Mesoscopic simulation studies on the aggregation behaviour of glycyrrhizin micelles for drug solubilization

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Abstract

Glycyrrhizin is a kind of natural surfactant with the micelle structure for drug solubilization and low toxicity in the human body. In this study, dissipative particle dynamics (DPD) and mesoscopic dynamics (MesoDyn) were carried out to elaborate the aggregation behaviour of glycyrrhizin micelles. Baicalin were selected as the poorly water-soluble drugs. It has been observed from DPD that glycyrrhizin molecules formed core/shell structured spherical, cylindrical and lamellar aggregates with the increase of concentration. Baicalin molecules are solubilized in the hydrophobic core. Glycyrrhizin molecules were easier to form micelles with the addition of drugs. Mesoscopic simulations based on experimental data provided more detailed information for the investigation of solubilization.

Keywords: mesoscopic simulation, dissipative particle dynamics, glycyrrhizin micelle, drug solubilization, aggregation behaviour

1 Introduction

Poor solubility ingredients carry a high risk of failure during the development since insufficient solubility may affect both the bioavailability and clinical effect, and finally affect the develop ability of the compound [1]. Glycyrrhizin, the main constitute of Glycyrrhiza uralensisб which is frequently used in Chinese prescriptions, has been reported to be a kind of natural surfactant with good properties. It has attracted a lot of interest for it can aggregate in water with the core/shell structure for encapsulating the poorly water-soluble drug and has low toxicity to human body [2-5]. It is necessary to investigate the solubilization mechanism of glycyrrhizin for its reasonable development and drug application. However, it is difficult to study the formation process of micelle by common experimental methods, for the solubilization process takes only milliseconds to reach equilibrium. As a result, the underlying mechanism still needs to be further studied, even though a number of experiments have been carried out to investigate the solubilization of saponins.

Mesoscopic simulation provides a powerful tool for the structure study and the performance prediction of surfactants [6-18]. In this work, DPD and MesoDyn mesoscopic simulation methods coupled with experimental studies, were employed to investigate the aggregation behaviour of glycyrrhizin micelles with baicalin, which is the poorly water-soluble drugs, to illustrate the solubilization mechanism of glycyrrhizin.

2 Material and experimental methods

2.1 MATERIALS

Glycyrrhizin (95%), and baicalin (98%) were purchased from Nanjing ZeLang Medical Technology Co., LTD. The drug-loaded glycyrrhizin solution was prepared by dissolting 5mg baicalin in 0.1% glycyrrhizin solution at 25±1°C for 24h, then filtered with 0.45μm filter membrane.

2.2 TEM EXPERIMENTS

The aggregate morphologies of 0.1% glycyrrhizin solution, baicalin solution and drug-loaded glycyrrhizin solution were observed on a transmission electron microscope (TEM, JEOL JEM-1230 microscope) operated at an acceleration voltage of 80 kV. The solution samples were deposited onto copper grids that had been percolated with a thin film of formvar and then coated with a thin carbon film. The liquid was blotted off with filter paper after a few minutes, and the grids were air-dried.

3 Simulation methods

3.1 DPD THEORY

Dissipative particle dynamics (DPD) is a mesoscopic simulation technique, which is suitable to study the collective behaviour of complex fluids. A DPD bead represents a small region of fluid matter and its motion is assumed to be governed by Newton's laws [19]:

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$$\frac{d\mathbf{r}_i}{dt} = \mathbf{v}_i \ , m_i \frac{d\mathbf{v}_i}{dt} = \mathbf{f}_i \ , \qquad (1)$$

where, $\mathbf{r}_i$, $\mathbf{v}_i$, m_i and $\mathbf{f}_i$ denote the position vector, velocity, mass, and total force acting on particle *i*, respectively.

The force $\mathbf{f}_i$ between each pair of beads contains three parts: a harmonic conservative interaction force $\left(\mathbf{F}_{ij}^{C}\right)$, a dissipative force $\left(\mathbf{F}_{ij}^{D}\right)$ and a random force $\left(\mathbf{F}_{ij}^{R}\right)$. The expression is given as $\mathbf{f}_i = \sum_{j \neq i}\left(\mathbf{F}_{ij}^{C} + \mathbf{F}_{ij}^{D} + \mathbf{F}_{ij}^{R}\right)$. All forces are short-range with a fixed cutoff radius r_c, which is usually chosen as the reduced unit of length $r_c \equiv 1$.

