L-Asparaginase

Subjects: Biotechnology

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Definition

L-Asparaginase (ASNase, EC 3.5.1.1) is a tetrameric aminohydrolase enzyme that catalyses the hydrolysis of the amino acid L-Asparagine into ammonia and L-aspartic acid. ASNase is present in different organisms such as bacteria, fungi, plant tissues and algae. ASNase is used in the pharmaceutical field as an anticancer drug for the treatment of acute lymphoblastic leukemia (ALL) and other malignant diseases such as Hodgkin's disease. In the food sector, ASNase is used to prevent the formation of acrylamide, a toxic compound formed when starch-rich foods are cooked at temperatures above 100 °C. ASNase can also be used as a biosensor for the detection of L-asparagine levels.

1. Introduction

ASNase can be produced by a wide variety of natural sources, namely microorganisms (bacteria, yeast, filamentous fungi, algae), plants and vertebrates. Microorganisms, such as *Aspergillus tamarii*, *Aerobacter* spp., *Bacillus* spp., *Photobacterium* spp., *Serratia* spp., *Xanthomonas* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris, Streptomyces griseus* and *Vibrio succinogenes* are preferred sources for ASNase production [1]. In 1967, two ASNase isozymes with different properties were discovered in *Escherichia coli*, namely type I and type II [2]. Type I ASNase is a homodimeric cytosolic constitutive enzyme, while type II ASNase, normally assuming a homotetrameric configuration, is located in the enzyme periplasm, being secreted only when exposed to low nitrogen concentrations. Even though both isozymes show enzymatic activity for L-asparagine and L-glutamine, the main difference between them is the specificity for L-asparagine [3]. Type II is known to have anti-tumour activity due to the higher specific affinity for L-asparagine [4].

The L-asparagine hydrolysis by ASNase occurs in two main steps (Figure 1). The first step involves the enzyme nucleophilic residue activation by NH_2, a powerful base, and the subsequent attack on the L-asparagine amide carbon atom, generating the beta-acyl-enzyme intermediate; the second one comprises the nucleophile activation by a water molecule, attacking the ester carbon successively, providing L-aspartic acid and liberating ammonia [5].

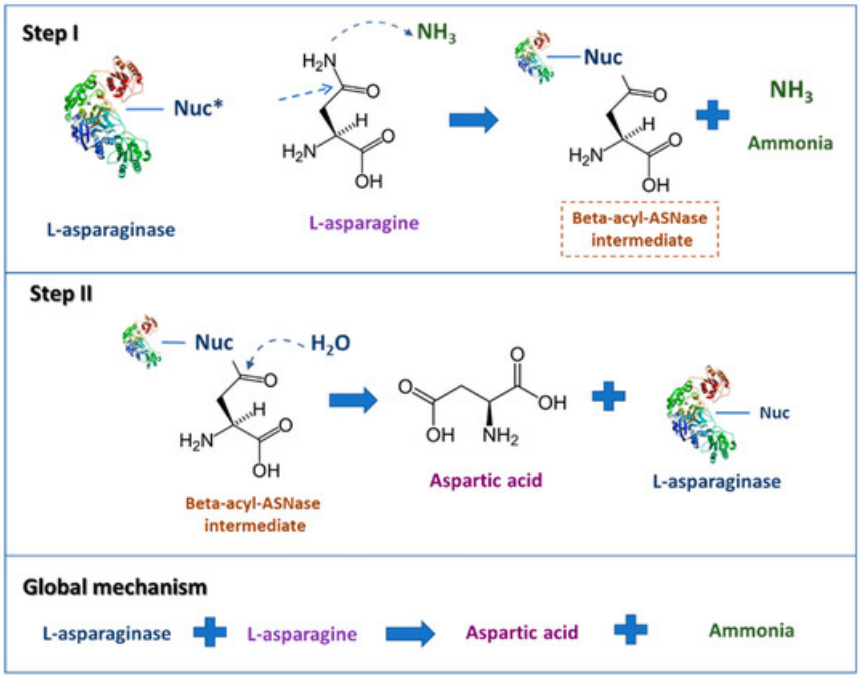

Figure 1. Scheme describing the L-asparaginase reaction mechanisms. * Nuc: nucleophilic residue (adapted from Hill et al. [6]).

