Dissipative Particle Dynamics Simulation of Ginsenoside Ro Vesicular Solubilization Systems

Xingxing Dai1, †, Xinyuan Shi1, 3, 4, *, †, Haiou Ding2, Qianqian Yin2, and Yanjiang Qiao1, 3, 4, *

1 Beijing University of Chinese Medicine, Beijing, 100102, China
2 School of Traditional Chinese Medicine, Capital Medical University, Beijing, 100069, China
3 Key Laboratory of TCM-information Engineer of State Administration of TCM, Beijing, 100102, China
4 Beijing Key Laboratory for Basic and Development Research on Chinese Medicine, Beijing, 100102, China

Ginsenoside Ro (Ro), a natural biosurfactant derived from ginseng, has been proven to form vesicles in aqueous solutions that enhance the solubility of the insoluble compounds. With the intention of expanding the applications of Ro in the pharmaceutical industry, we here examined the influence of drug additives on the solubilization of Ro vesicles. The effects of the compatibility between each drug and the Ro molecules and of the length of the hydrophobic and hydrophilic structure of drug molecules on the solubilizing capacity and solubilization site were here studied using dissipative particle dynamics (DPD). The results showed that the simple hydrophobic drugs lacking hydrophilic groups were mainly located on the hydrophobic layer of the vesicle, and the drugs containing both hydrophobic and hydrophilic structures were located on the palisade layer. There was no single, simple relationship between solubilizing capacity and drug properties. The solubility parameters of the hydrophobic structure, molecular size, changes in hydrophobic and hydrophilic properties attributed to changes in the length of the hydrophobic and hydrophilic structures, and the solubilization site were found to affect the molar solubilization ratio (MSR) of the Ro vesicles. These results provide insight into the vesicular solubilization system formed by saponins and may serve as guidelines for the further development and application of Ro and other saponins.

Keywords: Biosurfactant, Ginsenoside Ro, Solubilization, Vesicle, Mesoscopic Simulation.

1. INTRODUCTION

Although many drugs have been shown to have strong biological activity in vitro, many cannot be used in clinical treatments due to poor solubility.1–3 For this reason, solubilization is important for the preparation and absorption of insoluble drugs. Saponins are a class of biosurfactants; they are mainly derived from plants. They are glycosides containing a hydrophobic triterpene or steroid aglycone and one or more hydrophilic sugar chains. As solubilizers, they offer advantages over synthetic surfactants in that they are derived from renewable sources and are biodegradable, highly active, and either non-toxic.4 Therefore, saponins have been proposed as potential safe and effective adjuvants suitable for enhancing the absorption and dissolution of pharmacologically active substances and drugs through solubilization.5, 6

Ginseng (roots of Panax ginseng C. A. Mey) is a popular medicinal plant. Its main active compounds are a complex mixture of saponins, which have a variety of bioactivities, including anti-inflammatory, antioxidant, and anticancer effects.7, 8 Over 15,000 of the formulae in the Traditional Chinese Medicine (TCM) Database System (http://cowork.cintcm.com/engine/windex1.jsp) contain ginseng.9 In addition to its own pharmacodynamic efficacy, ginseng can also enhance the efficacy of other drugs. The solubilizing effect of saponins on insoluble compounds has been considered one possible mechanism of ginseng’s synergistic effects. For example, ginsenoside Ro (Ro) has been shown to markedly increase the solubility of saikosaponin a (SSa) which is the active ingredient in Radix Bupleuri (roots of Bupleurum Chinese DC. and Bupleurum scorzonerifolium Wild.), which is sparingly soluble in water.10 Our previous studies have proven that Ro can form vesicles in aqueous solutions and solubilize SSa molecules in palisade layers. It can also form mixed vesicles with the molecules to enhance the solubility of SSa.11, 12

Studies of solubilization mechanisms and laws governing interactions between insoluble drugs and saponin vesicle are of great significance. More information may expand
the applications of ginsenoside and similar saponins in medical situations and the pharmacological industry. However, in these studies, standard experimental methods can be very costly in both time and money, and it can be difficult to illustrate the solubilization mechanisms and the exact internal structures of the vesicles. Fortunately, powerful computer simulations can render the researches highly efficient. Many theories have been developed to study the organization of quantum matter. Dissipative particle dynamics (DPD) is one of the mesoscopic simulation methods used to study drug loading systems. This is a particle-based method which coarse-grains the familiar atomistic representation of the molecules to gain orders of magnitude in both length and time scale relative to traditional atomistic scale. DPD allows particles to interact through a simple-wise potential and accurately capture their hydrodynamic behavior and the underlying interactions. It can also depict the movement of meso-molecules. In this way, it offers advantages in elucidating the solubilization process.

