International Journal of Biological Macromolecules 108 (2018) 88–97

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Unique properties of arginase purified from camel liver cytosol

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ARTICLE INFO

Article history:
Received 26 July 2017
Accepted 21 November 2017
Available online 24 November 2017

Keywords:
Camel
Arginase
Purification

ABSTRACT

Arginase (ARG) is an enzyme involved in urea cycle, where it catalyzes the hydrolysis of L-arginine into L-ornithine and urea. Since there is no information about the isolation and purification of ARG from camel liver, this investigation was designed to purify and characterize ARG from camel liver and compare its molecular and kinetic properties with that reported from other species. Camel liver arginase (CL-ARG) was purified to homogeneity using heat denaturation followed by ammonium sulphate precipitation with a combination of DEAE-cellulose, SP-Sepharose and Sephadex G 100-120 chromatography columns. The specific activity of CL-ARG was increased to 18,485 units/mg proteins with 23.5-fold purification over crude homogenate. It was observed that CL-ARG showed a similarity with other species such as behaviour on DEAE-cellulose column, kinetics of inhibition, necessity for metal ions as cofactor, and alkaline optimum pH. On the contrary, CL-ARG differed in its molecular weight (180 kDa), oligomeric protein structure, slightly neutral-alkaline *p*I value (7.7), K_m value (7.1 mM), optimum pH (9, 10.7), and higher optimum temperature (70 °C). In conclusion, this study investigated the properties of CL-ARG via a simple and reproducible purification procedure and provided valuable information for its production from available source in Egypt for medical and industrial purposes.

1. Introduction

L-arginase (E.C 3.5.3.1, L-arginine amidinohydrolase, ARG), one of the urea-cycle enzymes, is a binuclear manganese cluster metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea [1,2]. Arginase has roots in early life forms and is widely distributed in the five kingdoms of organisms as diverse as bacteria, yeasts, plants, invertebrates and vertebrates [3]. Arginases have been purified and characterized from a wide variety organisms [4]. Also, the crystal structure of arginases from many species has been solved, including those from *Homo sapiens* [5], *Rattus norvegicus* [6] and *Bacillus caldovelox* [7]. Mammalian arginase is active as a trimer, but some bacterial arginases are hexameric [8]. The enzyme requires a two-molecule metal cluster of manganese in order to maintain proper function. These Mn^{2+} ions coordinate with water, orienting and stabilizing the molecule by allowing water to act as a nucleophile and attack L-arginine, hydrolyzing it into ornithine and urea [9]. In most mammals, two isozymes of this enzyme exist: cytoplasmic urea cycle arginase I (ARG I) or liver arginase which is highly expressed in the liver primarily to carry out ureagenesis via ammonia detoxification [10,11] and a second mitochondrial isoenzyme arginase II (ARG II) or nonhepatic arginase which is expressed in trace amounts in extra-hepatic tissues that lack a complete urea cycle, especially kidney, prostate gland, brain and lactating mammary gland [12] which is involved in L-arginine homeostasis [13] and regulating L-ornithine pools for subsequent biosynthetic transformations including the biosynthesis of polyamines, glutamate, proline [14] and controlling tissue level arginine for nitric oxide (NO) biosynthesis [15] by competing with inducible nitric oxide synthase (iNOS) for their common substrate, L-arginine, which is an important determinant of the inflammatory response in various organs and regulating nitric oxide-dependent apoptosis [14,16,17].

ARG I is one of the most important mammalian enzymes responsible for nitrogen metabolism since it comprises the main route for the elimination of excess nitrogen resulting from amino acid and nucleotide metabolism [18]. ARG I deficiency leads to hyperargininemia, characterized by progressive neurological and intellectual impairment, persistent growth retardation and infrequent episodes of hyperammonemia [19]. ARG I and ARG II possess the same catalytic function but differ with respect to tissue distribution, cellular localization, metabolic function, physicochemical and kinetic properties and immunological cross reactivity [4,20]. Arginases from eukaryotes have been under extensive study because they have important biological functions and are associated with a variety of diseases, such as diabetes [20], cardiovascular disorders and cancer progression [13,21]. Arginase, a powerful anti-

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https://doi.org/10.1016/j.ijbiomac.2017.11.141
0141-8130/

cancer enzyme, has been studied *in vitro* to treat several types of cancer, such as breast, rectal, and colon, to depletes blood L-arginine levels in order to starving cancer cells that are auxotrophic to L-arginine amino acid and argininosuccinate synthase-expressing tumors [22]. Many studies have shown that the increased stimulation of arginase expression in animal metabolism leads to the production of polyamines, which helps to stop tumor cell proliferation and wound healing [23].

Camel is one of the most common domestic mammals in Egypt, Arab world and the Middle East area, and arginase enzyme has never been purified from camel liver. Therefore, in this study, we attempted for the first time to set up a simple scheme to purify and characterize arginase from the camel liver cytosol to provide the optimal conditions for the production of arginase from camel liver as a locally available source for medical and industrial applications.

2. Materials and methods

2.1. Liver tissue

Fresh liver samples of adult male camel *Camelus dromedaries* obtained from Cairo Slaughterhouse, were collected from at least ten different animals. Liver samples were obtained within an hour from sacrifice and washed with cold physiological isotonic saline to remove contaminating erythrocytes.