In the pharmaceutical sector, type II ASNase has been applied in the treatment of lymphoproliferative disorders and lymphomas, namely ALL, T-cell lymphomas, subtypes of myeloid leukaemias and NK tumours [7]. Furthermore, due to its glutaminase activity, ovarian carcinomas and further solid tumours have been projected as ASNase additional targets [7]. In fact, in vitro ASNase sensitivity was exhibited for soft tissue sarcoma[8], β-catenin mutated hepatocellular carcinoma [8], hepatocellular carcinoma with low asparagine synthetase expression[9] and gastric adenocarcinoma [10]. ASNase can deplete L-asparagine, an essential amino acid to tumour cells. More specifically, healthy cells synthesise L-asparagine through transaminase enzyme, which converts oxaloacetate into an intermediate aspartate that subsequently transfers an amino group from glutamate to oxaloacetate, generating α-ketoglutarate and aspartate. They are then transformed into asparagine through asparagine synthase or glutamine-dependent asparagine synthetase via an ATP-dependent reaction, which takes advantage of the amido-N of L-glutamine in order to form the amido group of asparagine.

In the food industry, ASNase can prevent the acrylamide formation, a carcinogenic compound produced during the heat of food processed products [11]. Thus, the pre-treatment of starchy foods with ASNase, before heating, converts L-asparagine to aspartic acid, preventing the acrylamide formation by the Maillard reaction between L-asparagine and carbonyl compounds at high temperatures [11]. In 2003, Zyzak et al.[12] reported the ASNase application for acrylamide reduction in a potato matrix. This observation led to the inclusion of monographs on ASNase from *Aspergillus oryzae* and *Aspergillus niger* in World Health Organization (WHO) food additives series in 2008 (59th series)[13] and 2009 (60th series) [14], respectively. However, as the enzyme action could be affected by food composition, the ideal ASNase to be used in the food industry must be stable throughout the food processing and proteolysis and, once consumed, it should not cause allergic or toxic reactions.

The manufacture of ASNase-based biosensors to detect and/or quantify L-asparagine levels is also considered a promising technology in both clinical and food industries[15]. These biosensors' mechanism of action is due to the ASNase activity. Ammonium ions generated during the asparagine hydrolysis lead to a pH variation and subsequent change of colour and absorption wavelength [16].

2. Commercial ASNase

Currently, there are several type II ASNase commercially available, produced industrially for medical applications (detailed in Table 1): (i) native ASNase from *E. coli* (Elspar® from Ovation Pharmaceuticals, Illinois, IL, USA [17]; Leukanase® from Sanofi-aventis, New South Wales, Australia; Kidrolase® from EUSA Pharma, SAS, Lyon, France [18], etc.); (ii) PEGylated ASNase from recombinant *E. coli*, pegaspargase (Oncaspar® from Enzon Pharmaceuticals, Florida, FL, USA) [19]; (iii) native ASNase, but as a recombinant form, being produced in *E. coli* and *E. chrysanthemi* as host cells (Spectrila® from Medac Gesellschaft, Wedel, Germany [20] and Erwinase® (from *Erwinia chrysanthemi*) from EUSA Pharma, SAS, Lyon, France [21], respectively).

