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ORIGINAL PAPER

Molecular dynamics approach to investigate the coupling of the hydrophilic–lipophilic balance with the configuration distribution function in biosurfactant-based emulsions

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Abstract Emulsion stability has been characterized by macroscopic variables such as the hydrophilic–lipophilic balance, with the aim being to predict the surfactant properties of molecules. Nevertheless, this parameter does not take the topology of the molecule into account, as it only considers its lipophilic degree. On the other hand, the classical Derjaguin–Landau–Verwey–Overbeek approach (based on the continuum model), which has been widely utilized to evaluate the stabilities of colloids, polymers, and surfactants, takes some bulk macroscopic parameters such as the shear viscosity coefficient and the dielectric permittivity into account. In the work reported here, molecular dynamics simulations were used to elucidate the mechanism of layer formation and micellar structure for different combinations of valine–aspartic acid peptides in dodecane–water emulsions, as well as their associations with the hydrophilic–lipophilic balance. The peptide–dodecane radial distribution function showed that the first peak intensity was inversely correlated with the hydrophilic–lipophilic balance; moreover, the oscillatory structural forces became increasingly prominent when the hydrophilic–lipophilic balance was decreased. Our results seem to indicate that the radial distribution function could be utilized to evaluate the stabilities of emulsions of peptides via molecular simulations.

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Introduction

Emulsions constitute one of the main strategies used in the cosmetics and food industries to encapsulate active reagents. They consist of the suspension of small globules of one liquid in a second liquid, which is stabilized by the presence of molecules named surfactants. The main role of surfactants is to reduce the pressure difference between phases so that thermodynamic instability is decreased.

Biological surfactants have caught the attention of biomolecular engineers due to their reduced impact on the environment. They are "greener" to produce than other surfactants as they are derived from renewable (and cheaper) substrates, and are biodegradable, renewable, and ecologically friendly [1].

The self-assembly properties of peptides, and the fact that they can be built from twenty different naturally occurring amino acids, provide an infinite number of possibilities in terms of potential peptide functionality, three-dimensional structure, and responses to different physicochemical conditions. Specifically, by appropriately adjusting the amino acid sequence of the peptide, features such as the elasticity, thickness, and permeability of the fluid–fluid interface during emulsification can be controlled. In this regard, Jones et al. tested the abilities of three α-helix peptides (Lac21, Dan25, and DN1) to form force-transmitting networks at the air–water interface [2]. Parameters such as hydrophobicity, helix-forming tendency, and crosslinking capability were found to play important roles in force transmission during peptide design. Also, Dexter and Middelberg designed amphipathic peptides that were capable of converting a film into a cohesive

state, which could be used to stabilize foams and emulsions [3]. Among those amphipathic peptides, surfactin, V_6D_2 (where V is valine and D is aspartic acid), and eleven other derived molecules were evaluated in terms of their capacity to form nanotubes and nanovesicles.

Most of the approaches reported to date relate to the selection of the oligomer sequence, and are based on a trial and error method. Firstly, a starting sequence with an NMR- or X-ray-predicted structure (α-helix or β-sheet) is selected as a framework. This sequence is then purposely modified so as to tailor parameters such as hydrophobicity and crosslinking capability. After that, the sequence is synthesized and then tested to determine the stability of the emulsion, drop size, or interfacial stress [3]. However, this approach is expensive and time-consuming because it requires a considerable amount of laboratory time and equipment [4]. On the one hand, rational approaches for predicting emulsion stability have been proposed that take into account the Derjaguin–Landau–Verwey–Overbeek (DLVO) approach. This approach is based, in turn, on the DLVO theory, which explains the aggregation of aqueous dispersions by combining the effects of van der Waals attractions with electrostatic repulsion due to the double layer of counterions, volume exclusion effects, attractive depletion forces, and oscillatory structural forces (OSF) [5]. The modeling of OSF (based on the Ornstein and Zernike integral equation method [6]) to explore their role in emulsion stability has proven useful in food dispersion studies. Moreover, utilizing optical imaging, it is possible to calculate the radial distribution function (RDF) based on the probability of finding an oil droplet in the first and second shells. The relation between the potential of mean force and the RDF has been reported for large spheres immersed in colloidal particles [7]:

