International Journal of Biological Macromolecules 90 (2016) 81–88

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Catalase-only nanoparticles prepared by shear alone: Characteristics, activity and stability evaluation

Xiao-Nan Huang, Xin-Ying Du, Jin-Feng Xing, Zhi-Qiang Ge*

Department of Pharmaceutical Engineering, School of Chemical Engineering and Technology, Tianjin University, Education Ministry Key Laboratory of Systems Bioengineering, Tianjin 300072, PR China

ARTICLE INFO

Article history:
Received 25 May 2015
Accepted 24 August 2015
Available online 28 August 2015

Keywords:
Catalase
Nanoparticles
Shear stress
Conformation
Enzymatic activity
Stability

ABSTRACT

Catalase is a promising therapeutic enzyme; however, it carries risks of inactivation and rapid degradation when it is used in practical bioprocess, such as delivery *in vivo*. To overcome the issue, we made catalase-only nanoparticles using shear stress alone at a moderate shear rate of 217 s^{-1} in a coaxial cylinder flow cell. Properties of nanoparticles, including particle size, polydispersity index and zeta potential, were characterized. The conformational changes of pre- and post-sheared catalase were determined using spectroscopy techniques. The results indicated that the conformational changes of catalase and reduction in α-helical content caused by shear alone were less significant than that by desolvation method. Catalase-only nanoparticles prepared by single shear retained over 90% of its initial activity when compared with the native catalase. Catalase nanoparticles lost only 20% of the activity when stored in phosphate buffer solution for 72 h at 4 °C, whereas native catalase lost 53% under the same condition. Especially, the activity of nanogranulated catalase was decreased only slightly in the simulated intestinal fluid containing α-chymotrypsin during 4 h incubation at 37 °C, implying that the catalase nanoparticle was more resistant to the degradation of proteases than native catalase molecules. Overall, catalase-only nanoparticles offered a great potential to stabilize enzymes for various pharmaceutical applications.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Catalase is a well-known therapeutic enzyme because it can catalyze the decomposition of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) that is a typical free radical related to diseases, into molecular oxygen and water. It has been found that catalase can play a protective role in several pathological conditions, such as radiation, atherosclerosis, ischemia-reperfusion syndrome, viral infection and tumor progression [1], making catalase a promising therapeutic agent. However, it is challenging to retain its enzymatic activity during practical application, such as inactivation under intense conditions and rapid degradation in delivery process *in vivo*. To deal with the issues, it has been proposed to immobilize enzymes on the surface of materials [2] or encapsulated them in polymeric, proteinous or mesoporous materials in some delivery systems [3]. However, this approach cannot fully solve the issue as several shortcomings remain, such as low encapsulation efficiency, inactivation due to preparation processes or undesirable degradation products. Considering the values of the catalase, it is an urgent need to develop new strategies to fabricate enzymes delivery systems with high encapsulation and loading efficiency, uniform size, and adequate preservation of bioactivity.

Protein nanoparticles based on gelatin, collagen, casein, albumin and whey protein have been extensively investigated in the pharmaceutical industry [4]. However, proteins act only as carriers in the nanoparticle delivery systems. The changes of the secondary/tertiary structure and activity of the proteins do not affect efficacy and applications of the carried drugs. Methods for protein-based nanoparticles preparation can be classified in two major categories: emulsification and desolvation. The major disadvantage of emulsification method is that large amounts of organic solvents are required to remove the lipid residues and emulsifiers used in the process [5]. The desolvation method derived from the coacervation also has the disadvantages of requiring large amounts of organic solvents such as acetone, ethanol, ethyl acetate or isopropanol to act as anti-solvents during the desolvation process [6]. As it is well known that organic solvents are likely to induce conformational changes and subsequent denaturation of proteins, other alternative proposed is to employ pure physical process. In addition, as it is well known that perturbation of protein solution will cause aggregation [7], and is typically considered to be undesirable for therapeutic protein in manufacturing process because the

* Corresponding author.
E-mail address: gezhiq@tju.edu.cn (Z.-Q. Ge).

http://dx.doi.org/10.1016/j.ijbiomac.2015.08.056
0141-8130/© 2015 Elsevier B.V. All rights reserved.

aggregates may cause adverse events on administration [8]. But, not all protein aggregates are unacceptable, for instance, enzymes. When properly controlled, enzyme protein aggregation often results in novel and practical properties. For example, many kinds of enzyme aggregates have been proven that they can improve the activity and stability of the enzymes [9].

In this study, we report a one-step preparation method of catalase nanoparticles using shear alone at a moderate shear rate of 217 s^{-1}. The basic structural characteristics of catalase and corresponding nanoparticles were disclosed by spectroscopic examination. Biological activity and stability of the nanoparticles were also examined in the absence and presence of protease. To the best of our knowledge, catalase-only nanoparticles prepared by shear stress alone have not been thoroughly explored before. The present method thus may have significant values for enzyme formulations in the protein pharmaceutics.

2. Materials and methods

2.1. Reagents and chemicals

Catalase (EC 1.11.1.6) from bovine liver was purchased from Sigma–Aldrich (USA) and stored at −20 °C before use. Catalase solution was prepared in 0.05 M phosphate buffer solution (PBS, pH 7.0, mixture of $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}12H_2O$). All other reagents were of analytical purity. The deionized water used throughout the study was obtained from a Milli-Q system.

