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Influence of sodium alginate pretreated by ultrasound on papain properties: Activity, structure, conformation and molecular weight and distribution

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ABSTRACT

The aim of the study was to investigate the impact of sodium alginate (ALG) pretreated by ultrasound on the enzyme activity, structure, conformation and molecular weight and distribution of papain. ALG solutions were pretreated with ultrasound at varying power (0.05, 0.15, 0.25, 0.35, 0.45 W/cm^2), 135 kHz, 50 °C for 20 min. The maximum relative activity of papain increased by 10.53% when mixed with ALG pretreated by ultrasound at 0.25 W/cm^2, compared with the untreated ALG. The influence of ultrasound pretreated ALG on the conformation and secondary structure of papain were assessed by fluorescence spectroscopy and circular dichroism spectroscopy. The fluorescence spectra revealed that ultrasound pretreated ALG increased the number of tryptophan on papain surface, especially at 0.25 W/cm^2. It indicated that ultrasound pretreatment induced molecular unfolding, causing the exposure of more hydrophobic groups and regions from inside to the outside of the papain molecules. Furthermore, ultrasound pretreated ALG resulted in minor changes in the secondary structure of the papain. The content of α-helix was slightly increased after ultrasound pretreatment and no significant change was observed at different ultrasound powers. ALG pretreated by ultrasound enhanced the stability of the secondary structure of papain, especially at 0.25 W/cm^2. The free sulfhydryl (SH) content of papain was slightly increased and then decreased with the increase of ultrasonic power. The maximum content of free SH was observed at 0.25 W/cm^2, under which the content of the free SH increased by 6.36% compared with the untreated ALG. Dynamic light scattering showed that the effect of ultrasound treatment was mainly the homogenization of the ALG particles in the mixed dispersion. The gel permeation chromatography coupled with the multi-angle laser light scattering photometer analysis showed that the molecular weight (M_w) of papain/ALG was decreased and then increased with the ultrasonic pretreatment. Results demonstrated that the activity of immobilized papain improved by ultrasonic pretreatment was mainly caused by the variation of the conformation of papain and the effect of interactions between papain and ALG. This study is important to explain the intermolecular interactions of biopolymers and the mechanism of enzyme immobilization treated by ultrasound in improving the enzymatic activity. As expected, ALG pretreated by appropriate ultrasound is promising as a bioactive compound carrier in the field of immobilized enzyme.

1. Introduction

Ultrasound as a novel technology has attracted a wide range of interest from fundamental academic research to many different industrial applications in recent years. It has been proved to be an effective technique for the synthesis of nanomaterials as well as for the deposition and insertion of nano-particles on/into mesoporous ceramic, polymer supports, fabrics, and glass [1,2]. It can be divided into two intensity ranges. Low-intensity (high-frequency, 100 kHz–1 MHz, power <1 W/cm^2) ultrasound is most commonly applied as an analytical technique to provide information on the physicochemical properties of food such as firmness, ripeness, sugar content and acidity, etc. High-intensity (low-frequency,

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16–100 kHz, power in the range 10–1000 W/cm^2) ultrasound is suitable for the applications including homogenization, emulsification and extraction [3–6].

The effect of ultrasound on liquid system is mainly attributed to the cavitation bubbles generating intense shear stress [7]. The bubbles are rapidly formed and violently collapsed, leading to extreme temperature (5000 K) and pressure (120 MPa) which can produce very high shear energy waves and turbulence in the cavitation zone [8]. The energy released from collapsing cavitation bubbles can be transferred to the sonication matter through the appropriate choice of sonication medium and particle concentration [9]. Under such conditions, molecules trapped in the bubble (water vapor, gases and vaporized solutes) can be brought to an excited-state and dissociate [10]. The above mentioned factors of temperature, pressure and turbulence combined together to affect the ultrasound treated system. Moreover, hydroxyl free radicals could be generated by the rupture of molecules bond in aqueous solution, leading to the activation effect. There are some literatures focusing on studying ultrasound as an enzymatic pretreatment to reduce particle size or accelerate the reaction rate [11]. Bashari et al. found that ultrasound could improve the catalytic activity of immobilized dextranase [12]. Ultrasound is also known to perturb weak interactions and induce conformational changes in protein structures [13].