$$u(r) = -kT\ln g(r), \quad (1)$$

where $u(r)$ represents the potential of mean force in the colloidal system and $g(r)$ is the associated radial distribution function value. This equation demonstrates that the RDF can reproduce the oscillatory behavior of the forces; so it can be utilized to predict important aspects of the microstructures, and can be used to correlate the distribution of such microstructures with the stability.

On the other hand, surfactants are heuristically evaluated based on parameters such as the hydrophilic–lipophilic balance (HLB), which measures the affinity of the surfactant for one of the phases in the emulsion [8]. This parameter is determined at certain regions in the molecule. However, dissimilar proteins or peptides can present the same HLB due to specific microscopic peptide parameter restrictions. Thus, in order to perform molecular design using the multiscale approach, it is imperative to elucidate the mechanisms behind the macroscopic parameters and their relations to emulsion stability. In the work described in the present paper, we correlated the configuration distribution function, which describes how particles are distributed in a specific space, with the HLB, in order to assess its relevance from a molecular dynamics perspective.

Methodology

Molecular structure

Different valine–aspartic acid sequences were simulated while varying the hydrophilic–hydrophobic regions of conventional and unconventional biosurfactants (tail-head and random structures, respectively), which were considered in order to correlate the configuration distribution function with HLB (Table 1). In order to get the structure file for each element, we carried out energy minimization using, as a starting point, the β-sheet structure reported previously [9]. Energy minimization was carried out using the steepest descent algorithm, with the "one thousand steps of steepest descent" method employed to minimize the energy of the system before

Table 1 HLB values for MD simulations of different peptides in water/dodecane (V: valine, D: aspartic acid)

	Sequence	Molecular weight of hydrophilic chain (g/mol)	Molecular weight of lipophilic chain (g/mol)	HLB
V_1D_7	VDDDDDDD	932	117	18
V_2D_6	VVDDDDDD	799	234	13
V_3D_5	VVVDDDDD	666	351	10
V_4D_4	VVVVDDDD	532	468	8
V_5D_3	VVVVVDDD	399	586	5
V_6D_2	VVVVVVDD	266	703	2
VD_1	VDVVVVDV	133	117	8
VD_2	VDVVDVDV	266	117	11
VD_3	VDVDVDVD	399	117	13
VD_4	DVDDVDDV	532	117	15
VD_5	DDDVVDDD	666	117	16
VD_6	DVVVVVVV	799	117	17