Studies have shown that the solubilization effects of saponin are related to the structure and properties of insoluble drugs. We divided the insoluble drugs into two types according to their hydrophilic and hydrophobic structures. The first group of molecules has only hydrophobic parts. This group includes some free aglycones (such as quercetin and coumarin). The molecules in the other group contain both hydrophobic and hydrophilic parts. According to the number of hydrophilic parts, they can be subdivided further. This group includes some glycosides (such as baicalin and saikosaponin). These drug molecules are coarse-grained and their mesostructures are shown in Figure 1. By employing DPD simulation, the effects of hydrophilic and hydrophobic properties and the length of the hydrophobic and hydrophilic groups of drugs on solubilization are studied. These results can act as guidance for the further use of Ro and other saponins.

2. DPD SIMULATION METHOD

Dissipative particle dynamics (DPD) is a mesoscopic simulation technique suitable to the study of the collective behavior 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.

\[
\frac{d\mathbf{r}_i}{dt} = \mathbf{v}_i, \quad \frac{d\mathbf{v}_i}{dt} = f_i
\]

Here, \(\mathbf{r}_i, \mathbf{v}_i, m_i\) and \(f_i\) denote the position vector, velocity, mass, and total force acting on particle \(i\), respectively.

The force \(f_i\) between each pair of beads contains three parts: a harmonic conservative interaction force \(F^{C}_{ij}\), which is a soft repulsion acting along the line of centers; a dissipative force \(F^{D}_{ij}\), which represents the viscous drag between moving beads; and a random force \(F^{R}_{ij}\), which maintains energy input into the system in opposition to the dissipation. All forces are short-range with a fixed cutoff radius \(r_c\), which is usually chosen as the reduced unit of length \(r_c \equiv 1\). They are given as follows:

\[
F^{c}_{ij} = \frac{1}{\Delta t} \left( F^{c}_{ij} + F^{c}_{ji} \right)
\]

\[
F^{D}_{ij} = \begin{cases} a_{ij} (1 - r_{ij}) \mathbf{r}_{ij} & (r_{ij} < 1) \\ 0 & (r_{ij} \geq 1) \end{cases}
\]

\[
F^{R}_{ij} = -\gamma u^{D} (r_{ij}) (\mathbf{v}_i - \mathbf{v}_j) \mathbf{r}_{ij}
\]

\[
F^{R}_{ij} = \sigma w^{R} (r_{ij}) \xi_{ij} \frac{1}{\sqrt{\Delta t}} \mathbf{r}_{ij}
\]

Here, \(a_{ij}\) is the maximum repulsion between particles \(i\) and particle \(j\); \(r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|\); \(\xi_{ij}\) is a random number with zero mean and unit variance, and \(\Delta t\) is the time step of the simulation.
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Combined with the known compressibility of water and binodal data, the following expression can be obtained between $a_{ij}$ and the Flory-Huggins parameter $\chi$:

$$a_{ij} = a_i + 3.27\chi_{ij}$$  \hspace{1cm} (6)

Here, $a_{ij} = 25k_BT$ at a density $\rho = N/V = 3$, and the value of $k_BT$ is defined as 1.

