Advances in Colloid and Interface Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Historical perspective

Bromelain-loaded nanoparticles: A comprehensive review of the state of the art

Janaína Artem Ataide [a,c], Eloah Favero Gérios [b], Priscila Gava Mazzola [b,*,1], Eliana B. Souto [c,d]

[a] Graduate Program in Medical Sciences, School of Medical Sciences, University of Campinas, Brazil
[b] Faculty of Pharmaceutical Sciences, University of Campinas, Brazil
[c] Department of Pharmaceutical Technology of the Faculty of Pharmacy, University of Coimbra, Portugal
[d] REQUIMTE - Group of Pharmaceutical Technology, Coimbra, Portugal

ARTICLE INFO

Available online xxxx

Keywords:
Bromelain
Nanoparticles
Pharmaceutical nanotechnology
Stability enhancement

ABSTRACT

Stem bromelain is a common available cysteine protease derived from pineapple (*Ananas comosus* L.). Bromelain finds widespread applications in several areas, such as medicine, health, food, and cosmetics, and its strong proteolytic activity supports its future application in many additional fields. However, most proteins and/or enzymes are fragile, leading to important considerations about increase storage and operational stability to enable their practical application. In this scenario, the use of nanoparticles to deliver proteins is increasing exponentially, given that these systems are capable of enhance active's stability, solubility and permeability, and decrease toxicity. In the pharmaceutical nanotechnology field, bromelain has played different roles and thus this paper aims to review the available literature for the use of nanoparticles and bromelain.

Contents

1. Introduction

Bromelain is a crude extract derived from pineapple plant (*Ananas comosus* L.) and contains mixture of proteolytic enzymes and non-enzymatic substances [1]. It can be found in several parts of the pineapple plant, including its stem, fruit, leaves and peel [2,3]; only the stem and fruit, however, produce high amounts of bromelain [4,5]. Stem

* Corresponding author at: Faculty of Pharmaceutical Sciences, University of Campinas (Unicamp), Campinas, SP 13083-871, Brazil.
E-mail addresses: janaina.a.ataide@gmail.com (J.A. Ataide), pmazzola@fcf.unicamp.br (P.G. Mazzola).
[1] Postal Address: Faculty of Pharmaceutical Sciences, University of Campinas (Unicamp) - Cândido Portinari Street, 200, Cidade Universitária "Zeferino Vaz" - Campinas - SP - Brasil – zip code: 13083-871.

https://doi.org/10.1016/j.cis.2018.03.006
0001-8686/

bromelain (EC 3.4.22.32), found in the pineapple stem, has an isoelectric point (pI) of 9.5, and is the most abundant protease in pineapple tissue preparations. On the other hand, fruit bromelain (EC 3.4.22.33), which is found in the pineapple fruit, has a pI of 4.6, and is present in lesser amounts compared to stem bromelain [6,7]. Stem bromelain is economical to produce once pineapple stems are cheap compared with the fruit, which is normally consumed. Therefore, stem bromelain, which is composed of endopeptidases (ananain, comosain), phosphatases, glucosidases, peroxidases, escharase, cellulases, glycoproteins, proteinase inhibitors, calcium and carbohydrate, is the most commonly available commercial product [1].

Like other cysteine proteases, the spectral characteristics of stem bromelain suggest that this enzyme belongs to the $\alpha + \beta$ protein class, with 23% α-helix, 5% parallel β-sheet, 18% anti-parallel β-sheet, 28% turns and rest other secondary structures [8,9]. Stem bromelain contains 285 amino acids where the most abundant amino acids are alanine and glycine, while histidine and methionine are present in the lowest amounts [9–11], and its amino acid sequence has a high similarity to that of papain, actinidin, proteinase Ω and chymopapain [12].

Bromelain finds widespread applications in several areas, such as medicine, health, food, and cosmetics. Its applications have been widely reviewed in the literature: medical use [6], food industries and cosmetics [11], therapeutic applications [13], surgical care and related conditions [1], and bromelain commercialization for clinical and industry uses [5]. Literature shows that while the main application of bromelain continues to be in the pharmaceutical industry, the strong proteolytic activity of the enzyme supports its future application in many additional fields [13].

An increased storage and operational stability of an enzyme is an important consideration for its practical application [14], since most proteins and/or enzymes are fragile, and even small conformational changes may reduce their activity [15]. Several methods have been reported and used for enzyme stabilization, such as enzyme chemical modifications, protein engineering techniques, use of compatible osmolytes [14,16]. In addition, the use of nanoparticles (NPs) to deliver protein drugs is increasing exponentially, once these systems stabilize those actives against denaturation by enzymatic digestion, increasing their biopharmaceutical applications [17].

Besides stability enhancement, nanostructures as drug delivery systems (nanocarriers) are a key to overcome other challenges associated with drug therapy, including poor solubility, poor permeability and high toxicity [18,19]. Those nanometric carriers may also be used for the development of targeted delivery systems, which comprised a therapeutic agent, a targeting moiety, and a carrier system [20]. In the pharmaceutical nanotechnology field, bromelain has played different roles, such as surface modification [21–25], reducing and capping agent for gold NPs production [26–28], and immobilized or encapsulated active [29–36]. NPs have also been used as an alternative for bromelain purification [37–39] and quantification [40,41].