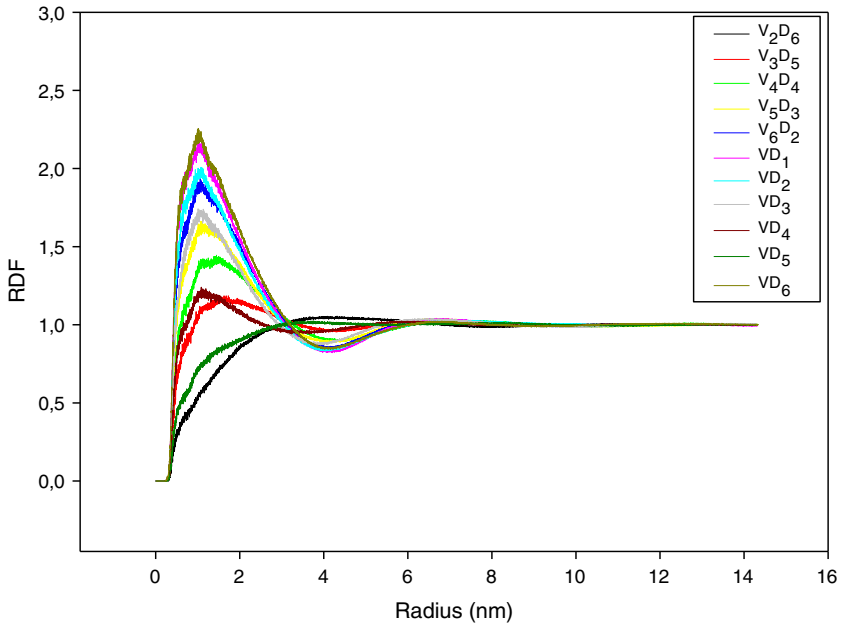

Fig. 1 Radial distribution functions obtained after 3000 ps in molecular dynamics simulations of different valine–aspartic acid peptides in water/dodecane

performing molecular dynamics simulations with a force constant of 10.0 kJ mol^{-1} nm^{-1}.

Molecular dynamics simulations

The simulations were carried out with GROMACS 4.5.4 using the GROMOS 53a6 force field [10], and the single point charge water model was assumed. The dispersed phase and continuous phase were oil and water, respectively (10 % dodecane, 4 % surfactant, and 86 % water); 1900 dodecane and 26,900 water molecules were simulated, with an average density of 973.14 kg/m^3. Eight hundred seventy-three peptides with a specific sequence (Table 1) were randomly placed into a 29-nm cubic box with periodic boundary conditions imposed. The system was equilibrated with NVT and then NPT simulations lasting 100 ps and including 50,000 steps were performed. A temperature of 291 K and a pressure of 0.75 atm were assumed, along with a 1.0-nm cutoff for the real space calculations. 1,500,000-step MD simulations were also carried out with time steps of 2 fs (thus, each simulation lasted 3000 ps), with a cutoff value of 0.9 nm applied for nonbonded interactions. The molecular system was placed in a 29-nm cubic box with periodic conditions imposed. The peptide–dodecane radial distribution function was evaluated at different time points in order to obtain the configuration distribution of the system. All simulations were run on 105 desktop computers, comprising 572 processing cores, 572 GB of RAM, 8 TB of storage, and 1 shared NFS storage of 1TB in total [11]. The processing time required for each simulation was between 1 week and 1 month.

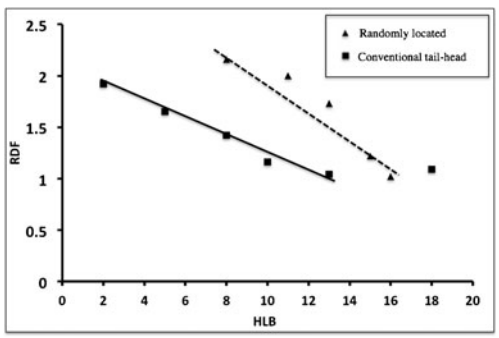

Fig. 2 Effect of HLB on the maximum value of the RDF in molecular dynamics simulations of conventional tail-head biosurfactants (*squares*) and surfactants with randomly located hydrophilic and hydrophobic regions (*triangles*) in water/dodecane

Hydrophilic–lipophilic balance

The HLB was evaluated in order to determine the correlation between the stability predicted by the atomistic model and the macroscopic behavior. The HLB is a simple parameter that indicates the relative affinities of the surfactant for the aqueous and oil phases [14]. In this work, the following definition of HLB reported by Kawakami et al. [12] was used:

$$\mathrm{HLB} = 11.7 \log \frac{W_\mathrm{h}}{W_\mathrm{l}} + 7, \qquad (2)$$

where W_h and W_l are the molecular weights of the hydrophilic and lipophilic chains, respectively.