2.2. Preparation of nanoparticles

Catalase-only nanoparticles were prepared by shear in the absence or presence of acetonitrile, respectively. First, we screened several processing parameters that have major influences on nanoparticles preparation, including shear rate, shear time and initial protein concentration that are important for aggregation procedure and aggregates properties [10]. Accordingly, different shear rates (167, 217, 267, 317 and 367 s^{-1}), shear time (5 and 10 min) and initial protein concentration (0.2 and 1.0 mg/ml) were evaluated to determine the parameters for preparing catalase nanoparticles with optimal particle size and polydispersity index (PDI). Since the objective of this research is to develop nanoparticles suitable for oral administration of catalase, rather than just carriers, the enzymatic activity of nanoparticles was also evaluated as a key criterion. The shear alone method can be simply described below. Five milliliters of catalase solution (concentration 0.2 or 1.0 mg/ml, respectively) was sheared in a coaxial cylinder flow cell at a certain defined shear rate for 5 or 10 min under ice-water bath. The prepared nanoparticles were purified through three rounds of centrifugation and dispersion. For each round, nanoparticles suspension was centrifuged at 12,000 × *g* for 10 min and the pellet was re-dispersed to the original volume with PBS at pH value of 7.0. Each redispersion procedure was performed in an ultrasonication bath [11]. The resulting nanoparticles were stored at 4 °C for characterizations. Based on the screening results as characterized in Fig. 1, protein concentration of 0.2 mg/ml, shear rate of 217 s^{-1} and shear time of 5 min were determined as the optimal parameters and used thereafter for preparing catalase nanoparticles in the below study.

Desolvation method employed in this study was approximately the same as the shear alone method described above except to the addition of acetonitrile along with the shear process. The final ratios of acetonitrile-to-PBS were 0.25 and 3 (final percentage of acetonitrile were 20% and 75%), respectively. Additional process was performed by syringe with a continuous dropwise that guaranteed a defined adding rate. Afterwards, the acetonitrile was removed from the solution by vacuum rotary evaporation at 37 °C, thus

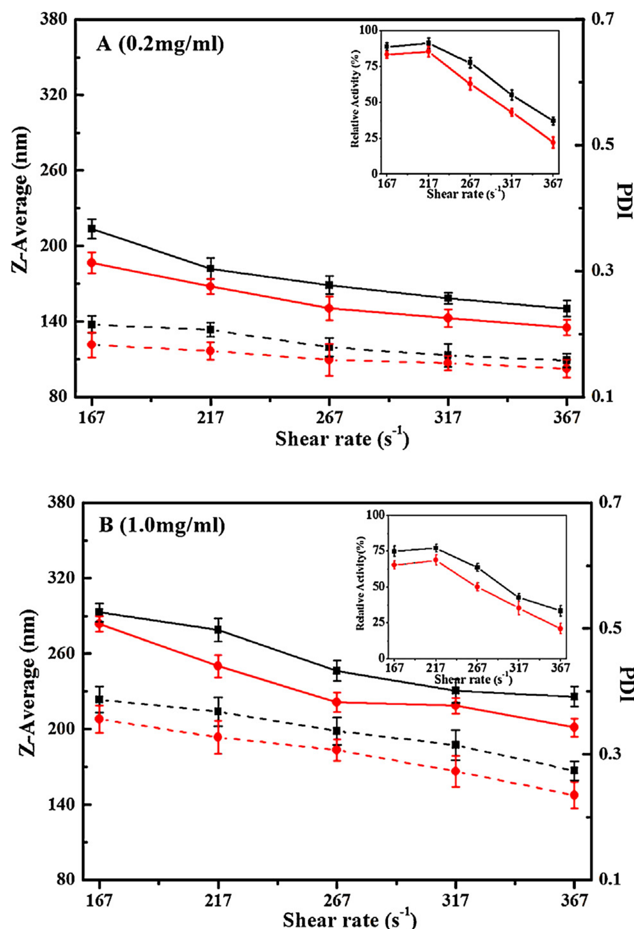

Fig. 1. Effects of catalase concentration, shear rate and time on parameters of nanoparticles. Data was expressed as mean ± SD (*n* = 3). (A) and (B) were catalase concentration of 0.2 mg/ml and 1.0 mg/ml, respectively. Inserts were effect of shear rates on relative activity. Solid line and dash line were Z-average and PDI, respectively. Black squares and red circles were shear times 5 min and 10 min, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ended up with a suspension of catalase nanoparticles. Nanoparticle purification was operated according to the above mentioned.

2.3. Determination of particle size, polydispersity index and zeta potential

The suspensions of catalase nanoparticles were diluted with PBS into a suitable concentration. The average particle size (Z-average) and polydispersity index (PDI) were determined at 25.0 ± 0.1 °C using a Malvern Zetasizer ZS (Malvern Instruments) with photon correlation spectroscopy at a fixed angle of 173°. Zeta potential was accessed by laser doppler electrophoresis (Zetasizer Nano ZS, Malvern, UK). Each sample was analyzed in triplicate.

2.4. Conversion efficiency

To measure the amount of catalase that transformed particles in preparation process, conversion efficiency (E_c) as an index was introduced. The concentration of initial catalase solution was expressed as C_{in}. Post-sheared solution was centrifuged for 10 min at 12,000 × *g*, and catalase concentration in supernatant was expressed as C_{sup}. Catalase concentrations were determined by

Download English Version:

https://daneshyari.com/en/article/1985590

Download Persian Version:

https://daneshyari.com/article/1985590

Daneshyari.com