The Flory-Huggins parameter $\chi_{ij}$ is often used to estimate the compatibility between drugs and solubilizer. Lower $\chi_{ij}$ values indicate better compatibility. It is the same for the repulsion parameter $a$, because it comes from $\chi_{ij}$. In DPD simulations, changes in $\chi_{ij}$ or $a_{ij}$ between two specific particles are a common means of studying the laws governing these interactions. However this perspective often ignores the interactions between these particles and other particles. The $\chi_{ij}$ values can be calculated from the solubility parameters $\delta$ using the following equation:

$$\chi_{ij} = \frac{(\delta_i - \delta_j)^3 V}{RT}$$  \hspace{1cm} (7)

$\delta$ itself can also be used to estimate the compatibility of different species. The similar the solubility parameters of the two species, the smaller the interaction parameters between them and the stronger their compatibility. We suggest that changing the $\chi_{ij}$ or $a_{ij}$ values by changing the $\delta$ of specific particles will produce more accurate results. In this way, the complex interactions between all the particles can be taken into consideration in simulations. $\delta$ depends on the chemical nature of the species and can be obtained through experimentation or molecular dynamics (MD) simulation.

3. SIMULATION DETAILS

Models. The DPD simulation system was composed of hydrophobic drugs, ginsenoside Ro (Ro), and water. Three different types of hydrophobic drugs were studied, and their mesostructures are shown in Figure 1(a). Bead $X$ and bead $G$ represent the hydrophobic and hydrophilic particles of drug, respectively. Model 1 represents drugs containing only hydrophobic beads $X$. Model 2 represents glycosides with hydrophobic aglycone $X_{ag}$ and single sugar chain $G_{s}$. Model 3 represents glycosides containing two sugar chains at the both end of aglycone. One end contains several sugars and the other end contains only one sugar. The mesostructure of Ro is shown in Figure 1(b). Ro is pentacyclic triterpenoid glycoside with two sugar chains. The aglycone is divided into five identical beads ($M$). Each sugar is represented by one bead ($G$ for glucose; $GA$ for glucuronic acid). One water molecule is represented by one bead ($W$ for water). All these beads have the same volume.

In our previous study, Ro molecules were shown to self-assemble into vesicles. The radius of each vesicle is about $5r_c$ (where $r_c$ is the DPD length scale). To study the solubilization mechanism by which hydrophobic drugs are loaded into vesicles, the Ro vesicle was placed in a watery environment to serve as the basis (Fig. 1(c)). The size of the cubic simulation box was 30 $\times$ 30 $\times$ 30 $\sigma$. The drug molecules were homogeneously distributed in the solvent. The molar concentration ratio of the drugs and Ro molecules was fixed at 2:1. The total simulation time was 50,000 steps, which was long enough for the simulation system to reach equilibrium. The spring constant was fixed at 4.0, which has been found to give reasonable results in this system. The simulation temperature was fixed at 25 °C. To show aggregate morphologies clearly, the display styles were chosen so that all water beads were hidden in the simulation.

Repulsion parameters. To calculate repulsion parameter $a_{ij}$, the solubility parameters $\delta$ of all beads were needed. In this study, solubility parameters of Ro were calculated using the Amorphous Cell module in Materials Studio 5.5 (Accelrys Inc., supported by CHEMICALCOMPUTING) with the COMPASS force field. The results are in Table I. To study the compatibility effects between the drug and Ro, we set the solubility parameter of $X$ ($\delta_X$) to 10, 15, 20, 25, and 30. The interaction parameters and repulsion parameters can be calculated using equations 6 and 7 and the results are shown in Table II.

