedure provided in the InsTAclone kit (Thermo Fisher Inc., catalog no. K1213). A single colony of BL21 cells harboring the target gene was inoculated overnight in LB broth medium containing ampicillin (100 μg/mL). One percent inoculum from the above overnight culture was used for the growth of bacteria and incubated in an incubator shaker at 150 rpm and 37°C to attain an optical density of 0.1 at 600 nm wavelength; 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was then added to the culture, and the culture was grown overnight at 20°C to induce gene expression.

2.4. Purification of recombinant enzyme

Bacterial cells were harvested by centrifugation and suspended in 45 mL of ice-cold 20 mM phosphate buffer, pH 7 (buffer A). The sample was sonicated in an ice box at moderate power for 10 min (2 min of rest followed after every 1 min of shock cycle). The homogenate was incubated at 70°C for 20 min followed by centrifugation at 12,000x *g* for 15 min at 4°C. The supernatant was precipitated using ice-cold acetone, and the precipitate was dissolved in buffer A and dialyzed overnight in 10 volumes of the same buffer at 4°C. Clear dialysate was obtained by centrifugation at 12,000 × g for 15 min at 4°C and subjected to a DEAE Sephadex column equilibrated with buffer A. The bound protein was eluted by a linear gradient of 0–0.5 M NaCl. Enzyme activity, specific activity, and other parameters were recorded at each purification step and tabulated.

2.5. Molecular weight of recombinant LDH

The purity and molecular weight of the recombinant enzyme were determined by 15% SDS-PAGE [22] and MALDI-TOF analysis. Two microliters of the purified recombinant enzyme solution (2 μg/μL) was mixed with 20 μL of matrix-B (5 mg of sinapinic acid in 1 mL of 35% acetonitrile containing 0.15% trifluroacetic acid [TFA]), and 6 μL of this mixture was spotted on a mass spectrometric plate, and the spot was air dried for 30–40 min. The spectrum was recorded with Bruker Autoflex MALDI-TOF (Bruker Daltonics Inc., Billerica, MAUSA).

2.6. Enzyme kinetics

The K_M value for pyruvate was determined by generating a Lineweaver–Burk double reciprocal plot by using a linear increase in substrate concentration, starting from 5 to 320 μM. The reaction consisted of 280 μM of NADH prepared in phosphate buffer, pH 7.0. The reaction mixture was adjusted at different temperatures to find out the optimum temperature for enzyme activity. Temperature stability of the enzyme was determined by incubating the enzyme sample at 40, 50, 60, 70, 80, and 90°C for 5 min followed by enzyme assay. Enzyme activity was also measured by using the reaction mixture adjusted at pH ranging from 4.0 to 8.5 to determine the optimum pH.

2.7. In silico protein modeling and validation

As the protein structure of LDH-GTD (LDH of *G. thermodenitrificans*

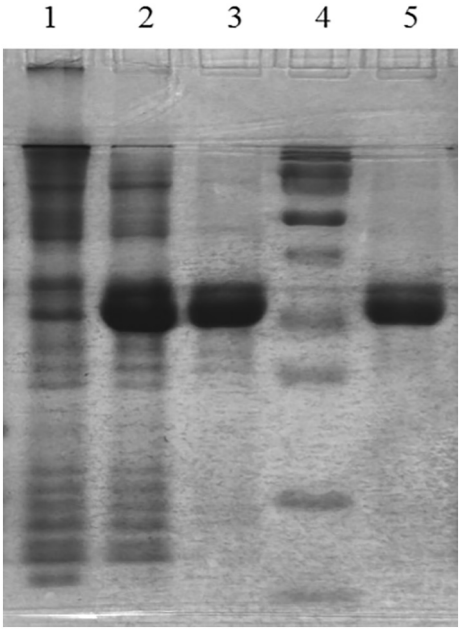

Fig. 1. Image of SDS-PAGE. Lane 1: Negative control, induced pET21a (+) proteins in *E. coli*, Lane 2: Induced pET21-GT proteins, Lane 3: Selective precipitate after heat treatment, Lane 4: Protein marker (Thermo Fisher catalog no. 26616), Lane 5: Purified LDH.

Download English Version:

https://daneshyari.com/en/article/6618482

Download Persian Version:

https://daneshyari.com/article/6618482

Daneshyari.com