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Lysozyme–magnesium aluminum silicate microparticles: Molecular interaction, bioactivity and release studies

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ABSTRACT

The objectives of this study were to investigate the adsorption behavior of lysozyme (LSZ) onto magnesium aluminum silicate (MAS) at various pHs and to characterize the LSZ–MAS microparticles obtained from the molecular interaction between LSZ and MAS. The results showed that LSZ could be bound onto the MAS layers at different pHs, leading to the formation of LSZ–MAS microparticles. The higher preparation pH permitted greater adsorption affinity but a lower adsorption capacity of LSZ onto MAS. LSZ could interact with MAS via hydrogen bonds and electrostatic forces, resulting in the formation of intercalated nanocomposites. The particle size, %LSZ adsorbed, and LSZ release rate of LSZ–MAS microparticles increased when the LSZ–MAS ratio was increased. The secondary structure of LSZ bound onto the MAS layers in microparticles prepared at various pHs was altered compared with that of native LSZ. Moreover, the LSZ extracted from microparticles prepared at pH 4 showed an obvious change in the tertiary structure, leading to a decrease in the biological activity of the LSZ released. These findings suggested that LSZ can strongly interact with MAS to form microparticles that may potentially be used as delivery systems for sustained protein release.

1. Introduction

Microparticles have been widely used as drug delivery systems for the continuous, targeted, sustained or controlled release of bioactive agents [1,2] because they can provide homogeneous drug absorption [3] and reduce local irritation at the adsorption site [4]. Moreover, they can also protect the incorporated bioactive substances against enzymatic degradation [5]. It is well known that polymers have been used to fabricate microparticles due to their biocompatibility, biodegradability, and bioadhesive properties. However, microparticles developed using polymers resulted in a high-burst drug release [5] and pH-dependent swelling and erosion [6,7], leading to undesirable drug release profiles. Additionally, the preparation of polymer microparticles is highly complicated because obtaining suitable microparticles necessitates the use of many reagents and processes. It is therefore of interest to develop new materials and methods for producing microparticles for use as drug reservoirs and drug delivery systems.

Magnesium aluminum silicate (MAS), a natural clay, has been widely used as a pharmaceutical excipient. It presents a silicate-layered structure that is composed of two tetrahedral silicate sheets that sandwich an alumina or magnesia octahedral sheet [8]. MAS has a large surface area with negative charges on its silicate layer surfaces, leading to high adsorption properties not only with positively charged substances via an ion-exchange mechanism but also with neutral and negatively charged substances by hydrogen bonding [9–11]. In addition, it can interact with anionic and cationic polymers, such as alginate [12] and chitosan [13], respectively. This interaction results in an improvement in the physicochemical properties of such polymers. One other application of clays is to adsorb protein macromolecules, such as enzymes, for improved biological activity [14,15].

Due to its interaction with drugs, MAS was widely used as a complexing agent. MAS interacted with cationic drugs, such as nicotine and propranolol, in dispersions, resulting in the formation of small flocculates of drug–MAS complexes [16,17]. The complexed particles improved the stability of drugs and provided a

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sustained release of drugs after an initial burst release. Recently, nicotine–MAS flocculates were prepared and dried by lyophilization, yielding dried nicotine–MAS microparticles with irregular shapes. Their physicochemical properties and drug release profiles were then investigated for use as drug delivery systems [18]. Using this method, it is thus possible to prepare microparticles formed of MAS and proteins.

Therefore, the objectives of this study were to investigate the adsorption behavior of protein onto MAS and to fabricate and characterize protein–MAS microparticles for use as drug reservoirs and drug delivery systems. Lysozyme (LSZ) was used as a model protein in this study. The adsorption isotherm of LSZ onto the MAS silicate layers at different pHs was investigated. The LSZ–MAS microparticles were prepared using molecular interactions between both substances, followed by drying via lyophilization. Conformational changes in LSZ, such as secondary and tertiary structures, and the biological activity of LSZ were determined. The thermal behavior and LSZ release from the microparticles were also examined.

2. Materials and methods

2.1. Materials

MAS (Veegum® HV) in granular form was purchased from R.T. Vanderbilt Company Inc., USA. Freeze-dried lysozyme from chicken egg white, BCA protein assay kit (QunatiPro™ BCA Assay Kit), and *Micrococcus lysodieikticus* were obtained from Sigma–Aldrich Co., Singapore. All other reagents used were of analytical grade and used as received.

