L -Arginase: An Enzyme of Therapeutic and Biomedical Importance

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Abstract

L-Ornithine plays an important role in cell proliferation, collagen formation, and other physiological functions. It is an excellent nutritional supplement for bodybuilders and sportsmen. Because of its several functions in health care, l-ornithine has a substantial global market; thus, a simple, efficient, and energy-saving technique for producing l-ornithine is required. Currently, several studies exploit arginase as an efficient catalyst for the sustainable synthesis of L-ornithine. Arginase cleaves L-arginine to form L-ornithine and urea and acts as a committed step in the urea cycle. It was also studied as an arginine-reducing agent to treat arginase deficiency and to treat arginine auxotrophic tumors. Many studies have been reported for the production of L-ornithine by microbial arginases, but the isolation of arginase from low-cost materials like plant biomass is still a field of study. This review focuses on the production of arginase from potent microbial strains for the cost-effective production of ornithine and the study of its therapeutic applications and emphasizes the development of robust microbial strains with high stability and productivity.

Keywords: L-ornithine, L-arginase, L-arginine, auxotrophic tumors, arginase deficiency

Introduction

L-arginase (EC 3.5.3.1) is a ureohydrolase enzyme that converts L-arginine to ornithine and urea. [1] It is a crucial enzyme in the urea cycle, which helps to remove ammonia as urea. [2] It was also studied as an arginine-reducing agent to treat arginase deficiency and to treat arginine auxotrophic tumors. [3] Thus, arginase has two important physiological functions: one is the detoxification of ammonia in the urea cycle, and the

second is the production of L-ornithine, which acts as a precursor of polyamines and L-prolines. [4, 5] These catalytic products perform various biological functions, including polyamines involved in cell physiology, protein and nucleic acid synthesis, regulation of ion channels, and protection from oxidative damage. [6] The prolines function in wound healing and neuroprotection or regeneration. [7] On the other hand, L-ornithine is a widely used industrial nutraceutical that is widely used in the food, pharmaceutical, and cosmetic industries. [8,9]. L-ornithine is an intermediate metabolite in the urea cycle, a crucial precursor for the biosynthesis of L-citrulline, L-proline, and polyamines, and a non-essential amino acid that is essential for the treatment of post-traumatic stress disorder, liver protection, and liver disease treatment. It also strengthens the heart and helps in immune system maintenance. In recent years, there has been a lot of focus on the affordable and effective synthesis of ornithine because of its significance in activities that promote human health. Focused on the enzymatic action of arginase on arginine and microbial fermentation, these two processes are the main methods for producing ornithine.

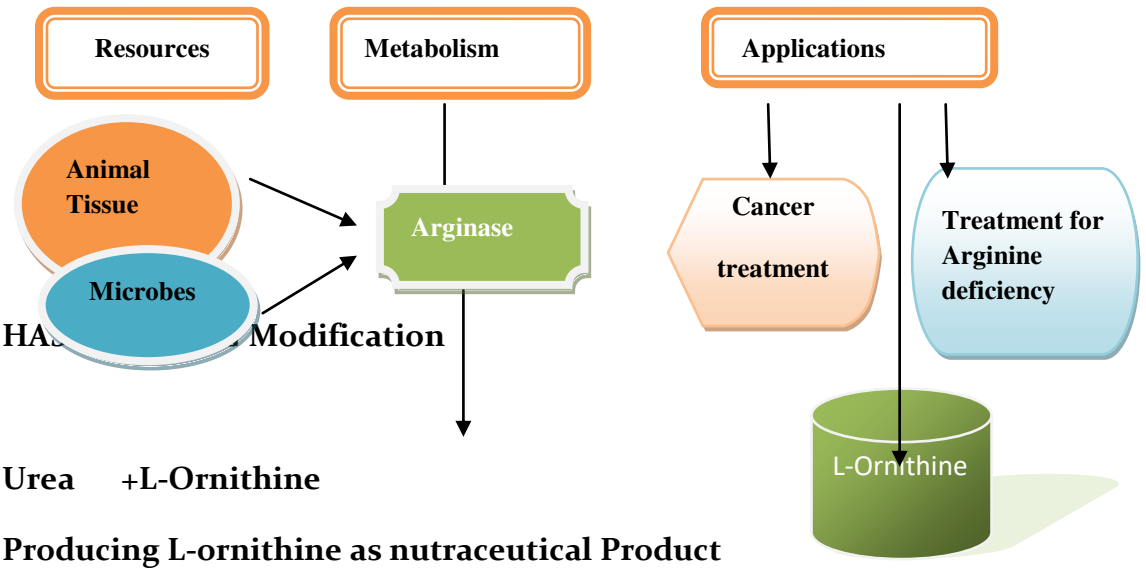

Fig.1:Arginase: uses in medicine and biotechnology:

Animal livers or microbiological sources can both be used to isolate arginine. A urea cycle enzyme called arginase breaks down L-arginine to produce urea and L-ornithine. Arginase has recently been employed extensively in medical applications to deplete arginine as a therapeutic technique for arginine-dependent malignancies or as an arginine-reducing drug in arginase-deficient patients. Arginase has also been shown to be a good catalyst for the environmentally friendly production of L-ornithine, an important component of nutraceuticals.