3.2 MESODYN THEORY

MesoDyn is based on a dynamic variant of mean-field density functional theory, which states that there is a one-to-one mapping between the distribution functions of the system, the densities, and an external potential field. The model used in the MesoDyn project consists of a variety of beads whose interactions are described by harmonic oscillator potentials for the intramolecular interactions. Each bead represents a certain group of atoms.

3.3 MODELS AND INPUT PARAMETERS

The coarse-grained models are shown in Figure 1. The saccharide and aglycone of glycyrrhizin were represented by two types of beads named A (red) and B (green). Baicalinand water were coarse grained by beads BA (yellow) and W (blue), respectively. To show the aggregate clearly, water molecules were not displayed.

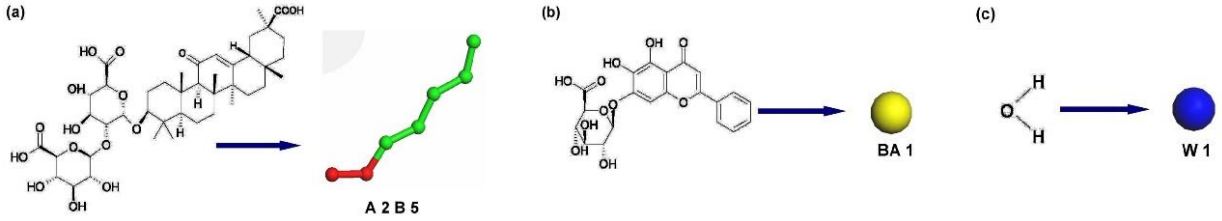

FIGURE 1 Coarse-grained models: (a) glycyrrhizin, (b) baicalin, (c) water

In the DPD simulations, a cubic simulation box with periodic boundary condition is applied in all three directions. A cubic box of 10×10×10 is sufficient to avoid the finite size effects. The grid spacing of 1.0nm is taken and the density is set to be 3.0. The integration time step of 0.05 and the simulation steps of 20000 are used in order to get thermodynamic equilibrium.

In MesoDyn simulations, a cubic simulation box with periodic boundary condition is applied. The dimensions of the simulation lattice are 32×32×32 and the grid spacing of 1.0nm is taken. To ensure isotropy of all grid-restricted operators, the bond length is set to be 1.1543nm. Bead diffusion coefficient is set to be 1.0×10-7cm^2·s^{-1} in order to ensure a stable numerical algorithm. The noise parameter is 75.002, the temperature is 298K and the time step is 50.0ns. Number of steps is 50000 (2.5ms) to ensure a complete process of micelle formation.

All the simulations are carried out using the DPD or MesoDyn module in the commercial software Materials Studio 4.1 from Accelrys Inc.

4 Results and discussion

4.1 AGGREGATION BEHAVIOR OF GLYCYRRHIZIN IN AQUEOUS SOLUTION

The DPD simulation results showed in Figure 2 indicated that glycyrrhizin molecules formed spherical aggregates at low concentration (0.9vol%-19vol%).

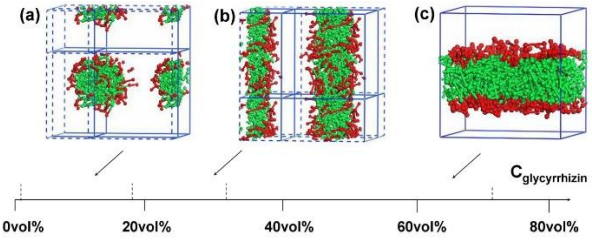

FIGURE 2 Aggregation behaviours of glycyrrhizin at different concentrations: a) sphere, b) cylinder and c) lamella

With the increase of concentration, small aggregates crashed and combined, then spherical aggregates with increased diameter formed. When the concentration of glycyrrhizin was between 20vol% and 32vol%, spherical aggregates disappeared and cylindrical aggregates began to form, whose radial diameter stretched with the increase of concentration. When the concentration of glycyrrhizin was from 33vol% to 72vol%, lamellar aggregates were observed and the lamella became thicker at higher concentration. Moreover, the aggregates did not change as the simulation time prolonged, indicating the above microstructures were the lease in local free energy minima [20].

