L-METHIONINASE: A COGENT ANTICANCER TOOL

Kavya Parnami
Scholar
Department of biotechnology and biochemical engineering
Indian Institute of Technology
New Delhi, India

ABSTRACT

In a spunk against cancer, Enzymo-therapy has emerged as one of the most effective strategy recently. Resistance to conventional anticancer therapies in patients with advanced solid tumors has prompted the need for alternative cancer therapies. Delving into novel pharmacological tools is one priority area of research in cancer biology due to the inefficiency of the existing therapeutic approaches toward this disease. Moreover, the success of novel cancer therapies depends on their selectivity for cancer cells with limited toxicity to normal tissues. L-methioninase enzymatic approach has shown considerable beneficial effects in reducing cancer burden in experimental models. L-Methioninase (MGL) has received much attention in the recent years, since it shows antiproliferative activity and methionine restriction has been known to arrest tumor growth. Methionine dependence is the only known general metabolic defect in cancers. Methionine is an essential amino acid necessary for normal growth and development in mammals. Normal cells have the ability to grow on homocysteine, instead of methionine, due to their active methionine synthase. Many human cancer cell lines and primary tumors have an absolute requirement for L-methionine, an essential amino acid. Thus, depleting cellular/plasma methionine levels using MGL seems to be a promising therapeutic approach to treat cancer. The present article is a brief attempt to witness the structure, prominent sources and mechanism of action of L-methioninase. It also catalogues the role of L-methioninase on tumor cells and highlights its future prospects.

KEY WORDS: L-Methioninase, Enzymo-therapy, anticancer, review

INTRODUCTION

Cancer is a cryptic disease that involves metabolic and behavioral changes in cells, causing them to proliferate uncontrollably. The disease process of cancer is also influenced by genetic, epigenetic, and other factors [1]. The scientific community faces a significant challenge in determining the response of cancerous cells to various treatment modalities. The traditional treatment methods of surgery, chemotherapy, ionization/radiation, and ultrasound treatments are however not very effective in reducing cancer and frequently may cause severe side effects in the body's healthy cells [2]. Thus, the scientific community is constantly in search of novel treatment modalities.

Enzymo-therapy is emerging as a promising therapeutic technology for disease treatment, over the last five decades. Owing to a few special characteristics, Enzymes are considered superior to other prevalent classes of drugs. Firstly, enzymes frequently bind and act on their targeted sites with high affinity and specificity, and secondly these are catalytic in nature and can convert numerous target molecules to the desired products. These particular features make them potential candidate to be used as specific and potent drugs for human therapeutic use [3]. L-Methioninase, one of such enzymes possess high therapeutic value, since it was reported as an effective anticancer agent *in vitro* as well as *in vivo* [4]. Late back in 1998, Anderson reported human cancer cell lines and primary tumors having an absolute requirement for L-methionine, an essential amino acid, to survive and proliferate [5]. Normal healthy cells do not require methionine to grow as they have active methionine synthase, and they survive on homocysteine, instead of methionine. The absence of methionine synthase in tumor cells, explains the inability of these cells to grow on homocysteine [4]. Therapeutic exploitation of L-Methioninase to deplete plasma methionine thus seems to be a promising strategy in curing tumors.

Structure of L-Methioninase

L-Methioninase is a pyridoxal phosphate (PLP) dependent hydrolytic enzyme and also known as L-Methionine-Ɣ-lyase, L-methionine-Ɣ-demethiolase, methionase, and L-methionine- methanethiol-lyase. It

is absent in mammalian system and intracellularly present in bacteria and extracellularly in fungi [6]. Over the past few decades, researchers all over the world have been thoroughly investigating the enzyme structure in various bacterial and fungal strains including Pseudomonas putida, Citrobacter freundii, Entamoeba histolytica, Micromonospora echinospora and Clostridium sporogenes. The detailed structure is as presented in Figure 1.