Microbial sources of L-Arginase

Arginase is found in various organisms in nature and is well studied in bacteria, fungi, [Table-1] lichens, plants, and higher mammals. The main function of arginase in microorganisms is to maintain L-arginine homeostasis and is involved in the regulation of many metabolic processes. [10] Many microbial strains have potentially produced enzymes, but the enzymes produced by several microbial strains may differ in physiological, biochemical, catalytic, and immunological characterization, which leads to continuous screening programmes to isolate the novel microbial strains that might produce effective enzymes with little limitation in the usage sector. [11] L-arginase is widely distributed and expressed in different organisms. [12]

Table-I: Microbial sources of arginase

Microbial source	Microbe	Reference
Bacteria	*SulfolobusAcidofilus*	[14]
	Pseudomonas sp. strain PV1	[15]
	Zymomonasmobilis	[16]
	Bacillus subtilis 168	[17]
	Bacillus anthracis	[18]
	Chlamydia pneumonia	[19]
	Cyanobacteria	[20]
	Helicobacter pylori	[13]
	Arthrobacter sp.KUJ 8602	[21]
	Cyanobacterium synechocystissp.strain PCC 6803	[22]
	Bacillus brevis	[23]
	Streptomyces clavuligerus	[24]
	Rhodobacter	[25]
	Bacillus caldovelox	[26]
	AgrobacterimTi plasmid C58	[27]
Fungi	*Candida albicans*	[28]
	Neurospora crassa	[29]
	Agaricusbisporus	[30]
	Trichoderma sp.	[31]
	Aspergillus nidulans	[32]
Yeast	*Evernia prunastri and Xanthoriaparietina*	[33]
	Schizosaccharomyces pombe strain 972	[34]
	Sacchromyces cerevisiae ATCC- 9763	[35]
Actinomycetes	*Actinomycetes KAR-73*	[36]

Relevance of Fungal enzymes over other microbial enzymes

Production of enzymes from microorganisms is faster, more cost-effective, scalable, and easier to manipulate genetically. [37] Among microbial enzymes, the fungal enzymes represent a vast range of industrially important enzymes [38] that are easy to recover due to their extracellular nature. Fungal enzymes contribute more than 50% of the total enzymes available on the market. [37] At the commercial level, a few species of *Aspergillus, Trichoderma, Rhizopus,* and *Penicillium* genera fulfill the requirements for enzyme production. The filamentous, thermophilic, psychrotrophic and white-rot fungi have the capability of producing various enzymes at optimum reaction. [39]

Arginine and its biosynthesis

Arginine is a conditionally essential amino acid that has been identified as playing an important role in a number of biological processes, including the normal function of the cardiovascular and immune systems. Many studies have revealed that arginine is necessary for cellular growth. [40] Arginine has been identified as the sole physiological precursor for NO, a key performer in many cellular regulatory functions. [41] Arginine is also a precursor for two important amino acids, proline and glutamate. [42] As arginine is a conditionally essential amino acid, the body can synthesize it as per the requirements of basal metabolic demands. In times of stress or rapid growth, like infection, wound healing, or neonatal development, arginine demand increases and availability becomes limited. [43] Inside microbial cells, arginine catalysis has two fates: the arginine urea pathway and the arginine deiminase pathway, both producing ornithine. [44]

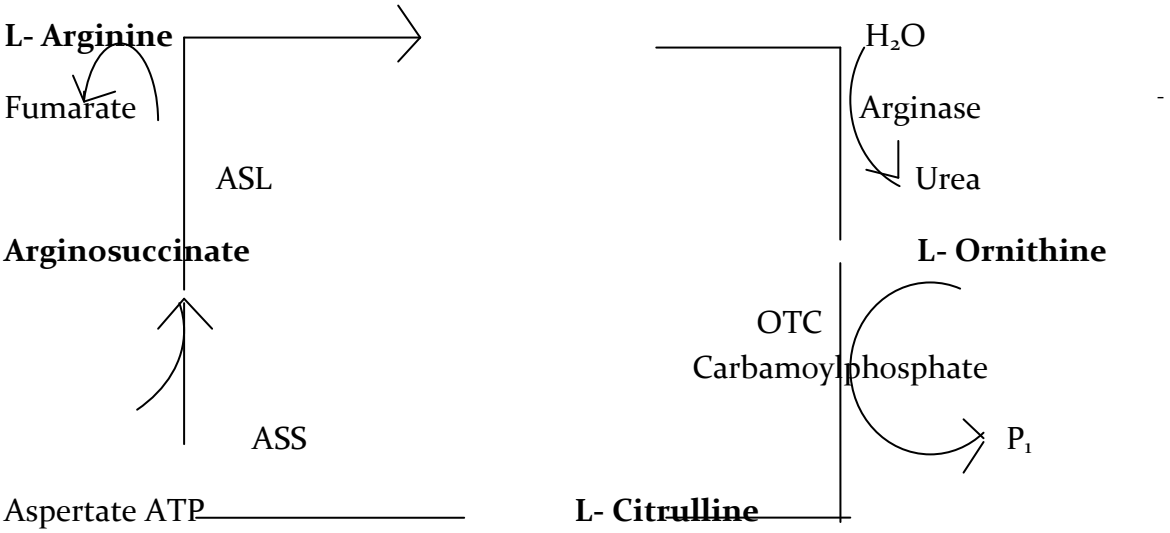

Fig.2: Hepatic Urea Cycle. CPS(Carbamoyl Phosphatase Synthatase), ASS (Arginosuccinate Synthatase), ASL(Arginosuccinate Lyase) OTC(ornithine transcarbamoylase).

Arginine and Cancer

Arginine is essential for cell growth, and its deficiency leads to retardation of cell growth, arrest of the cell cycle, and apoptosis in cancer cell lines. [45, 46] This antitumor property of the enzyme makes it a potent treatment for cancer. As compared to normal, healthy cells, cancerous cells require more energy and nutrients (amino acids) for their rapid growth. Keeping this point in mind, two strategies can be employed to stop their growth: targeting the cellular metabolism or manipulating the microenvironment around them. [47] Amino acid deprivation therapy is a well-established therapy based on targeting the cellular metabolism. It is well studied for asparaginase, methionase, glutaminase, and arginase enzymes. L-Arginine is involved in various cellular processes like the urea cycle, polyamine synthesis, nitric oxide formation, wound healing, and many more. In recent years, the demand for this amino acid has increased as it is studied for cancer treatment. [48]

Arginine decarboxylase (ADC; EC 4.1.1.19), Argininedeiminase (ADI; EC 3.5.3.6), and Arginase (EC 3.5.3.1) arethe three major enzymes that deplete arginine .As compared to these two enzymes, arginase act as a potential enzyme for efficienttreatment of hepatocellular carcinoma , human prostate cancer cells [49], pancreatic cancer [50],leukemia[51],glioblastoma [52],breast cancer [53], and non-Hodgkin'slymphoma.[54]