The spherical glycyrrhizin aggregates observed from DPD (Figure 3a) were further obtained by TEM experiment (Figure 3b). For further investigation the microstructure of glycyrrhizin aggregates, MesoDyn simulation was carried out to analyse the section view of

glycyrrhizin aggregates in concentration of 1.3vol%. Density profiles of beads A and B on the section passing through the centre of the aggregate were shown as Figure 3c. The hydrophilic A formed a peak existed in the position of the shell, while the hydrophobic B was more concentrated in the centre of the aggregate, producing a core/shell structured micelle. The sections formed by beads A and beads B were given as Figure 3d and e. The hydrophobic core formed for entrapping the poorly water-soluble drugs due to the hydrophobic interactions, while the hydrophilic shell provided a stable interface between the core and water.

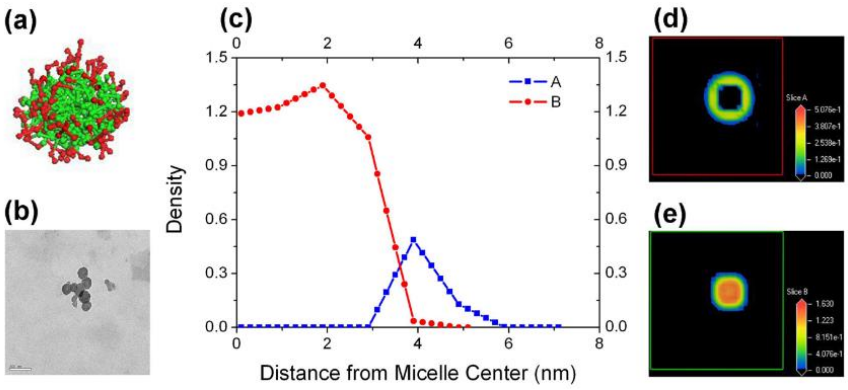

FIGURE 3 Morphological analysis of spherical aggregates of glycyrrhizin:
a) spherical glycyrrhizin aggregate observed from DPD, b) TEM image of spherical aggregates of glycyrrhizin, c) density distributions of beads A and B, and section views of beads A d) and beads B e)

4.2 INTERACTIONS BETWEEN GLYCYRRHIZIN AND POORLY WATER-SOLUBLE DRUGS IN AQUEOUS SOLUTION

Glycyrrhizin molecules can self-aggregate into micelles in aqueous solutions at the concentration above the critical micelle concentration (CMC). The core/shell structure provides a perfect environment for drug solubilization and eliminates the side effects caused by pharmaceutical excipients. Spherical aggregates of drug-loaded glycyrrhizin micelles were observed with TEM (Figure 4). It could be easily found from the simulation results that baiclin molecules were solubilized in the core of the aggregate formed by the hydrophobic beads B of glycyrrhizin due to the hydrophobic interaction, both in the spherical, cylindrical and lamellar aggregates.

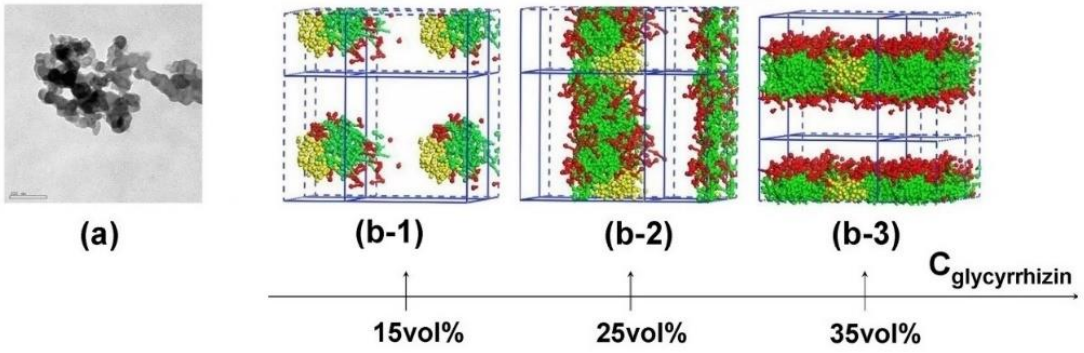

FIGURE 4 Aggregation behavior of baicalin/glycyrrhizin/water system from different methods:
(a) TEM; (b-1), (b-2) and (b-3) mesoscopic simulation