4. RESULTS AND DISCUSSION

4.1. Effects of Interaction Parameter $\chi$ Between Drug Beads and Ro Beads on 2 Solubilization

The interaction parameter between drug beads and Ro beads reflects the compatibility of drug and Ro molecules. Lower parameter values between two beads indicate stronger attractive interactions between them, and they are
tend to aggregate with each other. Therefore $\chi$ is very important to the study of the solubilizing capacity and solubilization sites of solubilization systems. $\chi$ can be calculated from solubility parameter $\delta$ as in Eq. (7). Here, Model 1 ($X_m$) and Model 2 ($X_mG_n$) drugs ($m = n = 1$) were used to study the effects of interaction parameters on the solubilization of Ro. The $\delta_X$ value was allowed to range from 10 to 30. The results are shown in Figures 2 and 3. Figures 2(a) and (b) show section views of Ro vesicles. They directly indicate the location of different drugs in these vesicles. Model 1 drugs containing only hydrophobic groups were mainly solubilized in the hydrophobic layer. With increased values of $\delta_X$, the location within the hydrophobic layer changed slightly, as indicated by the distribution probability of $X$ in Figure 2(c) (distribution probability $P = n/N$, $n$ is the number of $X$ beads on a certain distance away from the center of the vesicle, $N$ is the total number of $X$ beads in the vesicle). When $\delta_X = 10$, which was smaller than $\delta_M = 16.18$, the drug molecules tended to self-aggregate in the hydrophobic layer formed by the aglycones of Ro molecules, which was far from the external water solution environment (Fig. 2(a1)). The probability distribution curve formed a single sharp peak in the hydrophobic layer. This was because, at this time, the interaction parameter between $X$ and $M$ ($\chi_{XM}$) was much smaller than that between $X$ and $W$ ($\chi_{XW}$, $\delta_M = 48.37$), but it was larger than the one between $X$ and $X$ itself ($\chi_{XX}$). The compatibility between $X$ beads was the strongest, followed by $M$ beads. The strong repulsive forces between $X$ and $W$ forced them away from each other. When $\delta_X$ increased to 15 and 20, which was similar to $\delta_M$, the $\chi_{XM}$ decreased and approached $\chi_{XX}$. This indicated excellent compatibility between the drugs and Ro.
molecules. The former were distributed uniformly in the hydrophobic layer formed by the latter (Figs. 2(a2), (a3)). The probability distribution curve became wider. When $\delta_X$ increased to 25, the difference between $\delta_X$ and $\delta_M$ and between $\delta_X$ and $\delta_G$ (where $G$ is the hydrophilic group of Ro) were almost the same. That meant the strength of the interactions between the drugs and the hydrophobic and hydrophilic groups of Ro were largely the same. The drugs were mainly associated with the zone between the hydrophobic and hydrophilic layers. The probability distribution curve peaks appeared in both the inner and outer junction zones of the hydrophobic and hydrophilic layers.

Unlike Model 1 drugs, Model 2 drugs were solubilized in the palisade layer of the vesicles. The hydrophobic groups of the drug molecules were inserted into the hydrophobic palisade layer, and the hydrophilic groups of the drugs were located on the surface, where they interacted with the sugars of Ro through hydrogen bonding and dipolar interactions. There were no significant changes in the increases of $\delta_X$ from 10 to 25 (Figs. 2(b1)–(b4)). This was because the attractive interactions between the hydrophilic groups of drugs and Ro molecules were very strong. However, when $\delta_X$ increased to 30, the repulsive interactions between $X$ and $M$ beads were so large that they prevented the drug molecules from coming close to each other. The drug molecules could not then enter the hydrophobic layer and only adsorbed onto the vesicle surface by hydrophilic interaction (Fig. 2(b5)).

The solubilization capacity of each vesicle was also affected by the type of drug. The solubilization capacity of a surfact can be quantified by the molar solubilization ratio (MSR) which is often calculated as follows:

$$MSR = \frac{S - S_{\text{cmc}}}{C_s - C_{\text{cmc}}}$$  

(8)

Here, $C_s$ is the surfactant concentration, CMC is the critical micelle concentration of the surfactant, $S$ is the total apparent solubility of the additive, and $S_{\text{cmc}}$ is the apparent solubility of the additive at CMC. The MSR is a ratio of the moles of the additive solubilized to the moles of surfactant in micelle or vesicle. In DPD simulation, the moles of the solubilized additive and the surfactant forming the vesicle can be determined easily. As shown in Figure 3, when the solubility parameter was small ($\delta_X = 10$), the MSR for both Model 1 and Model 2 drugs was also small. That might have been because the molecules of the Model 1 group were located in the hydrophobic layer and those of the Model 2 were located in the palisade layer, which was smaller than the hydrophobic layer. At large values of $\delta_X$ ($\delta_X = 20$ and 25), the MSR of Model 2 was higher than that of Model 1. That might have been because of the repulsive interactions between $X$ and $M$ beads, which prevented the Model 1 drug molecules from entering the vesicle. However, Model 2 drug molecules were found to exist stably in the palisade layer, where their hydrophilic group $G$ interacted with the hydrophilic layer of the vesicle through hydrogen bonding or dipolar interactions.