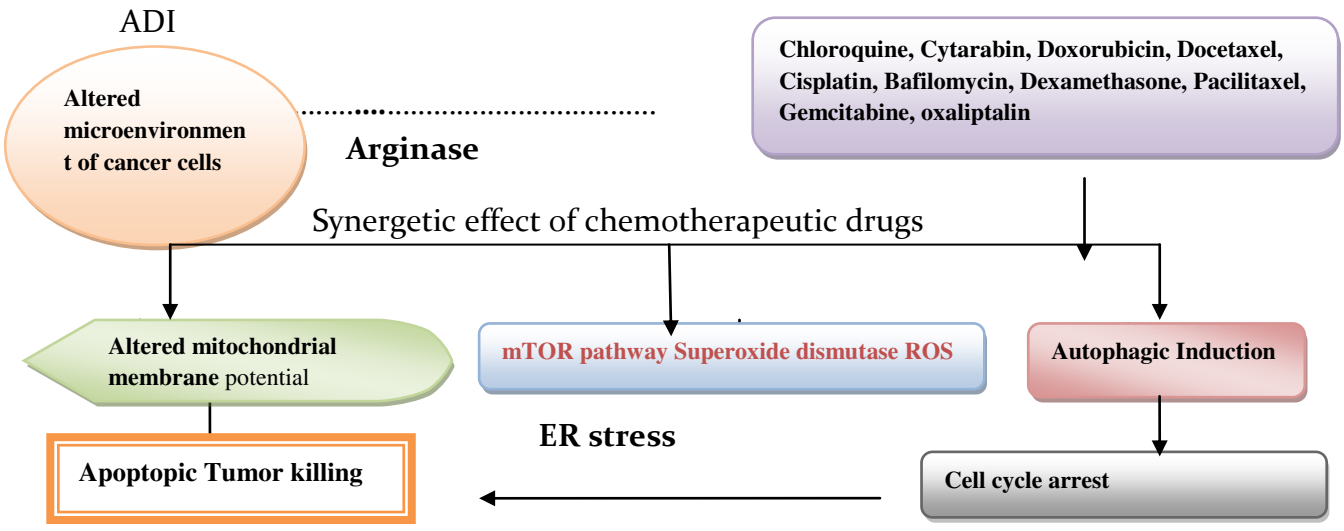

Fig.3: Arginine starvation on tumor cells:

Changes in the metabolic microenvironment of malignant cells cause cells to require more nutrients (such as arginine, asparagines, methionine, and energy fuel) to fight them considered likely candidates for treatment. The microenvironment is altered by

ADEs, which increases the activity of activated TAMS (Tumor Activated Macrophages), TRAIL (Tumor Necrosis Factor Related Apoptosis-inducing Ligand), and generation of ROS (Reactive Oxygen Species), a marker for apoptosis.[24] Fuel with low energy like Adenosine triphosphate (ATP) and nitric oxide (NO) activate enzymes such manganese superoxide dismutase, calreticulin, and glutathione peroxidase. They also stimulate the mTOR (mammalian target of rapamycin) pathway, which causes ER stress and, as a result, induces autophagic activity. At several checkpoints, long-term therapy causes cell growth to halt.

Arginine Metabolism

Arginine is synthesized in the liver via the urea cycle as well as in the kidney. [55] Arginine is a vital metabolite that acts as a precursor of many bioactive molecules like polyamines and proline via the ornithine decarboxylase (EC 4.1.1.17; ODC) and ornithine aminotransferase (EC 2.6.1.13; OAT) enzymes, respectively. The majority of tumor cells alters their metabolic cycles and requires additional amounts of polyamine, which is basically derived from arginine. Rapid growth and proliferation of cancer cells and their metastasis demand a high supply of arginine from external sources, thereby making them auxotrophic for arginine. [56]

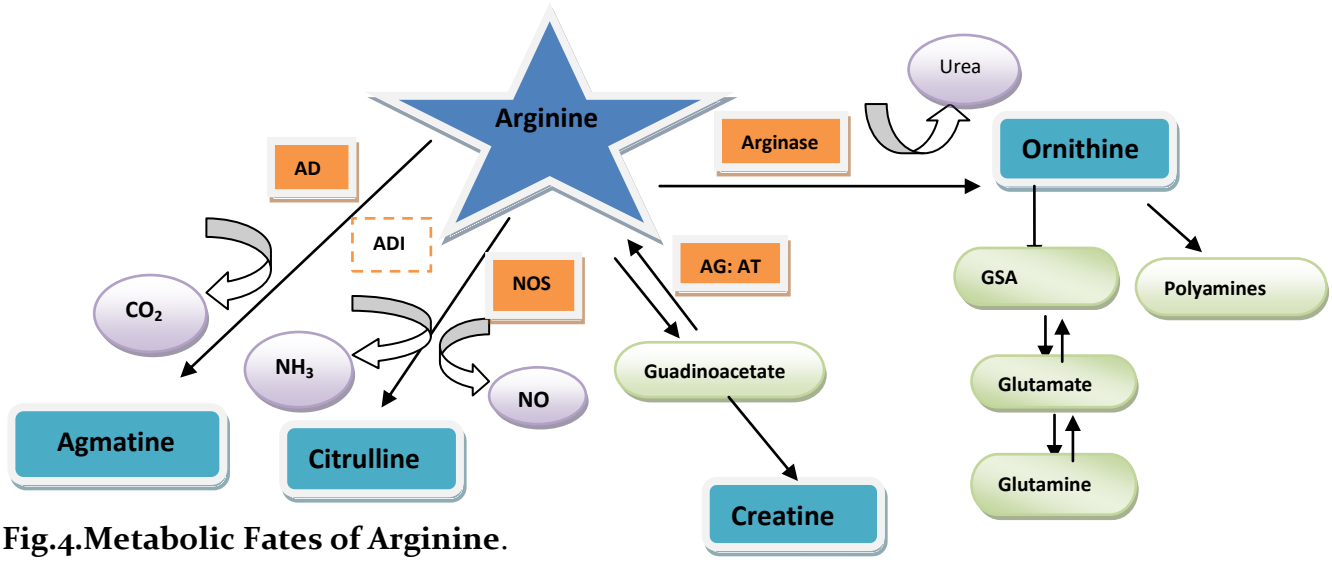

Fig.4.Metabolic Fates of Arginine.