Elspar® was the first ASNase to be available on the market and to be approved (1978) by the U.S. Food and Drug Administration (FDA) for use as a component of a multi-agent chemotherapeutic regimen for the treatment of patients with acute lymphoblastic leukaemia (ALL). In 1994, Oncaspar® received the same approval by FDA, but only for patients with hypersensitivity to native Elspar®. Only in 2006 it was approved as part of the first-line therapy for any ALL patient [22]. In November 2011, FDA approved Erwinase®, indicating its use as a component of a multi-agent chemotherapeutic regimen for ALL patients treatment who have developed hypersensitivity to either Elspar® or Oncaspar® [23]. Finally, in January 2016, the European Commission granted a marketing authorisation valid throughout the European Union for Spectrila® (from E. coli). However, all these ASNase products are associated with several noteworthy toxicities and should be used with care because of the possibility of severe reactions, including anaphylaxis and sudden death [24].

Commercially approved ASNases to be used in food industries (detailed in Table 1) comprise the fungal ones from *A. oryzae* (Acrylaway® from Novozymes A/S, Bagsvaerd, Denmark) and *A. niger* (PreventASe™ from DSM, Heerlen, The Netherlands) [25]. The US government attributed the status of “generally recognised as safe” (GRAS) to both ASNases, and in 2007-2008 they received a favourable evaluation as a food additive by the Joint FAO/WHO Expert Committee [26].

Table 1. Commercial ASNase for therapeutic and food applications.

ASNase Application	ASNase Form	Microorganism	ASNase Commercial Name	ASNase Manufacturer
Therapeutic/Pharmaceutical	Native ASNase	*E. coli*	Elspar®	Ovation Pharmaceuticals
			Leukanase®	Sanofi-aventis
			Kidrolase®	EUSA Pharma
	PEGylated ASNase	*E. coli*	Oncaspar®	Enzon Pharmaceuticals
	Native recombinant ASNase	*E. coli*	Spectrila®	Medac Gesellschaft
		E. chrysanthemi	Erwinase®	EUSA Pharma
		A. oryzae	Acrylaway®	Novozymes A/S
Food Industry	Native ASNase			

	A. niger	PreventASe™	DSM

3. Confined ASNase

The use of ASNase in its free form is challenging due to its unstable nature and limitation to a single use. Thus, the improvement of ASNase enzymatic and therapeutic properties has been achieved by introducing chemical modifications and physical integration within several supports. These techniques, if properly designed, can improve the stability of the enzymes and allow their reuse, also contributing to the reduction of operation costs [27]. Due to enzymes protection (enhanced activity and stability [28]) and expanded catalytic half-life [29], confined ASNase can find improved applications in the previous cited areas [30]. Nevertheless, as the enzymes confinement on support materials could result in several enzyme modifications, the changes in the enzyme structure and activity should be thoroughly studied and evaluated according to the target application [31]. Therefore, the choice of the support material and the confinement procedure are aspects of maximum importance. A high number of ASNase confinement possibilities have been recently developed, which may be grouped into three main approaches: (i) physical adsorption; (ii) covalent attachment; (iii) entrapment.

Even though in recent years numerous works about ASNase confinement have been published, in which the enhanced biochemical and pharmacological features of ASNase are reported, more work is still needed to fulfil the requirements of regulatory agencies and reach the biopharmaceutical industry. Within these recent reports, the ASNase entrapment displayed the most promising results for an intravenous application and *in vivo* safety. Although the ASNase entrapment into (nano)materials has been reported in the literature, there are no related commercial solutions currently in the market. Since the majority of ASNases are thermolabile and active in a narrow pH range, ASNase confinement for food applications has been recently investigated to improve the enzymatic stability and activity over a broad range of temperature and pH, in order to lower the processing time and costs. In recent times, confined ASNase also started to emerge in the biosensing technology, opening new possibilities up at the industrial level, namely in therapeutic/pharmaceutical and food industries due to its potential to monitor asparagine levels in blood serum samples of ALL and lymphosarcoma patients and to detect asparagine in food samples. The need for a thermostable ASNase to improve its catalytic performance reinforces the need for further research on the use of confined ASNase.

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Keywords

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