L-Asparaginase-Based Biosensors

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Contributor: Ana Paula Tavares, João C. F. Nunes, Raquel Cristóvão, Valeria Santos-Ebinuma, Joaquim Luís Faria, Cláudia Silva, Márcia C. Neves, Mara Freire

L-asparaginase (ASNase) is an aminohydrolase enzyme widely used in the pharmaceutical and food industries. Although currently its main applications are focused on the treatment of lymphoproliferative disorders such as acute lymphoblastic leukemia (ALL) and acrylamide reduction in starch-rich foods cooked at temperatures above 100 °C, its use as a biosensor in the detection and monitoring of L-asparagine levels is of high relevance. ASNase-based biosensors are a promising and innovative technology, mostly based on colorimetric detection since the mechanism of action of ASNase is the catalysis of the L-asparagine hydrolysis, which releases L-aspartic acid and ammonium ions, promoting a medium pH value change followed by color variation. ASNase biosensing systems prove their potential for L-asparagine monitoring in ALL patients, along with L-asparagine concentration analysis in foods, due to their simplicity and fast response.

Keywords: L-asparaginase ; biosensor ; L-asparagine ; monitoring ; ammonia ; pharmaceutical ; food ; industry

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Biosensors are analytical systems consisting of an immobilized biological element combined with a suitable transducer to quantify an analyte [1]. Depending on the biosensor purpose, the most common transducers are electrochemical, piezoelectric, optical, thermometric or magnetic [2]. Biosensors usually generate an electronic or optical signal proportional to the particular interaction between the analyte and the immobilized recognition compound. The low equipment costs, high sensitivity and precision and easy operation have led to increased demand for this type of systems compared to traditional analytical methods [3]. The biological component may be constituted by different bio-recognition elements, such as enzymes, bacteria, tissues, antibodies or nucleic acids, etc. Among them, enzyme-based biosensors have been increasingly used due to their recognized high specificity and exceptional biorecognition capabilities [4]. The enzyme immobilization procedure can influence the biosensor stability, specificity, sensitivity and reproducibility. Therefore, the method used to attach the enzyme onto the electrode must guarantee the stability of the active site and the biomolecule activity. The selection of a suitable technique depends on the biological component nature, the transducer type, the analyte characteristics and the operating conditions. The most used enzyme immobilization methods for the design and development of biosensors are classified into physical adsorption, covalent binding and entrapment [5]. Physical adsorption comprises low associated costs and improved enzymatic performance, whereas entrapment (within the framework of a support) allows enzyme preservation and high enzymatic activity levels. However, physical adsorption can lead to nonspecific adsorption and enzyme desorption, while entrapment can lead to mass transfer limitations and enzyme leakage. On the other hand, by applying a covalent bond between the enzyme and the immobilization support, the enzyme leaching is avoided, allowing for the recovery and reuse of the support/enzyme. Nevertheless, in this last option, the enzyme's active center amino acids should not be involved in covalent bonding to guarantee high levels of enzyme activity, which is defined by the enzyme binding orientation [6][7].

The enzyme L-asparaginase (E.C.3.5.1.1, ASNase) is a chemotherapeutic agent for the treatment of lymphoproliferative disorders, specifically acute lymphoblastic leukemia (ALL), lymphomas and natural killer cell tumors. The tumor-inhibitory characteristics of ASNase were first reported in animal trials in the 1950s [8][9]. ASNase catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia. Since healthy cells can produce L-asparagine internally, whereas leukemic cells depend on this crucial extracellular amino acid for their growth, prolonged deprivation of L-asparagine in blood leads malignant lymphoid cells into apoptosis [10]. L-asparagine concentration varies from 10^{-6} to 10^{-4} M in healthy blood serum samples and from 10^{-3} to 10^{-2} M in leukemia blood serum samples [11]. Thus, monitoring L-asparagine depletion in ALL patients is essential to assess the efficacy of ASNase therapy [12]. Full L-asparagine depletion and high ASNase activity are both associated with improved outcomes in ALL patients [13].

ASNase also has an important application in the food industry for acrylamide mitigation from heat-processed foods. Acrylamide is referred to as a Group 2A carcinogen ("probably carcinogenic to humans") by the International Agency for Research on Cancer (IARC) and by the World Health Organization (WHO) [14][15]. According to the Food and Drug Administration (FDA), the food products with the highest acrylamide concentrations (up to 8440 $\mu g.kg^{-1}$) are cereals, french fries, potato chips and cookies, whose average daily acrylamide intake varies from 0.03 to 0.05 $\mu g.kg^{-1}$ body

weight [6][16]. Nevertheless, the tolerable daily acrylamide intake to avoid carcinogenic risk is 2.6 μg kg^{-1} body weight [17]. The pre-treatment of starchy foods with ASNase before cooking transforms L-asparagine into L-aspartic acid, and the Maillard reaction takes place without the contribution of L-asparagine, avoiding the acrylamide formation in the final food product [18]. Since the amino acid L-asparagine is not mainly responsible for the taste and appearance of the processed foods, the desired organoleptic characteristics are preserved [19]. Thus, ASNase is already used to lower the acrylamide dosage in several food products, such as potatoes, bread, french fries, coffee and biscuits [6].

ASNase-based biosensors are a promising and innovative technology that can be used both to detect and monitor the level of L-asparagine in blood serum samples of leukemic patients and in different food samples [6]. The methods currently used to detect L-asparagine are based on spectroscopy and chromatography techniques [20], whereas the biosensor operation mode is mostly based on colorimetric detection. The L-asparagine hydrolysis releases ammonium ions, promoting, consequently, a change in the pH value of the medium followed by a variation in the color [21], being the mode of action of colorimetric ASNase-based biosensors (**Figure 1**). Although there are few reports on the development of ASNase-based biosensors, biosensing systems prove to be imperative for pharmaceutical/food industrial applications due to their simplicity and fast response, while allowing online L-asparagine monitoring.

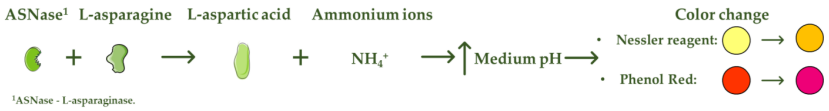

Figure 1. Mode of action of colorimetric ASNase based biosensors.

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