Arginase converts L-arginine to L-ornithine and urea. The nitric oxide synthase also converts it to L-citrulline and NO (NOS). The operations of argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) can recycle L-citrulline back to L-arginine (ASL). The enzymatic action of ornithine transcarbamylase can convert L-ornithine to L-citrulline (OTC). L-ornithine can be utilized to produce polyamines by ornithine decarboxylase (ODC). It can also be used to produce L-proline by ornithine aminotransferase (OAT).

Arginine biosensors

In the juice and wine industries, arginine detection is one of the most important steps in quality control, as in wine productions; L-Arginine degrades into urea and ornithine by the enzyme L-arginase. Some amount of this urea is absorbed by the yeast and some amount is released into the medium of fermentation. In the whole process, the excessive amount of arginine in grapes or other fruits, the Urea in the medium reacts with ethanol to form ethyl carbamates, a potent carcinogen. [57] A potentiometric L-arginine bi-enzyme biosensor has also been developed based on recombinant human liver arginase-1. [58]

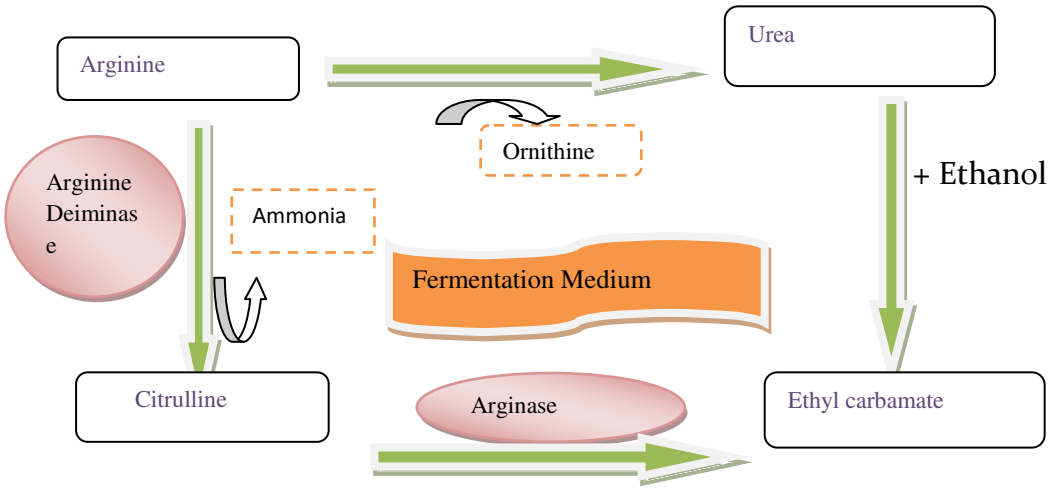

Fig.5:The bioprocess of ethyl carbamate synthesis from arginine during wine fermentation.

To avoid this potential health hazard detection of arginine levels by arginine biosensors has introduced. Arginine biosensors were developed by immobilizing two enzymes arginase (EC 3.5.3.1) and urease (EC 3.5.1.5). These enzymes catalyzed the hydrolysis of arginine to ammonium and bi-carbonate ions by two consecutive steps.

First Step

$$\text{L-arginine} \xrightarrow[+\ H_2O]{\text{Arginase}} \text{L-ornithine} + \text{Urea}$$

Second step

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_4^+ + HCO_3^-$$

Industrial applications of L-arginases
Production of L- ornithine.

L-ornithine is a non-protein amino acid formed by arginase and has enormous applications in the food and pharmaceutical industries. It acts as a precursor for the biosynthesis of polyamines. It helps in weight management; increases wound healing properties and enhance immunity. There is a steady demand for L-ornithine in the market for sports healthcare, nutrition supplements and drug treatment.[10] Keeping all these points, in recent years, the cost effective production of Ornithine has attracted much of attention.[59] Many microbial fermentation techniques with improved strains and recombinant arginase techniques are there to enhance the L-ornithine production at industrial level.[60] Ornithine production by arginase is more preferable over the other two methods, i.e., chemical and fermentative methods. Arginase from *Bacillus thuringiensis, Bacillus amyloliquefaciens, Bacillus caldoveloxand**Sulfobacillus acidophilus* are studied well for l-ornithine production. [61] L-ornithine taken orally travels from the intestines to the portal vein, where it is supplied to a variety of tissues, including the liver, kidney, and muscle.[62] Administration of L-ornithine has been shown to improve the ability of the liver to detoxify ammonia liver.By improving the effectiveness of energy use and encouraging ammonia excretion, l-ornithine exerts an antifatigue effect. [63] Due to the fact that L-ornithine is a free amino acid and is not abundant in meat or fish, it is challenging to consume enough of it through regular meals to support the ant fatigue impact. Therefore, it is advised to take L-ornithine as a nutritional supplement in cases of physical exhaustion. Ornithine is the most common nutraceutical to increase muscle strength and cardiovascular activity. It also helps to reduce fatigue if taken orally before exercise, as it increases energy consumption and excretes cellular ammonia efficiently. [64] Ornithine supplementation also led to enhanced wound-breaking strength and collagen deposition.It also finds applications in treating rheumatoid arthritis [65], as abiosensor to monitor levels of L-arginine in blood [59], and in fruit juices. L-ornithine acts as a precursor of polyamines. Studies on animals showed that polyamines are an important factor during intestinal maturation. Other research on children and lactating mothers found that high polyamine concentrations in mother's milk may protect children from food allergies until the age of five. [66] A study showed that the utilization of a mixture of glucose and sucrose increases L-ornithine production while improving L-arginine accumulation in *C. glutamicum*. During 72 h of batch cultivation, 40.82 g/L of L-ornithine was produced using isometric glucose and sucrose (1:1 weight ratio), which represents a 13.8% increase in the production titer compared to using glucose as the sole carbon source. These findings confirmed that glucose and sucrose co-utilization significantly promote L-ornithine accumulation, which further indicates that the production of L-glutamate family chemicals could be

improved by using glucose and sucrose as carbon sources. However, the yield of L-ornithine obtained using sucrose as the sole carbon source was only 33.96 g/L, which was lower than that produced by glucose alone or in combination with sucrose. [67]

