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Peptides design based on transmembrane *Escherichia coli*'s OmpA protein through molecular dynamics simulations in water–dodecane interfaces

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ABSTRACT

Recent research efforts have focused on the production of environmentally nonthreatening products, including identifying biosurfactants that can replace conventional surfactants. In order to utilize biosurfactants in different industries such as cosmetic, food or petroleum, it is necessary to understand the underpinnings behind the interactions that could take place for biosurfactants which display potential for interface activity. This work aimed to use molecular dynamics simulations to understand the interactions of rationally obtained peptide sequences from the original sequence of the OmpA gene in *Escherichia coli*, based on the free energy change (ΔG) during peptide insertion at the water–dodecane interface. Seventeen OmpA–based peptide sequences were selected and analyzed based on their hydropathy index profiles. We found that free energy change due to Columbic interactions and SASA ($\Delta G_{Coul/SASA}$), total free energy change and MW ($\Delta G_{/MW}$), and free energy change due to Coulombic and van der Waals interactions ($\Delta G_{Coul}/\Delta G_{vdW}$) ratios could provide a better understating in the contribution of the free energy decrease at the interface. The results indicated that the peptide sequences *GKNHDTGVSPVFA* and *THENQLGAGAFG* display biosurfactant potential based on low ΔG per square nanometer, high $\Delta G_{Coul}/\Delta G_{vdW}$ ratio, clearly defined moieties along its hydrophobic surface and sequence, and the presence of charged residues in the polar head. Clearly defined moieties and SASA were determinant for electrostatic interactions between oil–water interfaces. Experimental validations exhibited that the emulsions prepared remained stable between 3 and 27 h, respectively. Even though the peptide *GKNHDTGVSPVFA* displays strong interactions at the interface, stabilization times showed that the peptide *THENQLGAGAFG* exhibited the best performance suggesting that the stability can be better described by kinetic rather than thermodynamic criteria once the emulsion is formed.

1. Introduction

In recent years, research efforts in the industrial and scientific communities have focused on developing environmentally friendly technologies and products. Surfactants are widely used to stabilize oil-water systems such as emulsions in many products, including those involved in health, personal care, food, bioengineering and mineral and petroleum processing [1]. Adsorption of surfactants can modify the hydrophobicity, surface charge, and other key properties that govern interfacial processes such as dispersion/flocculation, flotation, detergency, enhanced oil recovery and even corrosion inhibition [2]. However, most commercial surfactants are derived from the oil industry and are environmentally toxic because they do not degrade easily [3]. Biosurfactants are surface–active compounds synthesized by microorganisms. They have a potential advantage over traditional surfactants for the use

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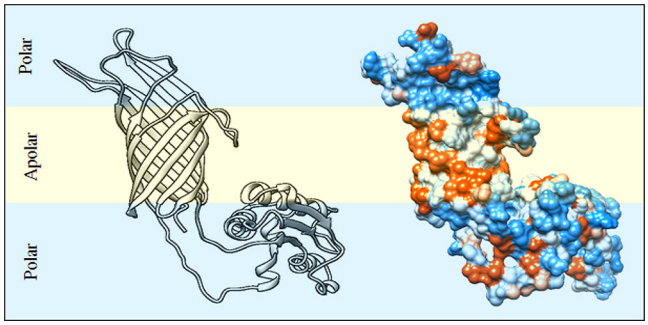

Fig. 1. The amphiphilic structure of OmpA, with overall hydrophobic β-barrel domain and hydrophilic periplasmic domain, mimics the amphiphilic structure of conventional surfactants, which display polar and non-polar groups at each extreme of the molecule (yellow region represents perisplasmic domain).

in many fields including environmental, food, biomedical, and other industrial applications [4]. Over the past few decades there has been a sudden interest in the development and discovery of new biosurfactants [5]. However, the process of development and selection of these compounds has followed a trial and error methodology. Molecular dynamics (MD) has been used recently as a rational tool for the study and development of biosurfactants. The self–assembly of the Lac21 and Lac28 peptides [6–8], the study of the interfacial properties of a surfactin micelle [9] and the development of coarse–grained models for studying this kind of systems [10] are indications of the growth of MD in the search for biosurfactants.