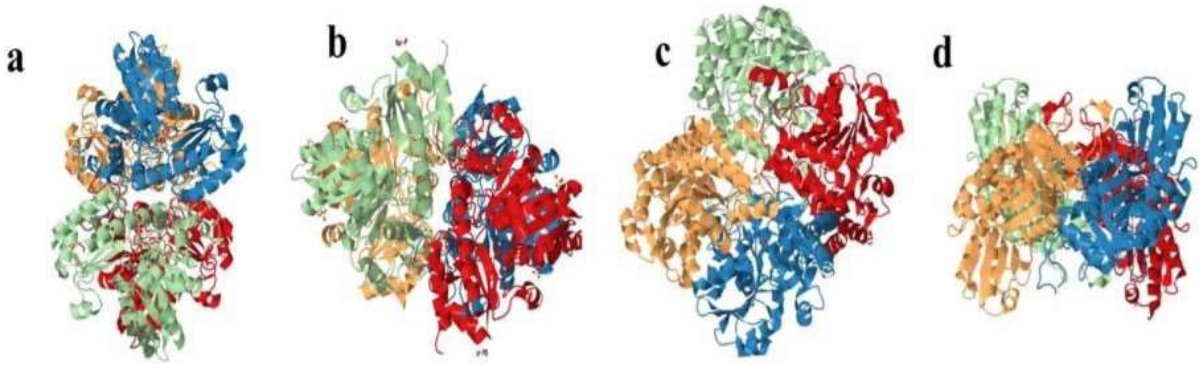


Figure 1: Three-dimensional structure of L-Methioninase. (a) Overall tetramer structure of Citrobacter freundii Methioninase and the subunits are color coded (b) Structure of Entamoeba histolytica Methioninase (c) Structure of Pseudomonas putida Methioninase (d) Structure of Micromonospora echinospora Methioninase

L-Methioninase occurs as a tetramer, a cytosolic enzyme logically formed by the addition of L-methionine to the culture medium. The molecular weight is between 149 and 173 kDa and consists of four subunits with identical molecular weights of about 41-45 kDa each. The purified enzyme has been reported to have a molecular mass of 47 KDa [7]. Structural analysis of L-Methioninase from Pseudomonas putida reveals the presence of six conserved amino acids residues, Tyr 59, Arg 61, Tyr 114, Cys 116, Lys 240 and Asp 241, which are present in the centre of the substrate, close to pyridoxal phosphate in tetramer fashion [8]. The mutational studies of Entamoeba histolytica from the L- Methioninase isozymes demonstrated that the cysteine residues directly contribute to the specificity of substrate [9]. The three-dimensional structure of the external aldimine of Citrobacter freundii of L-Methioninase with competitive inhibitor glycine has been determined at 2.45Å resolution [10].

Sources of L-Methioninase

Documented literature reports that L-Methioninase was initially characterized from the rumen bacteria around 1950's [11]. These preliminary studies formed a base for the later researchers to follow up, who focused on purification, biochemical characterization, and therapeutic evaluation of the enzyme as an anticancer agent toward various types of human cancer cell lines. Many species including bacteria, fungi, protozoa, and plants possess L- Methioninase and the various sources of the enzyme are compiled in the following table-

Table: Sources of L-Methioninase

Bacterial	Fungal	Protozoan	Plant Sources
Achromobacter starkey	*Aspergillus niger*	*Entamoeba histolytica*	*Arabidopsis thaliana*
Aeromonas hydrophila	*Aspergillus flavipes*	*Trichomonas vaginalis*	*Cucumis melo*
Arthrobacter sp	*Aspergillus ustus*		*Solanum tuberosum*
Bacillus subtilis	*Aspergillus parasiticus*		*Catharanthus roseus*
Brevibacterium linens	*Clonostachys rosea*		
Clostridium sporogenes	*Cladosporium cladosporoides*		
Citrobacter intermedius	*Cladosporium oxysporum*		
Citrobacter freundii	*Debaromyces hansenii*		

Clonostachys rosea	*Fusarium nivale*		
Fusobacterium nucleatum	*Saccharomyces cerevisiae*		
Ferroplasma acidarmanus	*Geotrichum candidum*		
Idiomarina sps.	*Trichoderma koningii*		
Micromonospora echinospora			
Porphyromonas gingivalis			
Pseudomonas putida			
Streptomyces sp			
Trichomonas vaginalis			
Treponema denticola			