4.2. Effects of Length of Hydrophobic Structures of Drugs on Solubilization

The length of hydrophobic structure can affect the molecular size and polarity of the drug, which can greatly influence the solubilizing capacity of the Ro vesicle. A Model 2 drug was used in further study ($n = 1$, $m = 1, 2, 3, 4, 5$). $\delta_X$ was fixed at 15, which produced peak MSR. The results are shown in Figure 4. Here, the MSR of drugs were not found to increase or decrease monotonically with increases in the length of the hydrophobic group. Instead, two decreasing processes were observed. When the number of beads, here given as m, increased from 1 to 3, the MSR decreased from 155.3% to 100.0%. As shown in Figures 4(a1)–(a3) the drug molecules were mainly located in the outer palisade layer. (Few of entered the inner layer.) Typical molecules are highlighted in the figure. However, longer hydrophobic structures produced larger molecules. This made it difficult for the molecules to incorporate into the vesicle because both the superficial area and the volume of the vesicle were limited. When $m$ increased to 4, the MSR increased to 136.7%. Because the length of the hydrophobic of the drug was similar to that of the Ro molecule (the length of Ro hydrophobic structure was...
5 beads), the drug molecules had a chance to interact with the inner palisade layer of the vesicle. In this case, the drug molecules almost formed a mixed vesicle with the Ro molecules, considering that the molecules of this length may have surface activity. This phenomenon has been observed in interactions between Ro and saikosaponin a molecules whose hydrophobic structure length was also 5 beads.\textsuperscript{11} When $m$ increased to 5, the drug molecules could still form mixed vesicles with Ro molecules (Fig. 4(a5)). However, due to the limited vesicle space, the drugs with larger molecular sizes could not easily incorporate into the vesicle. The MSR decreased to 98.7%. Although the efficiency of solubilization fluctuated with increases in the length of the hydrophobic structure, the vesicle size increased smoothly. This indicates that the overall volume of solubilized drugs also increases smoothly.

4.3. Effects of Length of Hydrophilic Structure of Drugs on Solubilization

The length of the hydrophilic structure of the drug can also affect the drug molecular size and polarity and so influence the solubilization behavior of Ro vesicles. Here, Model 2 and Model 3 ($G_1X_mG_n$) drugs were employed to study the effects ($m = 3$, $n = 1, 2, 3$). The $\delta_X$ was still fixed at 15. As shown in Figure 5, when the number of $G$ beads increased from 1 to 2, the MSR increased for both Model 2 and Model 3 drugs. This might because the solubilization site of both types of drugs was the palisade layer (Fig. 6), where the hydrophobic and hydrophilic structures of the drugs interacted with the hydrophobic and hydrophilic layers of the vesicle, respectively. Because the hydrophobic structure of each drug remained unchanged, the hydrophilic interactions became the biggest influence on solubilization capacity. Increases in the length of the hydrophilic structure caused increases in the strength of the hydrophilic interactions between the drug and Ro molecules and stabilized the drug in the vesicle. However, when $n$ increased to 3, the greater size of the drug molecules prevented them from entering the vesicle. Increases in the length of the hydrophilic structure also increased the polarity of each drug molecule. The
hydrophilicity of these drugs increased, and they began to show surface activity. The drug molecules were found to self-aggregate in aqueous solution to form stable micelles or vesicles. This decreased solubilization efficiency.

