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A new bioavailability enhancement strategy of curcumin via self-assembly nano-complexation of curcumin and bovine serum albumin

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ABSTRACT

Amorphous drug nanoparticles have recently emerged as a superior bioavailability enhancement strategy for poorly soluble drugs in comparison to the conventional microscale amorphous solid dispersions. In particular, amorphous drug nanoparticle complex (or nanoplex) represents an attractive bioavailability enhancement strategy of curcumin (CUR) - a medicinal herb known for its wide-ranging therapeutic activities - attributed to the high payload, cost-effective preparation, and supersaturation generation of the nanoplex. To address the poor colloidal stability of conventional nanoplex formulations, we herein developed a new class of CUR nanoplex by complexation of CUR with bovine serum albumin (BSA). The effects of two key variables in drug-protein complexation, i.e. pH and mixing ratio ($M_{BSA/CUR}$), on the physical characteristics and preparation efficiency were investigated. While the CUR-BSA nanoplex preparation was found to favor acidic pH and $M_{BSA/CUR}$ below unity, the nanoplex's physical characteristics were minimally affected by pH and $M_{BSA/CUR}$. At the optimal condition, CUR-BSA nanoplex with size ≈90 nm, zeta potential ≈27 mV, and payload ≈70% were produced at nearly 100% CUR utilization rate and ≈80% yield. The nanoplex produced a prolonged supersaturation level at ≈9× of the saturation solubility for 4 h. The dissolution rate could be modulated by thermal treatment of the nanoplex post its preparation. The long-term amorphous state stability, storage colloidal stability, and preserved bioactivity of the nanoplex were successfully established. Lastly, the CUR-BSA nanoplex was found to be superior to the conventional nanoplex in its size, supersaturation generation, colloidal stability, and yield.

1. Introduction

The therapeutic properties of curcumin (CUR) - a natural flavonoid extracted from turmeric plants - have been well established ranging from anti-inflammatory and antimicrobial to anticancer and anti-diabetic [1]. The importance of CUR as therapeutics was also evident from the extensive research efforts in the development of novel nanomaterials for accurate determination of the amount of CUR in the plasma [2]. The effectiveness of CUR therapy, however, is limited by its low oral bioavailability caused by its poor solubility in the gastrointestinal fluid and rapid degradation at the intestinal pH [3]. While nanoencapsulation has been extensively investigated as a bioavailability enhancement strategy of CUR, CUR nanocapsules exhibit two major drawbacks in (a) their costly and intricate preparation, and (b) low CUR payload [4,5].

To address these issues, we previously developed amorphous CUR-chitosan nanoparticle complex (or nanoplex in short) exhibiting a high payload (>80%) prepared by a simple, highly efficient, cost-effective method based on drug-polysaccharide complexation [6]. Most importantly, the nanoplex was capable of producing high apparent solubility of CUR upon dissolution as a result of the supersaturation generation of its amorphous state, thus making it an ideal bioavailability enhancement strategy [6]. The CUR-chitosan nanoplex, however, exhibited a tendency to agglomerate in its aqueous suspension form during short-term storage (i.e. after 6 h), hence necessitating its immediate transformation to dry powders [6].

Herein we hypothesized that the use of proteins, in place of polysaccharides, as the complexation partner for CUR could improve the colloidal stability of the resultant CUR nanoplex. Bovine serum

Abbreviations: AA, acetic acid; BSA, bovine serum albumin; CE, complexation efficiency; CFU, colony forming unit; CHI, chitosan; CUR, curcumin; FESEM, field emission scanning electron microscope; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; HPMC, hydroxypropylmethylcellulose; $M_{BSA/CUR}$, mixing ratio of BSA to CUR (w/w); $M_{CHI/CUR}$, mixing ratio of CHI to CUR (w/w); MHB, Mueller Hinton broth; MIC, minimum inhibitory concentration; MW, molecular weight; OD_{600}, optical density at 600 nm; PBS, phosphate buffered saline; PCS, photon correlation spectroscopy; pI, isoelectric pH; UV–vis, ultraviolet visible.

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albumin (BSA) was used as the model protein because of its low cost, biodegradability, non-toxicity, and wide acceptance for pharmaceutical uses [7–9]. Most importantly, several studies have reported the ability of BSA to provide excellent steric and electrostatic stabilization of nanoparticles by virtue of the presence of adsorbed protein corona on the nanoparticle's surface [10–12]. Furthermore, BSA has been known to possess high binding capacity for various drugs, including CUR and other flavonoids [13,14], where CUR binding with serum albumins (including BSA) has been found to improve the chemical stability of CUR in physiological condition [15–17].

The schematic of the CUR-BSA nanoplex formation is presented in Fig. 1. The high affinity of CUR to BSA attributed to the hydrophobic binding of CUR to the hydrophobic cavities of BSA [18] led to the formation of soluble CUR-BSA complex. Depending on the preparation pH, the electrostatic binding between BSA and the oppositely charged CUR could also play a role in the CUR-BSA complex formation [16]. Similar to the particle formation mechanism in drug-polysaccharide or protein-polyelectrolyte complexation [19,20], aggregates of the soluble CUR-BSA complex subsequently formed due to the hydrophobic interactions among the bound CUR and BSA molecules (Fig. 1). The aggregates of CUR-BSA complex then precipitated upon reaching a critical concentration to produce the CUR-BSA nanoplex.