[Adapted from Suganya et al, 2017]

Mammals however, lack the enzyme [12]. The enzyme is formed by most bacterial organisms as an intracellular enzyme. L-Methioninase has also been known to be produced both by gram-positive and gram-negative bacteria [13]. The enzyme is widely distributed in bacteria, especially in Pseudomonas species, and is induced by the addition of L-methionine to the culture medium. The review of literature till date, affirms the bacteria to be the potent prokaryote for synthesis of enzyme.

L-Methioninase mechanism of action

This enzyme catalyzes the direct conversion of L-methionine ($C_5H_{11}NO_2S$) into α- ketobutyrate, ammonia, and methanethiol [14] by eliminating it's α ɣ-group as can be seen from figure 2.

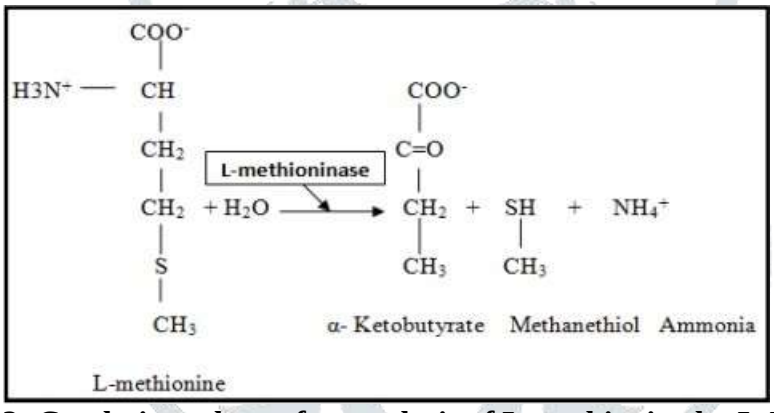


Figure 2: Catalytic pathway for catalysis of L-methionine by L-Methioninase

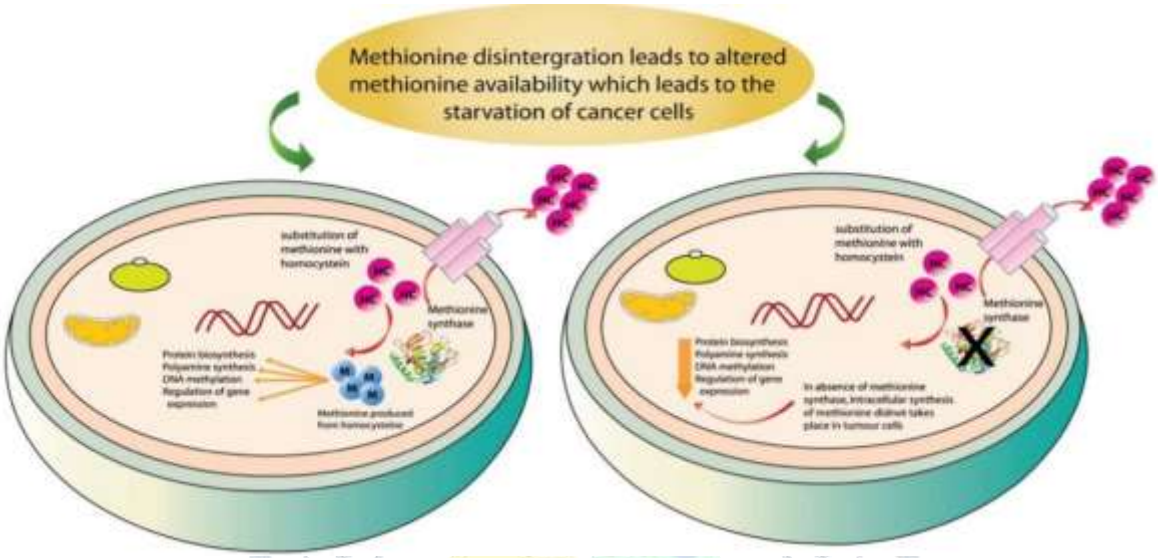
The hydrolytic activity of L-Methioninase can be described in the following steps-