2.2. Reagents and chemicals

Bovine serum albumin (BSA), L-arginine hydrochloride, thiosemicarbazide, diacetylmonoxime, urea, DEAE-cellulose (preswallen), gel filtration molecular weight marker protein kit (12.4–200 kDa), molecular weight SDS marker proteins (6.5–66 kDa) and isoelectric focusing protein marker kit (*p*I 3.2-9.3) were purchased from Sigma-Aldrich. Sephadex G 100-120 and Sulphopropyl Sepharose were purchased from Pharmacia. All other commercially available chemicals were of analytical grade and highest purity.

2.3. Assay of arginase activity

Arginase activity was assayed by the colorimetric determination of urea released from the hydrolysis of L-arginine by arginase enzyme using diacetylmonoxime and thiosemicarbazide reagents according to Geyer and Dabich [24] as modified by Dabir et al. [25]. Arginase reaction started by adding the enzyme solution to a reaction mixture (1 ml) containing 50 mM carbonate-bicarbonate buffer (pH 9.5) containing 25 mM L-arginine and 6 mM $MnCl_2$. The assay reaction mixture was incubated at 37 °C for 30 min then the reaction was stopped by adding 10% TCA. The mixture was centrifuged to remove the denatured protein and the produced urea was determined in the supernatant by adding 2 ml of the color reagent (3.6 mM diacetylmonoxime and 61.7 mM thiosemicarbazide) followed by 3 ml of acid solution (20% sulphuric acid, 56.7% o-phosphoric acid and 0.12 M ferric chloride) to 1 ml of the supernatant. The reaction mixture was placed in a boiling water bath for 10 min then ice cooled. The optical density of the solution was measured against a reagent blank at 520 nm. Urea standard curve was constructed using standard urea to cover the range from 500 to 5000 μM. The activity unit is defined as the amount of enzyme that catalyzes the release of 1 μmole of urea per minute at 37 °C and pH 9.5.

2.4. Protein quantification

The total protein concentration was determined according to Lowry et al. [26] as modified by Cooper [27], using bovine serum albumin (BSA) as a standard protein.

2.5. Purification of cytosolic camel liver arginase

2.5.1. Preparation of crude extract

Twenty g of liver were homogenized on ice in 100 ml of 100 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MnCl_2$ and 100 mM KCl. The liver homogenates were centrifuged at 4 °C at 15,000 *xg* for 30 min to collect a clear supernatant (cytosol). The clear supernatants containing most of the enzyme activity were saved and the pellets were discarded.

2.5.2. Heat treatment

Liver crude extract was incubated at 60 °C for 20 min then ice cooled. The denatured proteins were removed by centrifuging the protein solutions at 5000×*g* for 10 min and the collected supernatants were designated as heat-treated enzyme solutions.

2.5.3. Ammonium sulphate fractionation

The supernatants of heat-treated sample was adjusted to 35% saturation with ammonium sulphate by the addition of solid ammonium sulphate in a 4 °C ice bath with continuous stirring using magnetic stirrer for 30 min and the mixtures formed were centrifuged at 5000×*g* for 10 min at 4 °C. The precipitate was discarded and the collected supernatant was raised to 75% saturation with ammonium sulphate. After one hour, the mixture was centrifuged at 10,000×*g* for 10 min at 4 °C to collect the precipitated proteins. The precipitate was completely dissolved in 15 mM Tris-HCl buffer (pH 8) containing 5 mM $MnCl_2$ and 1 mM β-mercaptoethanol, using a magnetic stirrer for 15 min in a 4 °C ice bath. The supernatant was collected after centrifugation of the dissolved protein solutions at 4 °C for 5 min at 5000×*g*. The collected supernatant was dialyzed overnight against 15 mM Tris-HCl buffer (pH 8) containing 5 mM $MnCl_2$ and 1 mM β-mercaptoethanol at 4 °C using dialysis cellophane bag. The dialyzed protein solution was centrifuged at 3000×*g* for 5 min at 4 °C. This protein solution was designated as dialyzed ammonium sulphate fraction.

2.5.4. Diethylaminoethyl (DEAE) cellulose column chromatography

The dialyzed ammonium sulphate fraction was mounted on the top of a DEAE-cellulose column previously equilibrated with 15 mM Tris-HCl buffer (pH 8) containing 5 mM $MnCl_2$ and 1 mM β-mercaptoethanol. After washing the column, the adsorbed proteins were eluted using an equilibration buffer containing 0–1 M sodium chloride gradient at a flow rate of 30 ml/h. Fractions containing most of the enzyme activities were pooled (DEAE-cellulose pooled fraction) and dialyzed overnight at 4 °C against 15 mM Tris-HCl buffer (pH 5.6) containing 5 mM $MnCl_2$ and 1 mM β-mercaptoethanol. The dialyzed DEAE-cellulose pooled fractions were concentrated using Amicon ultrafiltration apparatus YM10 membrane.

2.5.5. Sulphopropyl (SP) sepharose column chromatography

The concentrated dialyzed DEAE-cellulose fraction was mounted on the top of SP-Sepharose column, which was previously equilibrated with 15 mM Tris-HCl buffer (pH 5.6) containing 5 mM $MnCl_2$ and 1 mM β-mercaptoethanol. After washing the column, the adsorbed proteins were eluted using an equilibration buffer containing 0–1 M sodium chloride gradient at a flow rate of 20 ml/h. Fractions containing most of the enzymes activities were