When \( n = 1 \) or 2, the MSR of two-sugar-chain drugs (Model 3) was higher than that of single-sugar-chain drugs (Model 2). The morphology and sectional views of the vesicles may provide us with explanations. Vesicles containing solubilized single-sugar-chain drugs continued to take the shape of spheres (Fig. 6(a1)). These drug molecules were mainly located on both the outer and inner palisade layers with the hydrophobic structures embedded in the hydrophobic layer (see the highlighted molecules in Fig. 6(b1)). However, the vesicles increased in size and became spheroid as they incorporated two-sugar-chain drugs (Fig. 6(a2)). The axial section view of shows that the drug molecules interacted with vesicles in three different ways (see highlighted molecules in Fig. 6(b2)):

1. Solubilized in outer palisade layer. The hydrophilic groups at both ends of drug molecules interacted with the hydrophilic layer of the vesicle, and the hydrophobic structure bent in the hydrophobic layer.
2. Solubilized in inner palisade layer. The behavior of molecules were similar to those in (1). However, they were located near the internal hydrophilic region.
3. Solubilized throughout whole the layer of the vesicle.

The hydrophobic structures were embedded in the hydrophobic layer while two sugar chain interacted with outer and inner hydrophilic layer respectively. In this way, the drug molecules acted as composites with Ro molecules and solubilized more drug molecules. As a result of these three different means of solubilization, vesicle size increased significantly and the solubilization was highly efficient.

However, this was not the case when \( n = 3 \). The MSR for both single- and especially two-sugar-chain drugs decreased. This was because the hydrophilicity of both drugs increased significantly. Only a small number of molecules interacted with the vesicle. Most tended to self-assemble in aqueous solution. Figures 6(a3) and (b3) showed the aggregate morphology and section view of vesicles with two-sugar-chain drugs solubilized. Only a few drug molecules were solubilized in the outer palisade layer. No molecules entered into the internal palisade layer of the vesicle. Most of the molecules self-assembled in aqueous solution to form micelles and even vesicles. That led to a very low solubilization efficiency of 54.7%.

### 4.4. Solubilization Processes of Drugs by Ro Vesicle

The mechanism by which the drug molecules enter the vesicle is also significant. Evaluations of molecular
5. CONCLUSION

Every given surfactant system is influenced by many factors, such as the size, shape, polarity, and branching of the target molecules, which can significantly influence the solubilizing behavior of the surfactant. Extensive experimental and theoretical work have been performed on micellar and vesicular solubilization systems formed by synthetic surfactants and their relationships with additives. However, little work has been done on vesicular solubilization systems of saponin. Ginsenoside Ro has been found to form vesicles and to solubilize insoluble drugs. This indicates that this kind of vesicular solubilization system merits further study and may have significant applications. Here, by using DPD simulation method, the effects of hydrophobicity, topological structure, and the length of hydrophobic and hydrophilic structures were studied. Both the site of solubilization and capacity were found to be significantly influenced by these factors. Simple hydrophobic drugs without hydrophilic groups were mainly located on the hydrophobic layer of the vesicle, and drugs containing both hydrophobic and hydrophilic structures were located in the palisade layer. When the length of both hydrophobic and hydrophilic structures were short \((m = 1, 2, 3, n = 1)\), the drug molecules were only located in the outer palisade layer. However, when the length of the drug molecule approached that of the Ro molecule, some of them became distributed in both the inner and outer palisade layers to form mixed vesicles with Ro molecules. The effects of the study factors on MSR were found to be complex. The similarity between the hydrophobic structures of drugs and Ro \((\delta_x = \delta_m)\) could increase the MSR above that of drugs with the same topological structure. This may be used to guide the structural modification of drugs to enhance their solubility in this solubilization system. For a given solubilization site, lower MSR were observed on larger molecules as the result of the limited space. More molecules were found to enter the vesicle if there were many possible sites at which they might do so. The drugs were also found to incorporate into the vesicle through both single-molecule and aggregate morphology. However, whenever the hydrophobicity or hydrophilicity of the drug was too extreme, the molecules tended to self-aggregate in aqueous solution instead of entering the vesicle. The MSR values of these drugs were consequently very low.

In this study, DPD simulation has been proved to be an applicable tool to study the organization behavior of nanoaggregations. It can provide high-efficiency prediction to experiments in both structures and dynamic processes. Our simulation results not only provide insight into the vesicular solubilization system formed by saponin, but may also facilitate the construction of guidelines for the safe and effective use of Ro as a solubilizer and the further development and application of Ro in the pharmaceutical industry.

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