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GLUTAMINASE: IT'S POTENTIAL FROM CELLS TO INDUSTRY

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ABSTRACT

Abstract: Glutaminase is an enzyme catalyzing the hydrolysis of L-Glutamin to L-Glutamic acid and ammonia. It holds a very important position among commercially important enzymes. This is due to its established role as antileukamic and as food flavoring agent. The list of both the industrial and therapeutic uses of glutaminase is increasing continuously. Glutaminase has shown its profound effect an antiviral activity and HIV associated dementia and multiple sclerosis. In meat, milk and dough processing industry it has made its position. It has been used for protein deamidation and soy food production. The present review discusses some of important proven uses and some yet to be commercially established application of glutaminase.

Keywords: L-glutaminase, food flavor, Glutamine, Glutamic acid, Antileukamic, anti HIV

INTRODUCTION

Industrial Applications of L-glutaminase

Therapeutics: Glutamine and glutamic acid are non-essential amino acid so generally present in all living cells. Glutamine is synthesized primarily in skeletal muscle, lungs, and adipose tissue in these cells it is a major respiratory fuel (1) as it is converted in glutamate by glutaminase, which is further used in TCA cycle for ATP generation. Glutamate feeds other biosynthetic pathways as precursors too.

Anti-tumor activity: In order to full-fill their bioenergetic and biosynthetic requirements cancer cells re-program their metabolic machinery. This results in change in glycolytic pathway which is known as the "Warburg effect". In this, obtained product pyruvate converts into lactic acid, which further changes into acetyl-CoA and ultimately, contributes as

anaplerotic source of citrate in the citric acid cycle. To balance these changes and to maintain a functioning of citric acid cycle, elevated glutamine metabolism and glutaminase activity is the savior for cancer cells. (2). Cancer cells are also known as "glutamine addicted". Glutaminase converts glutamine to glutamate and glutamate oxaloacetate transaminases that convert glutamate to α-ketoglutarate. Oxidative conversion of glutamine gives 40% of TCA cycle intermediates and 30% of the ATP generated (3). L-glutaminase, in combination with or as an alternative to asparaginase, shows significance in enzyme therapy for cancer especially in acute lymphocytic leukemia. As cancerous cell gets highly dependent on glutamine for energy, the Cancer cells are extremely sensitive to glutamine level so glutamine withdrawal results in the death of some cancer cells. This is surprising because being a non-essential amino acid that can be synthesized from glucose. But the strict requirement of glutamine for tumor cells makes glutaminolysis enzymes as an attractive anticancer target (4).

There are many factors including myc and Rho GTPase that affect glutaminase expression and it's regulation. In human B lymphoma and prostate cancer cells, the up-regulation of glutaminase expression is in a c-Myc-dependent manner. Myc promotes glycolytic metabolism as well as works as proto-oncogene and major regulator of cell proliferation. It stimulates other metabolic genes in cell including glutamine uptake and metabolism. Basically Myc enhances mitochondrial respiration, all of which contribute to the overall increased metabolic function of cancer cells (5). So it is a factor for oncogenic cell's glutamine dependency. Glutamine metabolism stimulation by Myc occurs in both direct and indirect ways. As a transcription factor, Myc directly binds the promoters and stimulates expression of glutamine metabolism genes, such as the transporter Slc1a5. Myc also promotes glutaminase activity indirectly by repressing expression of miR-23a/b, which targets kidney-type glutaminase. RNAi-mediated suppression of kidney-type glutaminase leads to an increase in ROS levels and cell death, associated with diminished glutathione levels. Glutamine's anaplerotic role is critical for supporting cell survival in Myc-transformed cells. Furthermore, In Myc-driven cells, carbon derived from glutamine was shown to be preferentially used for glutathione synthesis, in contrast to carbon originating from glycolysis. These results suggest that glutamine's antioxidant roles are important for survival in Myc-driven cells (6).

Rho GTPases regulate glutamine metabolism and in cancer cells displays higher glutaminase activity which is regulated in an NF-κB-dependent manner. To weaken the growth and invasive potential of Rho GTPase-driven tumorigenesis, glutaminase is a potential target (6). Altered energy balance is hallmark in cancer metabolism. For viability and growth a cancerous cell can be governed by glucose and glutamine both (7).

Anti-HIV property: HIV infected macrophages are glutamine dependent. It's main cellular energy source is extracellular glutamate. HIV infected patients have significantly higher concentrations of glutamate in plasma (8, 9) which induces neuronal damage (10, 11, 12, 13). So the inhibition of glutaminase can reduce the problem.