In the present work, the first objective was to investigate the effects of the two key variables in drug-protein complexation (i.e. pH and mixing ratio of BSA to CUR) on the (1) physical characteristics (i.e. size, zeta potential, payload, and amorphous state) and (2) preparation efficiency (i.e. curcumin utilization rate and production yield) of the CUR-BSA nanoplex produced, from which the optimal preparation condition was determined. Subsequently, the second objective was to analyze and compare the (1) physical characteristics, (2) preparation efficiency, (3) colloidal stability, (4) dissolution rate, and (5) supersaturation generation capability of the CUR-BSA nanoplex prepared at the optimal condition, with those exhibited by the conventional CUR-chitosan nanoplex. In addition, we assessed whether the bioactivities of CUR were adversely affected by its complexation with BSA by using antimicrobial assay as the representative bioactivity test among the many bioactivities of CUR.

Lastly, the third objective of the present work was to equip the CUR-BSA nanoplex with modulated release functionality achieved by post-preparation thermal treatment of the nanoplex. As proteins, BSA unfolds upon heating causing the disruption of its compact helical structures [21,22]. We hypothesized that the conformational change of BSA in the nanoplex after the thermal treatment would enable us to modulate the release of CUR from the nanoplex. For this purpose, the effect of heating on the nanoplex's structural integrity was examined first in order to determine the onset of thermal denaturation of BSA that led to the structural collapse of the nanoplex.

2. Materials and methods

2.1. Materials

CUR (≥98% purity) and Mueller Hinton broth (MHB) were purchased from Alfa Aesar (USA) and BD Diagnostics (USA), respectively. BSA (MW = 69 kDa, ≥96% purity), chitosan (CHI) (MW =

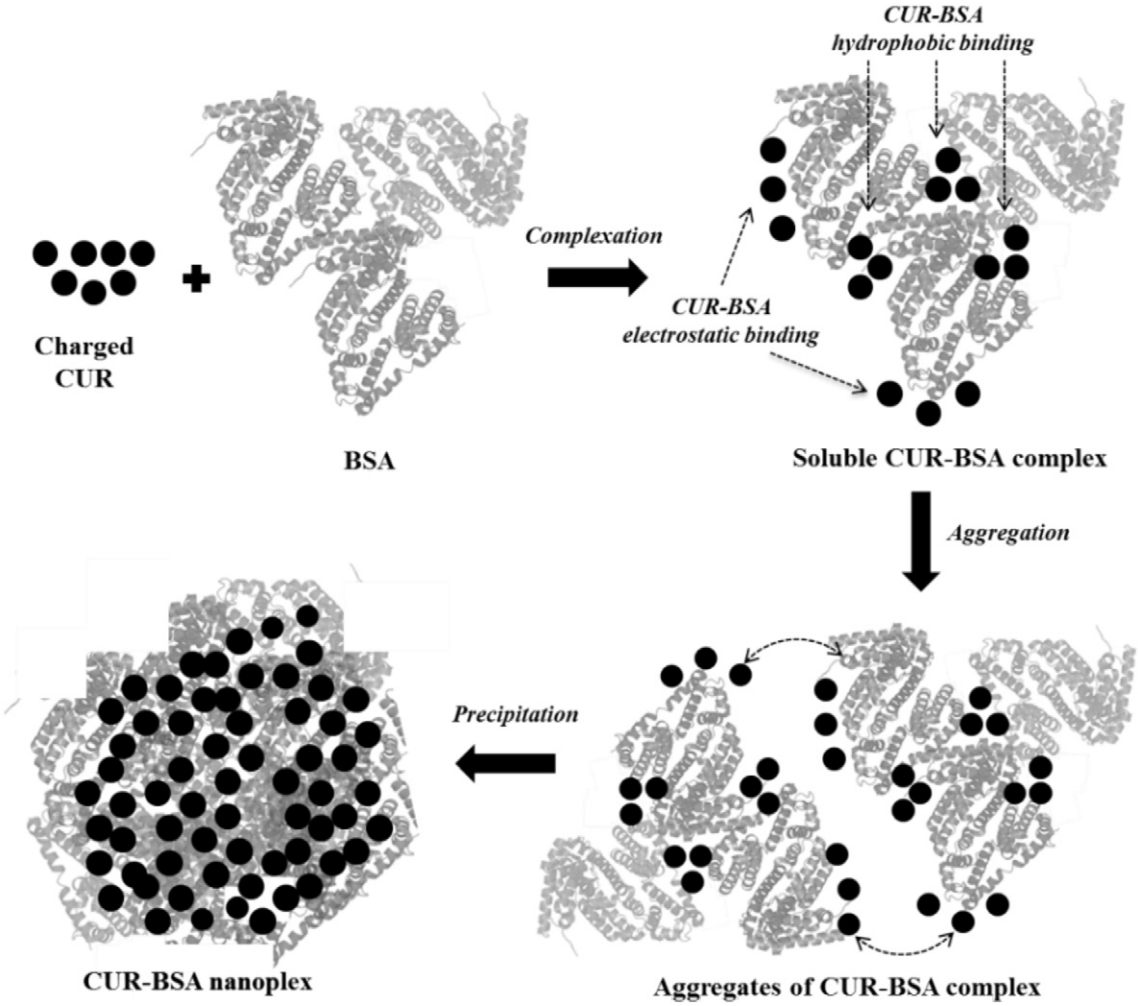

Fig. 1. Schematic of CUR complexation with BSA driven by their hydrophobic and electrostatic interactions resulting in the formation of CUR-BSA nanoplex.