The aim of this paper was to review the available literature for the use of NPs and bromelain in pharmaceutical area. For this purpose, Web of Science and PubMed databases have been used, crossing the terms "bromelain" and "nanoparticle", resulting in 21 papers. Out of these, only 16 papers have been thoroughly revised according to the NP composition, excluding those 5 reporting bromelain purification and quantification.

1.1. Inorganic compounds

1.1.1. Silica

Mesoporous silica nanoparticles (MSNs) are biocompatible materials, with large surface area and pore volume, providing great potential for drug absorption and loading within the pore channels [42]. MSNs mesoporous structure and adjustable pore size enable better control of drug loading and releasing [43]; and their surface can be easily modified for controlled and target drug delivery, enhancing drug therapeutic efficacy and reducing toxicity.

Parodi, Haddix [21] developed MSNs with a proteolytic (bromelain) surface, to enhance NPs diffusion features upon contact with tumor extracellular matrix. First non-modified MSNs were produced and characterized by TEM analysis, which showed a uniform size around 50 nm and spherical shape. Pore size and surface area were also analyzed, and found to be around 2.3 nm pore size and 650 m^2/g of surface area to mass ratio [21]. Surface functionalization of MSN with bromelain was achieved and proven by FTIR spectroscopy. Size and zeta potential were determined for MSN and bromelain-conjugated MSN by DLS, and found to be 173.7 nm and 213.6 nm, +3.89 mV and −2.01 mV, respectively. The mass of bromelain linked to the MSN surface was quantified with Bradford protein assay [44], revealing 126.0 μg of protein extract/mg of unmodified particle was bound and absorbed after subtraction of the MSN background [21].

In this study, authors also showed that bromelain modification increased particles affinity for tumor extracellular matrix, and showed a minor impact on cell viability and cell endolysosomal activity. Bromelain–MSN demonstrated efficient digestion and diffusion in matrigel, as well as inhibition of the organization of endothelial cells into tube-like structures when plated upon matrigel. Upon direct administration within the tumor, modified particles diffused to a greater extent within 1 h than non-proteolytic MSN [21].

Silica supports are of particular interest for enzyme encapsulation in solid supports, due to their inert and stable matrix characteristics, with a pore size that can be tailored to the specific dimensions of the enzyme [45]. To enhance encapsulation efficiency, a bioinspired silicification technique has been used, wherein the enzymes are entrapped within silica nanosphere aggregates. Baker, Patwardhan [31] used sodium metasilicate as a silica precursor and ethyleneamines of different chain lengths as initiators to encapsulate papain, bromelain, and trypsin in silica nanosphere aggregates.

Overall, an encapsulation efficiency >70.0% was observed for each protease, independent of ethyleneamine chain length, and thus pentaethylenehexamine (PEHA) was chosen as the initiator, due to its ability to provide rapid silica formation [31]. Morphology and particle size of the bioinspired silica aggregates were analyzed using field-emission scanning electron microscopy, showing smooth spherical particles with diameters ranging from 125.0 to 325.0 nm in the absence of proteases. Particles formed in the presence of either bromelain had morphology and size distribution similar to blank particles. Enzyme encapsulation was also confirmed by FTIR characterization and porosity measurements [31].

After encapsulated, hydrolysis and aminolysis were evaluated to determine if proteins remained their activities in comparison with free proteases. In casein assay, an activity reduction >60.0% was observed when 2.0 mg of protein was used in the enzymes encapsulation process, and for bromelain only 12.1% of activity remained after encapsulation. This activity was partially recovered by increasing the total mass of the protease encapsulated in the silica aggregates from 2.0 mg to 10.0 mg, achieving 61.7% of remained activity for bromelain. To evaluate the effect in aminolysis, L-amino acid ethyl esters were used as substrate. Encapsulation did not change the percent yield of poly-L-leucine, but increased 10 °C in optimal temperature for production of poly-L-leucine, which was found to be 40 °C for free enzyme and 50 °C for encapsulated form [31]. Thermal stability of free and encapsulated enzymes where also compared through enzymatic activity evaluation in temperatures ranging from 20 to 70 °C. Encapsulation process leads to an increase of 10 °C in optimal thermal activity to bromelain. In addition, encapsulated proteases retained a higher relative hydrolytic activity then free enzymes at temperatures above that for optimal activity [31].

1.1.2. Gold

Gold NPs present various biomedical applications and present the possibility of surface functionalization, which has opened new perspectives for anticancer and antimicrobial drugs [46]. However, the most

Please cite this article as: Ataide JA, et al, Bromelain-loaded nanoparticles: A comprehensive review of the state of the art, Adv Colloid Interface Sci (2017), https://doi.org/10.1016/j.cis.2018.03.006