Medical applications of arginase

Application in cancer treatment

For HCC treatment, arginase has emerged as one of the most promising drugs, due to the l-arginase auxotrophic nature of the hepatocellular carcinoma cell. [68] Many cell line studies have been conducted that show arginase as a highly specific and efficient agent with no side effects in cancer treatment. [69] Autophagy (programmed cell death) induced by arginase leads to increased membrane potential, ROS, the Akt/mTOR signaling pathway, Erk1/2 activation, activation of tumor-associated macrophages, and various pro-apoptotic factors. Due to these events, cancer cell death takes place. [70]

Application in Alzheimer's disease treatment

Damaged neurons are regenerated by the action of arginase, which acts as an agent for the protein that is degraded after axon injury. It is useful in the treatment of Alzheimer's disease as it increases polyamine levels and repairs damaged axons. [1]

Application in muscular performance improvement

L-arginine administration has been studied to promote an increase in blood perfusion in the active muscle in humans. It increases the availability of substrates necessary for improving muscular recovery and protein synthesis during and/or after exercise. It also works to remove metabolites such as lactate and ammonia [71], which are responsible for muscle fatigue during intense physical exercise.

Application in reproductive health improvement

Supplements containing arginase play an important role in improving sperm count and motility. Prostate function is also improved by the administration of arginase. [72]

Arginase assay

Quantitative and qualitative assays are both available for determining arginase activity (Table II). Thin layer chromatography (TLC) is one of the most common types of assay and is based on the use of the reagent ninhydrin, which produces an orange color when combined with ornithine and has a specific retention factor (Rf). It is a qualitative method, but it can also be used for quantitative analysis. [74] Another quantitative and qualitative analysis is the spectrophotometric method, which is based on forming a colored complex by recording absorbance at a specific wavelength. [73] High-Performance Liquid Chromatography (HPLC) [74] and biosensors are used for

quantitative analysis as they are more sensitive and reliable. [34] The urea detection method is also there to determine its activity.

Table II: Comparison of different types of assay for arginase

Types of assay	Substrate used	Analyzed products	Principle	Major outcomes	Shortcomings	References
Spectophotometric	Arginine	Urea	Urea develop a colored complex	Specific towards Urea	Volatile nature of the reagent Low sensitivity	[73]
Spectophotometric	Arginine	Urea	Urea reacts with α-nitrosopropiophenone reagent to form red color, which showed maximum absorbance at 540 nm	Very much specific towards urea	Dark conditions required to maintain red color complex	[73]
Spectrophotometric	Arginine	Ornithine	Reaction between ornithine and ninhydrin at a very low pH results in an orange color complex (read at 515 nm). The	Specific towards ornithine	Hinder by reaction conditions like pH as the reaction is pH specific	[73]

			Amount of orange complex formed is correlated with arginase activity			
TLC	Arginine	Ornithine	ornithine has a specific Rf value visualized as an orange spot when sprayed with ninhydrin	Qualitative analysis is done	Quantitative determination is not possible	[74]
HPLC	Arginine	Ornithine	Reverse-phase chromatography was employed to elute bounded ornithine	Both qualitative and quantitative analysis is done	Expensive	[74]
Potentiometric Biosensors	Arginine	Ammonia	Biosensors based on bi-enzyme system arginase and urease having an ammonia detecting electrode giving out the signal	Gives signal over a wide range	Expensive	[58]

Relevance of Arginase over Arginine Deiminase (ADI)

ADI (arginine Deiminase) is mainly of bacterial origin, and due to this, its administration leads to eliciting an immune response as it is recognized as a foreign molecule. Another drawback is that the action of the ADI enzyme releases citrulline and ammonia as by-products, which can be toxic and lead to hyper ammonia (psychological problems). Arginase is universal in nature; it is also of human origin, so there is no problem of immunogenicity. Its end product is also non-toxic. Pegylation increases its efficacy and catalytic property and makes it a more valuable therapeutic agent for the treatment of cancer.

Conclusion and Future Outlook

Arginase has been employed as an efficient catalyst for the ecologically friendly synthesis of L-ornithine, a plentiful non-protein amino acid that has been widely used as a dietary supplement and nutrition product in recent decades. L-ornithine is a non-essential amino acid that has great therapeutic and commercial utility in the treatment of complex liver illnesses. It is widely used as a food additive and a chemical pharmaceutical intermediary. [36] Massive demand has necessitated the immediate expansion of production capacity. To lower costs, the microbial fermentation technique has significant potential for L-ornithine production. As a result, effective strategies for developing strains capable of producing abundant L-ornithine are required. The present review sums up the enzyme arginase, its role inside the human cell, its significance in cancer treatment, its wide applications, and its products. The studies on arginase as a therapeutic enzyme and its various sources and the work done on them are also mentioned in the article. Medical research on arginase and the biological uses of L-ornithine has received attention. However, arginase still has significant flaws as a protein that restricts the range of its potential applications. These flaws include poor stability at physiological pH, antigen reactivity, ease of hydrolysis by protease in vivo, and a brief half-life in vivo. A thorough understanding of the sequence-structure-function relationship of arginase and optimization of the enzyme's functional characteristics through computer simulation of molecular design, combined with techniques like site-directed mutagenesis, directed evolution, and enzymatic glycosyl transfer technology, may offer crucial application indicators for the preparation of L-ornithine and the treatment of targeted tumor cells.