HIV-associated dementia and multiple sclerosis: Glutaminase has central role in the generation of excitotoxic glutamate in central nervous system disorders such as HIV-associated dementia and multiple sclerosis. Glutaminase is responsible the generation of glutamate which is a key excitatory neurotransmitter in the CNS. Excessively activated macrophages and microglia releases elevated level of glutamate which correlates with upregulated glutaminase contribute to neuroinflammation, a hallmark of several neurodegenerative diseases. (14).Two methods, glutaminase siRNA and glutaminase inhibition have been shown to be effective in in-vitro models of cancer and HIV-associated dementia, suggesting a potential role for small molecule glutaminase inhibitors. However, there are no potent, selective inhibitors of glutaminase currently available. The two prototypical glutaminase inhibitors, BPTES (bis-2-(5-phenylacetimido-1,2,4-thiadiazol-2-yl)ethyl sulfide) and DON (6-diazo-5-oxo-Lnorleucine), are either insoluble or non-specific. It was reported ebselen, chelerythrine and apomorphine appear to be significantly more efficient than either DON or BPTES (bis-2-(5-phenylacetimido-1, 2, 4-thiadiazol- 2-yl) ethyl sulfide) for glutaminase inhibition (14).

Anti-viral therapy: In Human Cytomegalovirus Infection (HCMV) infection, infected cells become dependent upon glutamine for ATP production and viral production. It was observed that glutaminase and glutamate dehydrogenase gets increased within 24 h of infection, glutamine becomes main substrate for ATP production and this increases significantly by 48 h post infection. The inhibition of glutamine uptake or glutaminolysis may be an effective antiviral therapy for Human Cytomegalovirus Infection (15).

In food industry: There are four classic taste: sweet, salty, sour and bitter. The fifth taste discovered by K. Ikeda in 1908 (umami taste) appears to be the natural taste of the foods available to early man such as human milk, fish, chicken, sea-foods, sea weeds, all rich in glutamate proving the glutamate taste (16). Glutaminase converts glutamine into glutamate. The 'umami' taste includes 'delicious', 'umami' and 'brothy'. Glutamate is most important amino acids and the main active component that improve the taste of meat. Therefore, more the concentration of glutamate in meat gives better taste of meat. Free glutamate stimulates the salivation which is essential for mastication and swallowing. Glutamate evokes the cephalic phase of food digestion, such as an indication of pancreatic juice secretion (15). In fermented food, the concentration of glutamate increases and gives it palatable taste. By increasing the level of free glutamate in meat or tissues by feeding animals diets containing glutamate-rich feeds or by directly administration can rise the flavor of meat. Glutamate is not directly absorbed in many tissues because it is predominantly used as an energy source by intestinal cells. It has reported that high protein diet in broiler chicks regulates muscular glutamate metabolism (17).

Glutamate is present in some vegetarian food supplements as Soyabeans, beans, corn, green peas, tomato spinach, cabbage, mushroom, onion, sea-weeds, dried lever, kelp (konby) and some non-vegetarian food supplements as beef, fish, chicken, chees, human breast milk, sea foods, crabs, scallop etc. Food additives such as monosodium glutamate or hydrolyzed vegetable protein are also significant sources (15).

Flavor enhancer of Meat: Glutamine is claimed to increase muscle protein. Increased concentration of Glutamate in meat improves the taste of meat. The free Glutamate content in pork and chicken meat is increased by aging for 6 or 2 days post-mortem, respectively. Because of dietary Glutamate is not directly absorbed in many tissues of body, so muscle free Glutamate content, which is an active taste component of meat, was significantly increased by short-term feeding of a short-term high-protein diet. Lysine α-ketoglutarate reductase and glutaminase appear to be involved in the increase in the muscle free Glutamate level (17).

Milk Production: Glutamine and its counterpart Glutamic acid are most abundant in milk. Ruminents have low plasma glutamine level because of low glutamine synthatase capacity as compare to monogastic animals. Glutamine is

limiting amino acid because of high metabolic stress of milk production the uptake of glutamine by mammary gland is 100 %.

fermented foods: In addition to nutritional benefit, protein associated glutaminase gives texture and sensory properties to the foods. In dough softening in baking industry, enhancement of 'umami' taste in hydrolyzed vegetable protein and increase protein digestibility foods. Microorganisms are sources of glutaminase, *Lactobacillus spp*. are probiotic bacteria and used in the food fermentation industry for manufacturing cheeses, buttermilk, sauerkraut, and yogurt (18).

Protein deamidation: Protein deamidation technology by glutaminase leads to production of protein containing foods with improved functional properties, especially protein solubility, and potentially decreased flavor fade problems associated with flavor-protein interactions, especially with carbonyl containing flavor compounds (19). Protein-glutaminase converts glutaminyl residues into glutamyl residues which results in increase of negative charge in food proteins (milk casein, wheat gluten, rice glutelin etc.) due to increase in negative charge the isoelectric point of deamidated protein gets lowered and gives good solubility of product in acidic pH. Protein foldings gets changed by deamidation due to newly form negative charges and exposure of hydrophobic region, results in a protein with improved amphiphillic character. This character is responsible for making the protein an ideal emulsifier or foaming agent (20).