Several snapshots of configurations during the DPD simulation were carried out to study the dynamic formation process of drug-loaded glycyrrhizin micelles. In order to observe the process clearly, the layered aggregate of glycyrrhizin was taken as an example. All components distributed randomly in water in the beginning stage of the simulation. Then some glycyrrhizin molecules aggregated together and small aggregates formed. With the increase of simulation time, larger aggregates formed and the aggregate changed from cylindrical to lamellar aggregates. In baicalin/glycyrrhizin/water system, baicalin first spread around the surface of glycyrrhizin micelles and then dispersed into the core gradually as the simulation time prolonged. After the simulation of 20000 steps, baicalin molecules dispersed into the core totally and a stable drug-loaded micelle formed (Figure 5). Similar process was observed to puerarin.

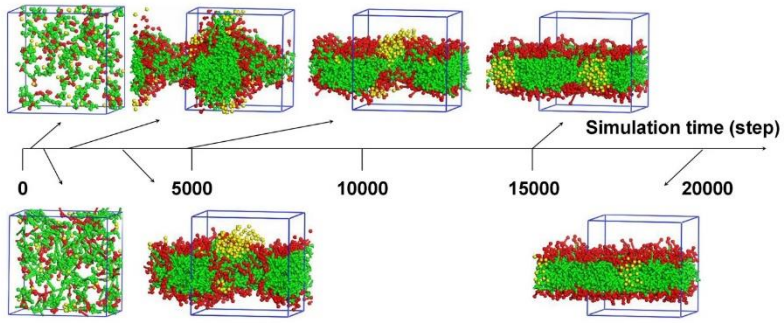

FIGURE 5 The configurations of baicalin/glycyrrhizin/water system at different simulation steps

The micellar property of surfactant is always a research hotspot in the process of preparations. Additionally, the poorly water-soluble drug has effect on the micellar property of surfactant. Figure 6 indicated that the addition of poorly water-soluble drug could facilitate the aggregation of glyrrhizin. As the concentration of the poorly water-soluble drug increased, the time needed for the aggregate formation decreased.

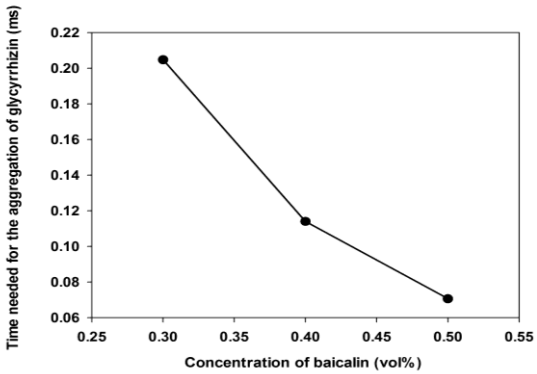

FIGURE 6 Time needed for the aggregate formation of 0.8vol% glycyrrhizin with the addition of poorly water-soluble drugs

5 Conclusion

Glycyrrhizin molecules formed core/shell structured spherical, cylindrical and lamellar aggregates with the increase of concentration. Baicalin, a kind of poorly water-soluble drug, turned to mainly distributed in the hydrophobic core of glycyrrhizin micelle. The solubilization process went through the diffusion from the surface to the core. The addition of poorly water-soluble drug can facilitate the aggregation of glyrrhizin.

Mesoscopic simulations, as the extension of experiments, could provide more details to the aggregation behaviour of glycyrrhizin micelles for drug solubulization, which was a powerful tool for the investigation of solubilization properties and mechanism.

Acknowledgments

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