1. Attacking internal aldimine structure with the amine group of the methionine.
2. Formation of external aldimine by transformation of Schiff's base and release of enzyme from lysine group.
3. The released enzyme would further attack on tyrosine moiety and removes α-position of methionine from the hydrogen group resulting in the formation of ketoimine.
4. Quinonoid would be further formed by releasing thiol group after donating hydrogen group to the β- positions from the tyrosine moiety of hydroxyl group.
5. The imine bond would be further attacked by the water moiety and releases the α-keto acid.
6. The Internal aldimine formation would be further occurred by attacking lysine moiety of amine group from L-Methioninase on amine bonds with release of ammonia [15].

How does L-Methioninase works against Cancer cells

The availability of nutrients highly forms the basis of a link between metabolic regulation and cancer progression [16]. Thus, to properly elucidate the nutritional dependency of cancer cells, it becomes a requisite to analyze the metabolic role of the enzyme [17]. However, the metabolism of cancer is unique because Cancer cells have a much higher demand for nutrients for their growth and are often starved of nutrients and oxygen which are needed in abundance for rapid growth. L-Methionine not only is essential for methylation of DNA, polyamine synthesis and mammalian protein synthesis, but also plays a central

role in the metabolism of all macromolecules, control of gene expression, cytoprotection and membrane integrity [18]. Methionine restriction is a principal approach in metabolic control of cancers. In the presence of L-Methioninase, malignant cells are deprived of vital growth factors that result in exhaustion of methionine and eventually tumor cells die off. In the absence of methionine, the enzyme helps in thriving the normal cells on nutrients supplemented with homocysteine, folic acid and cobalamin (B12). Methionine deprivation affects tumor cells with the propensity to divide and causes them to arrest predominantly in the late S/G2 phase of the cell cycle and to eventually undergo apoptosis [19]. Cells that are arrested in late-S/G2 phase are more susceptible to spontaneous death and are hypersensitive to chemotherapeutic agents. Therefore, methionine restriction with methioninase is stated to have a comprehensive selective strategy for many cancers in vitro as well as a high activity for killing cancer cells. The process is explained in the Figure below-



The normal cells have active methionine synthase and so can grow on a medium supplemented with homocysteine

Cancer cells lack active methionine synthase and so exhibit inability to grow with the external supplement of homocysteine

Figure 2: Mechanism of action of L-Methioninase normal and cancer cells in the presence and absence of Methionine synthase

FUTURE PROSPECTS

L-methioninase has attracted a great deal of attention due to its potential application as an active therapeutic agent against different types of cancer in human beings. The enzyme is characterized by its specific catalytic reaction on tumor cells. The distribution is restricted only in few pathogens and completely absent in mammals, which make this enzyme a promising target to design novel chemotherapeutic drugs. Tumor cells show enhanced methionine dependence/requirement in comparison to the normal cells. Thus, the forced restriction of methionine may be an important strategy in cancer growth control particularly in malignant/cancers that exhibit dependence on methionine for their survival and proliferation. L-Methioninase also has been used in fusion proteins (linked to human annexin- V). This approach has an advantage over other approaches in that they specifically target tumor cells without affecting the normal cells.

CONCLUSION

The documented review literature supports the efficacy of L-Methioninase in treating somatic malignancies. Considerable data has been generated over a span of more than last 50 years establishing the role of L-Methioninase in curing tumors. Since, the enzyme is widely distributed among various life forms including bacteria, fungi, protozoa and plants, this has significantly drawn considerable attention of the scientific community for a cost-effective remedy which can be produced effortlessly. In the fight against cancer, enzyme-therapy using L-Methioninase appears to the most effective strategy recently. Unlike

traditional approaches, it seems to be a promising therapeutic technology owing to its high specificity and affinity towards a clue substrate on specific metabolic pathway. The enzyme may enhance drug efficacy directed to cancer treatment and may diminish chemotherapy toxicity.

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