Results and analysis

We simulated, using MD, various combinations of valine–aspartic acid residues to evaluate how the lengths of the hydrophobic and hydrophilic regions and their

locations in the molecule influence the HLB on a microscopic scale. Moreover, the sequences modeled covered the entire HLB range observed in biosurfactants (Table 1); HLB values from 7 to 11 indicate water-in-oil and values from 12 to 16 indicate oil-in-water emulsifiers [12]. Interestingly, the biosurfactants with conventional tail-head structures and low HLB values showed the presence of a maximum in the RDF curve, possibly indicating close dodecane packing (Fig. 1). Furthermore, we found a negative correlation between the HLB and the RDF, demonstrating a positive effect of the hydrophilic region on the dodecane packing or density inside the droplet (Fig. 2). The HLB was additionally assessed by placing the hydrophobic and hydrophilic residues at random positions in order to explore the behavior of nonconventional head and tail biosurfactants. Likewise, upon decreasing the HLB value of the peptide, the first peak in the radial distribution increased; nevertheless, these peptides seem to be more efficiently packed than conventional biosurfactants. Moreover, the first valley in the RDF is deeper when the RDF peak is higher (Fig. 1). Also, we found that peptides with HLB values above 16 (recommended for

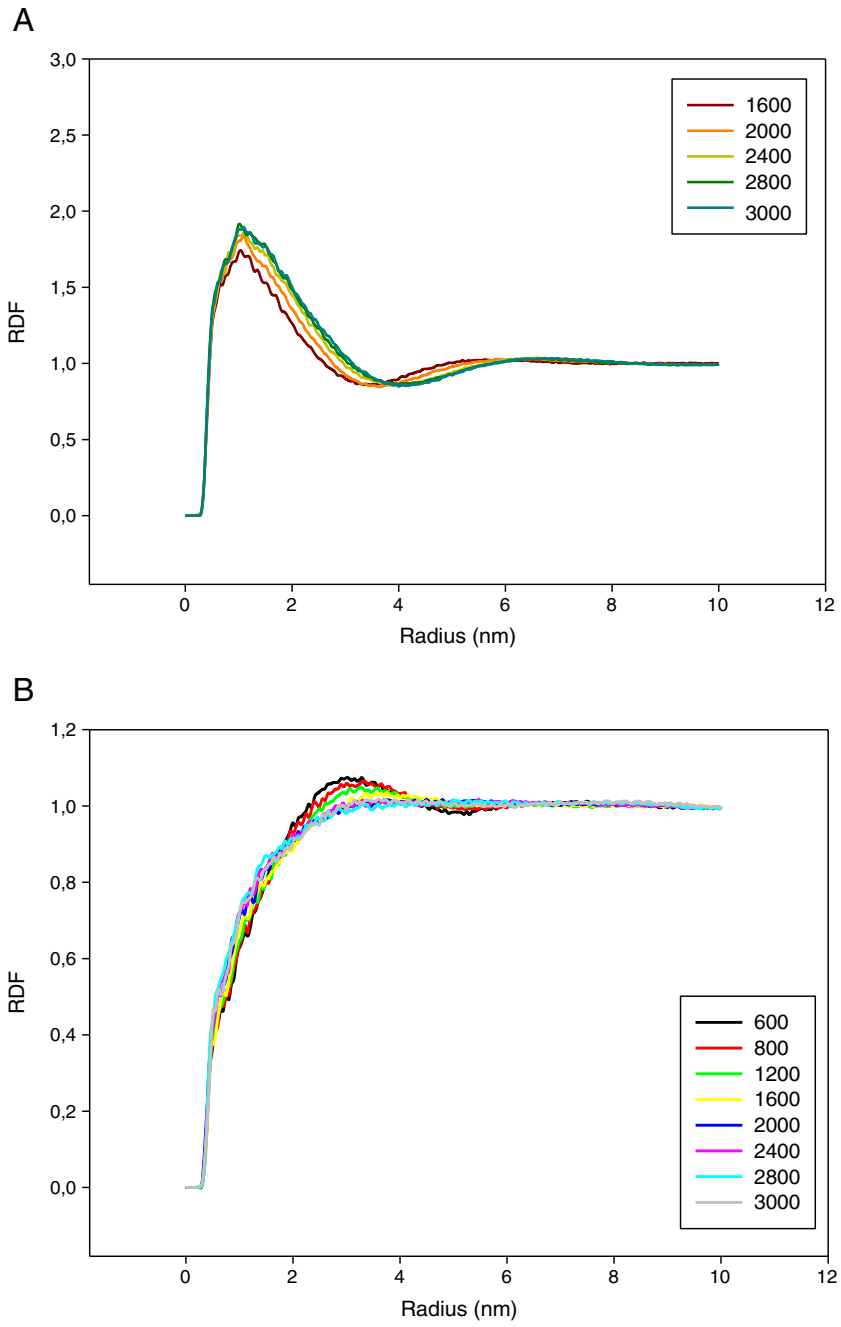

Fig. 3a–b Time evolutions of the RDFs of a low-HLB peptide (V_6D_2, **a**) and a high-HLB peptide (VD_5, **b**) observed in molecular dynamics simulations performed in water/dodecane

solubilizers) do not display the same trend, possibly indicating that our simulations could not adequately represent the behavior of long-chain hydrophobic peptides.

Asakura and Oosawa [13] describe an attractive force between particles suspended in a solution of macromolecules when there are no direct interactions; this depletion force could lead to phase separation when the structural factor becomes divergent. This is reflected in the OSF when the separation distance between the surfaces of two suspended spheres is on the order of the size of several colloidal particles. Taking into account the potential mean force equation (Eq. 1), our results show that the OSF become more prominent for low-HLB peptides (V_6D_2, V_5D_3, V_4D_4, VD_1) due to the presence of the higher peak and deeper valley in the RDF. Wasan et al. [5] studied the positive effect of OSF on emulsion stability by modeling it with the Ornstein and Zernike integral equation. Our results seem to indicate that these peptides are more capable of stabilizing water–dodecane systems in the presence of OSF. We did not find a significant difference in the position of the first peak from the peptide when we varied the HLB and, accordingly, the droplet diameter at 3000 ps was similar in all of the simulations when an RDF peak was present. Furthermore, the distance of the minimum RDF from the peptide corresponded to the average droplet diameter (4 nm).