References:

1. Caldwell RW, Rodriguez PC, Toque HA, Narayanan SP, Caldwell RB. Arginase: a multifaceted enzyme important in health and disease. Physiological reviews. 2018 Apr 1;98(2):641-65.
2. Patil MD, Bhaumik J, Babykutty S, Banerjee UC, Fukumura D. Arginine dependence of tumor cells: targeting a chink in cancer's armor. Oncogene. 2016 Sep;35(38):4957-72.
3. Al-Koussa H, El Mais N, Maalouf H, Abi-Habib R, El-Sibai M. Arginine deprivation: A potential therapeutic for cancer cell metastasis? A review. Cancer cell international. 2020 Dec;20(1):1-7.
4. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW. Arginase: an old enzyme with new tricks. Trends in pharmacological sciences. 2015 Jun 1;36(6):395-405.
5. Harada D, Nagamachi S, Aso K, Ikeda K, Takahashi Y, Furuse M. Oral administration of l-ornithine increases the content of both collagen constituting amino acids and polyamines in mouse skin. Biochemical and biophysical research communications. 2019 May 14;512(4):712-5.
6. Pegg AE. Functions of polyamines in mammals. Journal of Biological Chemistry. 2016 Jul 15;291(29):14904-12.
7. Olin-Sandoval V, Yu JS, Miller-Fleming L, Alam MT, Kamrad S, Correia-Melo C, Haas R, Segal J, Peña Navarro DA, Herrera-Dominguez L, Méndez-Lucio O. Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism. Nature. 2019 Aug 8;572(7768):249-53.
8. Salvatore F, Cimino F, d'Ayello-Caracciolo M, Cittadini D. Mechanism of the protection by L-ornithine-L-aspartate mixture and by L-arginine in ammonia intoxication. Archives of biochemistry and biophysics. 1964 Sep 1;107(3):499-503.
9. Shi HP, Fishel RS, Efron DT, Williams JZ, Fishel MH, Barbul A. Effect of supplemental ornithine on wound healing. Journal of Surgical Research. 2002 Aug 1;106(2):299-302.
10. Li M, Qin J, Xiong K, Jiang B, Zhang T. Review of arginase as a promising biocatalyst: characteristics, preparation, applications and future challenges. Critical reviews in biotechnology. 2022 Jul 4;42(5):651-67.
11. Unissa R, Sudhakar M, Reddy AS. Evaluation of in vitro anti-proliferative activity of L-arginine deiminase from novel marine bacterial isolate. British Microbiology Research Journal. 2016 Jan 1;13(5):1.
12. Maharem TM, Zahran WE, Hassan RE, Fattah MM. Unique properties of arginase purified from camel liver cytosol. International journal of biological macromolecules. 2018 Mar 1;108:88-97.
13. Zabaleta J, McGee DJ, Zea AH, Hernández CP, Rodriguez PC, Sierra RA, Correa P, Ochoa AC. Helicobacter pylori arginase inhibits T cell proliferation and

reduces the expression of the TCR ζ-chain (CD3ζ). The Journal of Immunology. 2004 Jul 1;173(1):586-93.

14. Huang K, Zhang S, Guan X, Liu J, Li S, Song H. Thermostable arginase from Sulfobacillus acidophilus with neutral pH optimum applied for high-efficiency L-ornithine production. Applied Microbiology and Biotechnology. 2020 Aug;104:6635-46.
15. Nadaf P, Vedamurthy AB. Optimization of l-arginase production by Pseudomonas sp. Strain PV1 under submerged fermentation. Int. J. Scient. Technol. Res. 2020;9(1):4390-94.
16. Hwangbo SA, Kim JW, Jung SJ, Jin KS, Lee JO, Kim JS, Park SY. Characterization of a dimeric arginase from Zymomonasmobilis ZM4. Frontiers in Microbiology. 2019 Nov 26;10:2755.
17. Yu JJ, Park KB, Kim SG, Oh SH. Expression, purification, and biochemical properties of arginase from Bacillus subtilis 168. Journal of Microbiology. 2013 Apr;51:222-8.
18. Viator RJ, Rest RF, Hildebrandt E, McGee DJ. Characterization of Bacillus anthracis arginase: effects of pH, temperature, and cell viability on metal preference. BMC biochemistry. 2008 Dec;9(1):1-4.
19. Hartenbach S, Daoud-El Baba M, Weber W, Fussenegger M. An engineered L-arginine sensor of Chlamydia pneumoniae enables arginine-adjustable transcription control in mammalian cells and mice. Nucleic acids research. 2007 Nov 1;35(20):e136-.
20. Herrero A, Muro-Pastor AM, Flores E. Nitrogen control in cyanobacteria. Journal of bacteriology. 2001 Jan 15;183(2):411-25.
21. Arakawa N, Igarashi M, Kazuoka T, Oikawa T, Soda K. d-Arginase of Arthrobacter sp. KUJ 8602: characterization and its identity with Zn2+-guanidinobutyrase. Journal of biochemistry. 2003 Jan 1;133(1):33-42.
22. Quintero MJ, Muro-Pastor AM, Herrero A, Flores E. Arginine catabolism in the cyanobacterium Synechocystis sp. strain PCC 6803 involves the urea cycle and arginase pathway. Journal of Bacteriology. 2000 Feb 15;182(4):1008-15.
23. Kanda M, Saito Y. Purification and properties of arginase from gramicidin S-producing Bacillus brevis. Infaseb Journal 1997 Jul 31 (Vol. 11, No. 9, pp. A1020-A1020). 9650 Rockville Pike, Bethesda, Md 20814-3998 Usa: Federation Amer Soc Exp Biol.
24. Fuente Jl, Martín Jf, Liras P. New type of hexameric ornithine carbamoyltransferase with arginase activity in the cephamycin producers Streptomyces clavuligerus and Nocardia lactamdurans. Biochemical Journal. 1996 Nov 15;320(1):173-9.
25. Blasco RA, Castillo FR. Characterization of a nitrophenol reductase from the phototrophic bacterium Rhodobactercapsulatus E1F1. Applied and Environmental Microbiology. 1993 Jun;59(6):1774-8.