Outer membrane protein A (OmpA) of *Escherichia coli* (*E. coli*) contains hydrophobic and hydrophilic chains and has been found to play a major role during biofilm formation in *E. coli* [11]. As verified experimentally, OmpA displays a significant role as a potential biosurfactant since it increases the stability of dodecane–water emulsions [12]. Its amphiphilic structure, with overall hydrophobic β-barrel domain and hydrophilic periplasmic domain, mimics the amphiphilic structure of conventional surfactants, which contain polar and non-polar groups at each end of the molecule (Fig. 1). Deeper inspection of OmpA's structure reveals that the extracellular loops of the transmembrane domain, along with the periplasmic domain, remains hydrophilic at neutral conditions.

Rational modifications can be performed over OmpA's sequence, including the cleavage of the extracellular loops or other sections of the protein to rearrange the hydrophobicity of each moiety. Here, we departed from shorter OmpA fragments inside the sequence with similar hydrophobic-hydrophilic regions in order to analyze the interactions at water–dodecane interfaces and evaluate a correlation between those interactions and the stability of the fragments in real emulsions. The latter approach allows for better control over the polar and non-polar characteristics of each moiety, since it is easier to monitor the presence of just a few amino acids with the same characteristics in each moiety than in a whole assembly of β-sheets or α-helices.

To date, different products and process have been designed based on MD as a tool to design including products such as functionalized materials or drug delivery systems [13,14]. Specifically, our group has demonstrated that MD is a useful tool for exploring the interactions of proteins and peptides in oil–water emulsions and evaluating the correlation of macroscopic variables such as lipophilic–hydrophilic balance and emulsion stability [15,16]. Nevertheless, MD presents space scale limitations and challenges when resolving system dynamic properties such as droplet size or polydispersion. The surface tension at the interface between two immiscible fluids arises from the imbalance in their respective intermolecular cohesive forces. As such, surface tension has an ever–present effect on the dynamics of interfaces, but it is especially central to understanding such fluid phenomena as the formation of fluid droplets [17]. In the emulsification process, when a surface active ingredient is added, its molecules will tend to be oriented between the two faces with the polar ends in the polar phase and the non-polar ends in the non-polar phase, which will lower interfacial tension. This results in a reduction in the surface tension [18].

It is already reported that interaction of surfactants on the surface is reflected on a Gibbs free energy decrease per surface area and that Gibbs free energy interface is related to its interfacial tension [16–19]. Taking into account that the kinetics and the thermodynamics play a role in emulsion stabilization we selected a free energy criterion to study the interactions of the peptides and proteins at the interface, by focusing on their ability to decrease the system's free energy. Therefore, the aim of this work was to study peptides designed based on OmpA via free energy (FE) and MD. We also contrasted our experimental results with dodecane–water emulsions obtained from chemically synthesized peptides.

2. Materials and methods

2.1. Peptide sequences and structures

The sequences of seventeen peptides (Table 1) were extracted from the overall OmpA sequence by following the hydrophobicity/hydrophilicity patterns in the hydropathic graph of OmpA (Supplementary material, Fig. S1- S18). It was possible to locate

Table 1
Sequences of the sixteen extracted models along with their physical–chemical characteristics. Molecular weight (MW), isoelectric point (pI), and charge (Ch).

Model	Length	Sequence	MW	pI	Ch
s1	35	IYTRLGGMVWRADTKSNVYGK NHDTGVSPVFAGGV	3.7	9.52	+2
s2	32	VVVLGYTDRIGSDAYNQGLSERRAQSVVDYLI	3.5	6.48	−1
s3	26	WRADTKSNVYGKNHDTGVSPVFAGGV	2.7	8.50	+1
s4	21	NNNGPTHENQLGAGAFGGYQV	2.1	5.24	−1
s5	19	AHTIGTRPDNGMLSLGVSY	2.0	6.79	0
s6	17	PVVAPAPAPAPEVQTKH	1.7	7.17	0
s7	14	YQWTNNIGDAHTIG	1.6	5.08	−1
s8	13	PKDNTWYTGAKLG	1.4	8.90	+1
s9	13	GKNHDTGVSPVFA	1.3	6.74	0
s10	13	MLSLGVSYRFGQG	1.4	8.50	+1
s11	12	THENQLGAGAFG	1.2	5.21	−1
s12	12	GAGAFGGYQVNP	1.1	5.52	0
s13	12	TRPDNGMLSLGV	1.2	5.50	0
s14	11	ALIDCLAPDRR	1.2	6.00	0
s15	11	IATRLEYQWTN	1.4	6.00	0
s16	10	KLGWSQYHDT	1.2	6.74	0
s17	10	SVVVLGYTDR	1.1	5.55	0
R	325	$OmpA_{325}$	35	5.60	−5