We analyzed the time evolution of the RDF (Fig. 3) in an attempt to understand the forces that govern droplet formation. Our results indicate that peptides that display RDF peaks are OSF-driven once the droplet begins to form, because the RDF peak increased over time (Fig. 3a). In contrast, high-HLB peptides showed decreasing RDF peaks over time, so they appear to oppose droplet formation (Fig. 3b). The atom–atom radial distribution function allows us to evaluate the invariant expression for the entropy of a multicomponent fluid of N particles enclosed in a volume V at temperature T [14]:

$$\frac{S}{Nk_B} = -2\pi\rho\sum_{\alpha\beta} x_\alpha x_\beta \int_0^\infty \left\{ \mathrm{RDF}_{(r)} \ln\left(\mathrm{RDF}_{(r)} - \left[\mathrm{RDF}_{(r)} - 1 \right] \right\} r^2 dr, \quad (3)$$

where x_α and x_β are the mole fractions of each component in the mixture and ρ is the number density of the fluid. Consequently, considering the dramatic decrease in entropy (according to the RDF) and the small change in enthalpy associated with these interactions [15], the effect of the HLB on the droplet formation process appears to be an entropy-driven phenomenon that is caused by the increase in the hydrophilic area exposed to water.

Conclusions

In this work, through MD simulation, we found a correlation between HLB and the configuration distribution function based on the RDF. Then, using the RDFs obtained from MD simulations, we demonstrated that it is possible to predict the affinity of a molecule for each phase during the formation of an emulsion. Nevertheless, when the molecular behavior of these systems was explored, the behavior of the macroscopic parameter HLB appeared to contradict previously recommended HLB values for oil-in-water and water-in-oil emulsions. We corroborated the notion that the presence of OSF is correlated with the spontaneous formation of micelles, as our simulations did not impose any particular external shear rate. Furthermore, we found that the period of the forces is not affected by the HLB, nor by the height of the peak and the depth of the valley in the RDF curve, so we can conclude that the HLB indicates the dodecane packing inside the droplet and the attractive and repulsive forces present.

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