26. Patchett ML, Daniel RM, Morgan HW. Characterisation of arginase from the extreme thermophile 'Bacillus caldovelox'. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 1991 Apr 29;1077(3):291-8.
27. SCHRELL A, ALT-MOERBE J, LANZ T, SCHROEDER J. Arginase of Agrobacterium Ti plasmid C58: DNA sequence, properties, and comparison with eucaryotic enzymes. European journal of biochemistry. 1989 Oct;184(3):635-41.
28. Wagener J, MacCallum DM, Brown GD, Gow NA. Candida albicans chitin increases arginase-1 activity in human macrophages, with an impact on macrophage antimicrobial functions. MBio. 2017 Mar 8;8(1):e01820-16.
29. Turner GE, Weiss RL. Developmental expression of two forms of arginase in Neurospora crassa. Biochimica et Biophysica Acta (BBA)-General Subjects. 2006 Jun 1;1760(6):848-57.
30. Wagemaker MJ, Welboren W, van der Drift C, Jetten MS, Van Griensven LJ, den Camp HJ. The ornithine cycle enzyme arginase from Agaricusbisporus and its role in urea accumulation in fruit bodies. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression. 2005 Jan 11;1681(2-3):107-15.
31. Schuster A, Kubicek CP, Friedl MA, Druzhinina IS, Schmoll M. Impact of light on Hypocreajecorina and the multiple cellular roles of ENVOY in this process. BMC genomics. 2007 Dec;8(1):1-7.
32. Dzikowska A, Le Caer JP, Jonczyk P, Węgleński P. Purification of arginase from Aspergillus nidulans. Acta Biochimica Polonica. 1994 Dec 31;41(4):467-71.
33. Legaz ME, Fontaniella B, Millanes AM, Vicente C. Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. European journal of cell biology. 2004 Jan 1;83(8):435-46.
34. Choi GW, Um HJ, Kim MN, Kim Y, Kang HW, Chung BW, Kim YH. Isolation and characterization of ethanol-producing Schizosaccharomyces pombe CHFY0201. Journal of microbiology and biotechnology. 2010;20(4):828-34.
35. Chan PY, Cossins EA. Arginine metabolism in Saccharomyces cerevisiae. Some general properties of yeast arginase. Plant and cell physiology. 1973 Aug 1;14(4):641-51.
36. Gumashta R, Jain R, Pandey A, Tiwari P, Jain A. Applicability of Native L-Arginase produced by Streptomyces plicatus KAR73 as Antineoplastic Agent. Journal of Scientific and Industrial Research (JSIR). 2021 Nov 11;80(10):841-9.
37. Kango N, Jana UK, Choukade R. Fungal enzymes: sources and biotechnological applications. Advancing Frontiers in Mycology &Mycotechnology: Basic and Applied Aspects of Fungi. 2019:515-38.
38. Singh N, Kumar A, Sharma B. Role of fungal enzymes for bioremediation of hazardous chemicals. Recent Advancement in White Biotechnology Through Fungi: Volume 3: Perspective for Sustainable Environments. 2019:237-56.

39. Sharma A, Sharma A, Singh S, Kuhad RC, Nain L. Thermophilic Fungi and Their Enzymes for Biorefineries. Fungi in Extreme Environments: Ecological Role and Biotechnological Significance. 2019:479-502.
40. Tong BC, Barbul A. Cellular and physiological effects of arginine. Mini reviews in medicinal chemistry. 2004 Oct 1;4(8):823-32.
41. Allerton TD, Proctor DN, Stephens JM, Dugas TR, Spielmann G, Irving BA. l-Citrulline supplementation: impact on cardiometabolic health. Nutrients. 2018 Jul 19;10(7):921.
42. Kuo MT, Chen HH, Feun LG, Savaraj N. Targeting the proline–glutamine–asparagine–arginine metabolic axis in amino acid starvation cancer therapy. Pharmaceuticals. 2021 Jan 18;14(1):72.
43. Gilbreath KR, Bazer FW, Satterfield MC, Wu G. Amino acid nutrition and reproductive performance in ruminants. Amino Acids in Nutrition and Health: Amino Acids in the Nutrition of Companion, Zoo and Farm Animals. 2021:43-61.
44. M, Picossi S, Valladares A, Herrero A, Flores E. Catabolic pathway of arginine in Anabaena involves a novel bifunctional enzyme that produces proline from arginine. Molecular Microbiology. 2019 Apr;111(4):883-97
45. Singh R, Pervin S, Karimi A, Cederbaum S, Chaudhuri G. Arginase activity in human breast cancer cell lines: N ω-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. Cancer research. 2000 Jun 15;60(12):3305-12.
46. Leung SL, Ho MK, Lam YM, Chow HY, So YH, Leung YC. PEGylated recombinant human arginase as a drug for breast cancer. Hong Kong Med. J. 2019 Dec 1;25:28-31.
47. Roma-Rodrigues C, Mendes R, Baptista PV, Fernandes AR. Targeting tumor microenvironment for cancer therapy. International journal of molecular sciences. 2019 Feb 15;20(4):840.
48. Kumari N, Bansal S. Arginine depriving enzymes: applications as emerging therapeutics in cancer treatment. Cancer Chemotherapy and Pharmacology. 2021 Oct;88:565-94.
49. Yu KM, Pang TP, Cutler M, Tian M, Huang L, Lau JY, Chung SF, Lo TW, Leung TY. Rational design, engineer, and characterization of a novel pegylated single isomer human arginase for arginine depriving anti-cancer treatment. Life Sciences. 2021 Jan 1;264:118674.
50. Menjivar RE, Nwosu ZC, Du W, Donahue K, Espinoza C, Brown K, Velez A, Yan W, Lima F, Bischoff A, Kadiyala P. Arginase 1 is a key driver of immune suppression in pancreatic cancer. bioRxiv. 2022:2022-06.
51. Yang JS, Wang CC, Qiu JD, Ren B, You L. Arginine metabolism: a potential target in pancreatic cancer therapy. Chinese Medical Journal. 2021 Jan 5;134(01):28-37.