Papain is a kind of cysteine protease obtained from carica papaya. It is a compact globular protein (molecular mass = 21 kDa) containing 212 amino acid residues with three disulfide bonds. The active site of papain consists of Cys25–His159–Asn175 [14]. Sodium alginate (ALG) is one of the widely used polysaccharides as an enzyme carrier and composed of β-D-mannuronate residues and α-L-guluronate residues. It has been demonstrated that alginate is a promising bioactive compound carrier [15]. Recently, ALG immobilized papain has received a great deal of attention. However, the grid structure of polymer carriers may hinder the macromolecules' diffusion and restrain the release of substrate such as casein. It was reported that the activity of papain immobilized by ALG-chitosan was enhanced successfully by the ultrasound treatment and the optimal frequency was 135 kHz [16]. The activity of immobilized papain improved by ultrasonic treatment was probably caused by the increase of the diffusion properties of the casein at different frequencies and powers, respectively [17]. Meanwhile, the results could also be due to the variation of the secondary and tertiary structures of papain or the effect of interactions between papain and polysaccharides [18]. To the best of our knowledge, there are few published paper related to clearly demonstrate the latter reason. Additionally, few studies focused on investigating the enzyme-polysaccharide liquid system pretreated by ultrasound, especially the polysaccharide was pretreated by ultrasound.

Therefore, the purpose of this study was to investigate properties of papain mixed with ALG pretreated by ultrasound in liquid system rather than entrapped in the ALG gel. Comparison of papain features in ALG liquid system based on ultrasound eliminated the influence of casein diffusion. Hosseini et al. reported that ultrasonication promoted ALG-ALG interactions and decreased the interaction strength between β-lactoglobulin and ALG [19]. It was stated that ultrasound pretreatment is an efficient method in rapeseed proteolysis to produce peptides through its impact on the molecular conformation [20]. In our current study, it was hypothesized that ultrasonic pretreatment of ALG would affect the interactions between papain and ALG, molecular conformation of papain and further impact the immobilized enzyme activity. Based on our group previous study [16], the ultrasound pretreated conditions were carried out at different powers (0.05, 0.15, 0.25, 0.35, 0.45 W/cm^2), 135 kHz, 50 °C for 20 min.

To investigate the impact of ALG pretreated by different ultrasonic powers on enzymology characteristics of papain by using the dynamic light scattering (DLS), fluorescence spectroscopy, circular dichroism (CD) and gel permeation chromatography coupled with a multi-angle laser light scattering photometer analysis (GPC–MALLS). The aim is to evaluate enzyme activity, structure, conformation and molecular weight and distribution of papain impacted by ultrasound pretreated ALG which is to explain the mechanism of ultrasound-accelerated enzymolysis of papain immobilization in order to extend the application of ultrasound in the process of immobilized enzyme.

2. Materials and methods

2.1. Materials

Sodium alginate (ALG, $M_w = 1.93 \times 10^5$ g/mol, M/G = 1.51, 200 ± 20 mPa s viscosity) and 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Aladdin Reagent Company (Shanghai, China). Papain (21 kDa, 2.77×10^5 U g^{-1}) was purchased from Sigma Chemicals. Casein was purchased from Beijing Ao Bo Xing Bio-Tech. Co., Ltd. (Beijing, China). Folin phenol reagent and L-Glutathione were purchased from Sigma Chemicals. All other chemicals and solvents used were of analytical grade.

2.2. Ultrasound equipment

An assembled ultrasonic bath system equipment with two sets of JXD-02 multi-frequencies processing system and the low temperature circulating water tank was employed (JXD-02, Beijing Jinxing Ultrasonic Equipment Technology Co., Ltd., China).The experimental ultrasound apparatus used in this work has been described in detail in our previous work [17]. Ultrasonic intensity was measured by calorimetry using a thermo couple (model: TASI 601, TASI Ltd., Suzhou, China) and expressed in W/cm^2. The ultrasound generators probe could deliver a maximum power of 0.45 W/cm^2 and a maximum frequency of 135 kHz. The length, width and depth of the ultrasonic bath were 20, 20, and 15 cm, respectively.

2.3. Preparation of samples and ultrasound pretreatment

ALG solutions (0.95 wt%) were prepared in 0.1 M phosphate buffer solution at pH 7.0. The solutions were stirred with heating at 50 °C to ensure complete dispersion and hydration, and then cooled to the room temperature. Papain solution (0.48 wt%) was prepared in 0.1 M phosphate buffer solution at pH 7.0. ALG solutions prepared with ultrasound treatment were carried out at different powers (0.05, 0.15, 0.25, 0.35, 0.45 W/cm^2), 135 kHz, 50 °C for 20 min based on our group previous study [16]. After ultrasonic treatment, the papain diluted appropriate times was mixed with the ALG solution in a volume ratio of 1:1 and then the mixtures were analyzed according to the experiment requirement. In this study, papain was used without ultrasound treatment which is focus on the effect of ultrasound pretreatment of ALG on the properties of papain.

2.4. Enzyme activity measurement

The papain was mixed with the above ultrasound pretreated ALG (2.3) in a volume ratio of 1:1. After that, the concentration of papain was 0.024 mg/mL. The activity of papain was determined according to the method of Folin-phenol described by Li [21] and calculated by the standard curve of tyrosine solution obtained by Ultraviolet-visible spectrophotometer (UV-1240, Shimadzu, Co.,