The value of broken and debris rice or by-product of rice starch can be significantly improved by this method. Moreover, the resource waste and environment pollution could be minimized. These new features of deamidated rice glutelin suggested that glutaminase could be a potential tool for enhancing the usability of rice protein in the food industry (21). Soy-protein isolates enzymatic deamidation by protein-glutaminase, incereases functional properties of soy protein. That can be used for various purposes in the food industry, especially for use in acidic soy based beverages. However, studies on the conformational changes and other functional properties, such as impact on the flavor profile and flavor binding properties, are still needed (19).

For soy-food production: In Japanese soy sauce fermentation study Wakayama et al. (22) studied that glutaminase from *S. maltophilia* has high ability for glutamic acid production as compare to other microorganism such as *Aspergillus oryzae, Escherichia coli, Pseudomonas citronellolis,* and *Micrococcus luteus,*

indicating that this enzyme is suitable for application in Japanese soy sauce fermentation. The unique flavor of fermented soy sauce is credited mainly to glutamic acid (concentrations of 0.7 to 0.8% per total nitrogen) (23). Under optimal conditions the partial deamidation of soymilk by protein-glutaminase, enhances protein solubility under acidic conditions (pH 5.0) and decreased the flavor binding potential of the protein to both vanillin and maltol. These could benefit soy protein and soy-food manufacturers who intend to reduce the flavor fade problem in aqueous food products containing soy *proteins* (20).

Substitute of monosodium glutamate: The sodium salt of glutamic acid is monosodium glutamate. It has the nutrient property and salt substitute (16). Food industry has widely used monosodium glutamate as a flavor enhancer. However, monosodium glutamate shows negative side effects such as wheezing, heart-rate changes, and breathing difficulty in some people which is questionable on its safety. It can induce hypothalamic lesions and leptin resistance, possibly influencing energy balance, leading to overweight (16).

Vitamin B6 Synthesis: Plants and microorganisms have the capacity to synthesize vitamins through De Novo biosynthesis but animals must have to obtain it from diet. Glutaminase involve in vitamin B6 synthesis. Vitamin B6 is vital metabolite for all living organisms and for various biochemical reactions, it works as cofactor. In eubacteria, deoxyxylulose 5-phosphate dependent and in archaea, fungi, plants, protista, and most eubacteria deoxyxylulose 5-phosphate independent two distinct and mutually exclusive de novo pathway are present. In these organisms, pyridoxal 5'-phosphate (PLP) formation is catalyzed by a single glutamine amidotransferase (PLP synthase) composed of a glutaminase domain, PDX2, and a synthase domain, PDX1 (24).

Research and development: Glutaminase catalyzes the hydrolysis of glutamine to glutamate and plays a central role in the proliferation of neoplastic cells via glutaminolysis. For understanding the role of glutaminase in cancer cell metabolism to identify therapeutic targets (5). It is used as analytical agent for the determination of glutamine and glutamate. Study of brain-specific BNIP-2-homology protein Caytaxin relocalises glutaminase to neurite terminals and reduces glutamate levels (25). For L-glutaminase production partitioning studies has performed on Bacillus cereus MTCC 1305 in different PEG–salt/dextran (26) etc.

In Biosensor: In biosensor, for monitoring the glutamine level in mammalian and

hybridoma cell cultures without the need of separate measurement for glutamic acids. Simultaneous measurement of L-glutamine and L-glutamate, Integrated thin film biosensors were developed for the microbial micro-flow cell. Due to a novel glutaminase with an activity optimum in the neutral pH range, direct monitoring of glutamine in a mammalian cell culture medium could be performed (27).

Glutamine biosensor system based on a conductance-surface acoustic wave (SAW) frequency response, in which a SAW resonator oscillating at 61 MHz and a biosensor was developed for the determination of glutamine. In biosensor kidney cortex tissue (porcine) or Escherichia coliform Glutaminase was used as a biocatalyst of the hydrolysis reaction of glutamine (28).

Production of chemicals: Glutamic acid is a product of hydrolysis of glutamine by glutaminase. Theanine, which is a food additive and dietary supplement, can be synthesized efficiently by a ϒ-glutamyl transfer reaction using glutaminase. Generally, the production of theanine synthetase (EC 6.3.1.6) synthesizes theanine but Tachiki et al. (29) have developed a method of producing theanine from glutamic acid and ethylamine using a combination reaction of bacterial glutamine synthetase with a sugar fermentation reaction of baker's yeast as an ATP-regenerating system (30). Another method for production of L-theanine was done using glutaminase encapsulated in carbon-coated mesoporous silica. Glutamine, glutamate and proline are the precursor for the synthesis of arginine in the intestinal-renal axis in human and most other mammals (16). Pseudomonas nitroreductions IFO 12694 has been reported for the catalysis of ϒ-glutamyl transfer reaction and hydrolysis simontaniously in which the reaction mixture contains ϒ-glutamyl donor and ϒ-glutamyl acceptor (29).

Future prospect: Structural, regulatory and biochemical understanding will be helped by glutaminase understanding at the gene level from different microorganisms. Application study will increase the thrust in this area and open-up new ways to simplify or improve living beings life. It is required for further improvement in production.

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