52. Oriol A, Vives S, Hernández-Rivas JM, Tormo M, Heras I, Rivas C, Bethencourt C, Moscardó F, Bueno J, Grande C, del Potro E. Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA Study Group. Haematologica. 2010 Apr;95(4):589.
53. van der Vos KE, Abels ER, Zhang X, Lai C, Carrizosa E, Oakley D, Prabhakar S, Mardini O, Crommentuijn MH, Skog J, Krichevsky AM. Directly visualized glioblastoma-derived extracellular vesicles transfer RNA to microglia/macrophages in the brain. Neuro-oncology. 2015 Oct 3;18(1):58-69.
54. Zeng X, Li Y, Fan J, Zhao H, Xian Z, Sun Y, Wang Z, Wang S, Zhang G, Ju D. Recombinant human arginase induced caspase-dependent apoptosis and autophagy in non-Hodgkin's lymphoma cells. Cell death & disease. 2013 Oct;4(10):e840.
55. Morris Jr SM. Regulation of enzymes of the urea cycle and arginine metabolism. Annual review of nutrition. 2002 Jul;22(1):87-105.
56. Parker AL, Toulabi L, Oike T, Kanke Y, Patel D, Tada T, Taylor S, Beck JA, Bowman E, Reyzer ML, Butcher D. Creatine riboside is a cancer cell–derived metabolite associated with arginine auxotrophy. The Journal of Clinical Investigation. 2022 Jul 15;132(14).
57. Verma N, Singh AK, Singh M. L-arginine biosensors: A comprehensive review. Biochemistry and biophysics reports. 2017 Dec 1;12:228-39.
58. . Stasyuk N, Smutok O, Gayda G, Vus B, Koval'chuk Y, Gonchar M. Bi-enzyme L-arginine-selective amperometric biosensor based on ammonium-sensing polyaniline-modified electrode. Biosensors and Bioelectronics. 2012 Aug 1;37(1):46-52.
59. Jensen JV, Eberhardt D, Wendisch VF. Modular pathway engineering of Corynebacterium glutamicum for production of the glutamate-derived compounds ornithine, proline, putrescine, citrulline, and arginine. Journal of Biotechnology. 2015 Nov 20;214:85-94.
60. Zhan Y, Liu J, Mao P, Zhang H, Liu Q, Jiao Q. Biotransformation of L-ornithine from L-arginine using whole-cell recombinant arginase. World Journal of Microbiology and Biotechnology. 2013 Nov;29(11):2167-72.
61. Vaubourdolle M, Jardel A, Coudray-Lucas C, Ekindjian OG, Agneray J, Cynober L. Fate of enterally administered ornithine in healthy animals: interactions with alpha-ketoglutarate. Nutrition (Burbank, Los Angeles County, Calif.). 1989 May 1;5(3):183-7.
62. Krebs HA, Hems R, Lund P. Accumulation of amino acids by the perfused rat liver in the presence of ethanol. Biochemical Journal. 1973 Jul 15;134(3):697-705.
63. Sugino T, Shirai T, Kajimoto Y, Kajimoto O. L-ornithine supplementation attenuates physical fatigue in healthy volunteers by modulating lipid and amino acid metabolism. Nutrition research. 2008 Nov 1;28(11):738-43.

64. Majumdar R, Minocha R, Minocha S. Ornithine: at the crossroads of multiple paths to amino acids and polyamines. In: D'Mello, JPF, ed. Amino acids in higher plants. Osfordshire, UK: CABI: 156-176. Chapter 9.. 2015:156-76.
65. Rodriguez-Martínez L, Bang H, Regueiro C, Nuño L, Triguero-Martinez A, Peiteado D, Ortiz AM, Villalba A, Martinez-Feito A, Balsa A, Gonzalez-Alvaro I. Improved classification of rheumatoid arthritis with a score including anti-acetylated ornithine antibodies. Scientific reports. 2020 Nov 6;10(1):1-0.
66. Dandrifosse G, Peulen O, El Khefif N, Deloyer P, Dandrifosse AC, Grandfils C. Are milk polyamines preventive agents against food allergy?. Proceedings of the Nutrition Society. 2000 Feb;59(1):81-6.
67. Nie L, Xu K, Zhong B, Wu X, Ding Z, Chen X, Zhang B. Enhanced l-ornithine production from glucose and sucrose via manipulation of the fructose metabolic pathway in Corynebacterium glutamicum. Bioresources and Bioprocessing. 2022 Dec;9(1):1-2.
68. Chrzanowska-Wodnicka M, Kraus AE, Gale D, White GC, VanSluys J. Defective angiogenesis, endothelial migration, proliferation, and MAPK signaling in Rap1b-deficient mice. Blood, The Journal of the American Society of Hematology. 2008 Mar 1;111(5):2647-56.
69. Tsui SM, Lam WM, Lam TL, Chong HC, So PK, Kwok SY, Arnold S, Cheng PN, Wheatley DN, Lo WH, Leung YC. Pegylated derivatives of recombinant human arginase (rhArg1) for sustained in vivo activity in cancer therapy: preparation, characterization and analysis of their pharmacodynamics in vivo and in vitro and action upon hepatocellular carcinoma cell (HCC). Cancer Cell International. 2009 Dec;9(1):1-3.
70. Zou S, Wang X, Liu P, Ke C, Xu S. Arginine metabolism and deprivation in cancer therapy. Biomedicine & Pharmacotherapy. 2019 Oct 1;118:109210.
71. Schaefer A, Piquard F, Geny B, Doutreleau S, Lampert E, Mettauer B, Lonsdorfer J. L-arginine reduces exercise-induced increase in plasma lactate and ammonia. International journal of sports medicine. 2002 Aug;23(06):403-7.
72. Ahmadi S, Bashiri R, Ghadiri-Anari A, Nadjarzadeh A. Antioxidant supplements and semen parameters: An evidence based review. International Journal of Reproductive BioMedicine. 2016 Dec 10;14(12):729-36.
73. Yu JJ, Oh SH. Isolation and characterization of lactic acid bacteria strains with ornithine-producing capacity from natural sea salt. The Journal of Microbiology. 2010 Aug;48(4):467-72.
74. Hussein HA, Mekki BB, Abd El-Sadek ME, El Lateef EE. Effect of L-Ornithine application on improving drought tolerance in sugar beet plants. Heliyon. 2019 Oct 1;5(10):e02631.

75. Corraliza IM, Campo ML, Soler G, Modolell M. Determination of arginase activity in macrophages: a micromethod. Journal of immunological methods. 1994 Sep 14;174(1-2):231-5.