2.2. LSZ adsorption isotherm study

Adsorption isotherms of LSZ onto MAS were performed by mixing MAS dispersions with different concentrations of LSZ. MAS (100 mg) was accurately weighed and dispersed using hot water. The pH of MAS dispersions was adjusted to 4.0, 6.0 or 8.0 using 2 N HCl, and the 100 ml final volume was adjusted with distilled water to achieve a 0.1% (w/v) MAS dispersion. LSZ solutions were prepared by dissolving 50 mg of LSZ into distilled water, and the final volume was adjusted to 100 ml using distilled water to obtain a 0.05% (w/v) LSZ solution. Then, 0.05% (w/v) LSZ solution in the volumes of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, or 6.0 ml was incorporated into 1.25 ml of 0.1% (w/v) MAS dispersion in a 125 ml Erlenmeyer flask. The volume of the mixture was adjusted to 20 ml, and the pH of the mixture was adjusted to 4.0, 6.0 or 8.0. After that the samples were adjusted to a final volume of 25 ml to obtain the concentrations of 10, 20, 30, 40, 60, 80, 100, or 120 $\mu g\,ml^{-1}$ LSZ in the mixtures, respectively, and the concentration of MAS in the mixtures was 50 $\mu g\,ml^{-1}$. The mixtures were incubated at 37 °C with 75 oscillations min^{-1} for 24 h. Then, the LSZ concentration in the supernatant at adsorption equilibrium was determined using the BCA protein assay.

The adsorption of LSZ onto MAS was analyzed using the Langmuir models [19]. The Langmuir adsorption isotherm that assumes a monolayer coverage of LSZ at the surface of the silicate layer of MAS is represented as follows:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{K_L Q_m} \quad (1)$$

where C_e is the equilibrium LSZ concentration in dispersion ($mg\,ml^{-1}$), Q_e is the amount of LSZ adsorbed onto MAS at the equilibrium LSZ concentration ($mg\,g^{-1}$), Q_m is the monolayer capacity of MAS ($mg\,g^{-1}$), and K_L is the constant for the adsorption process. The Q_m and K_L can be estimated from the slope and the intercept of the linear curve between C_e/Q_e and C_e.

2.3. Preparation of LSZ–MAS microparticles

Briefly, 1% (w/v) MAS dispersion and 2% (w/v) LSZ solution were prepared and adjusted to a final pH of 4, 6 or 8 using 2 N HCl. LSZ solution of 2% (w/v) in the volumes of 12.5, 25, or 50 ml was dropped into 1% (w/v) MAS dispersion (500 ml) prepared at the same pH with continuous stirring at 300 rpm to obtain LSZ–MAS ratios of 0.05:1, 0.10:1, or 0.20:1 by weight, respectively. After mixing, the wet LSZ–MAS microparticles were formed immediately. The pH of the LSZ–MAS microparticle dispersions was adjusted to 4, 6 or 8 before incubation in a water bath at 37 °C for 24 h. After incubation, the wet LSZ–MAS microparticles were characterized. Then, the wet microparticles were collected by centrifugation for 10 min at 5000 rpm with the temperature controlled at 25 °C. The wet microparticles were washed twice using 25 ml of distilled water, and redispersed in 50 ml of distilled water before freezing at −20 °C for 24 h. The frozen microparticles were dried by lyophilization (FlexiDry™ MP, Stone Ridge, USA). The dry microparticles were gently sieved through a 180-μm sieve and kept in a desiccator over silica gel until analysis.

2.4. Characterization of wet microparticles

2.4.1. Particle size determination

Particle size of the wet LSZ–MAS microparticles in dispersions was measured using a laser diffraction particle size analyzer (Mastersizer2000 Model Hydro2000SM, Malvern Instrument Ltd., UK). The samples were dispersed in 70 ml of distilled water in a small volume sample dispersion unit with a stirring rate of 3000 rpm for 30 s before measurement. Particle size determination of each sample was run in triplicates. The volume weighted mean diameters of particles were reported.

2.4.2. Zeta potential determination

Zeta potential of the wet microparticles in dispersions was measured by using a laser Doppler electrophoresis analyzer. The temperature of samples was controlled at 25 °C. To obtain an appropriate count rate, the dispersions were diluted 10-fold with distilled water before measurement.

2.4.3. Determination of LSZ content in wet microparticles

The LSZ concentration in the supernatants of LSZ–MAS dispersions was determined using the BCA protein assay after adsorption equilibrium. The LSZ adsorbed (%w/w) onto MAS and entrapment efficient (EE) of LSZ were computed using following equations:

$$\text{LSZ adsorbed}\,(\%w/w) = \frac{W_i - W_e}{W_{MAS}} \times 100 \quad (2)$$

$$\text{EE}\,(\%) = \frac{\text{LSZ adsorbed}\,(\%w/w)}{\text{Initial LSZ content}\,(\%w/w)} \times 100 \quad (3)$$

where W_i was the initial LSZ amount (g), W_e was the remaining LSZ amount at equilibrium, and W_{MAS} was amount of MAS (g) used in the preparation. The initial LSZ content was the amount of LSZ used compared with the MAS amount before the adsorption process.

2.5. Characterization of dry microparticles

2.5.1. Particle size determination

The particle size of microparticles was measured using a laser diffraction particle size analyzer. Twenty milligrams of microparticles was immediately dispersed in 1.5 ml of distilled water by shaking. The procedures that followed were the same as those mentioned in Section 2.4.1.