Asian Journal of Chemistry; Vol. 26, No. 21 (2014), 7191-7195

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16559

Behaviour of Biosurfactant Iturin A at Liquid-Liquid Interface

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Received: 22 October 2013; *Accepted*: 14 January 2014; *Published online*: 30 September 2014; AJC-16100

ab initio molecular orbital calculations combined with molecular dynamics simulations have been performed on biosurfactant Iturin A. Intermolecular interaction results indicate that self aggregation of Iturin A is thermodynamically favored even in gas phase. In presence of a medium it will be further facilitated. Inverted micellar formation is predicted to be a spontaneous process and must occur at oil-water interface keeping polar heads towards water surface. Elucidation of this mode of action will complement research in designing pepfactants with synthetic ease and industrial applications. Biosurfactants are widely used in membrane stabilization studies and biotechnology experiments. Simulations carried out in this study indicate that these compounds can be used to enhance delivery of nuclear targeted drugs, in particular as anticancer agents, because they form a polar core and outer hydrophobic surface when self aggregating at lipid interface.

Keywords: Iturin A, Biosurfactant, Liquid-liquid interface.

INTRODUCTION

Surfactants are surface active agents generally amphiphilic in nature containing a hydrophilic head and a hydrophobic tail. These compounds provide wetting to inorganic and organic contaminants and result in their removal. These compounds are popularly used in soap and detergent industry[1]. Increasing environmental awareness has resulted in an interest in eco-friendly biosurfactants produced by microorganisms. These biosurfactants are a group of structurally diverse surface active substances amphiphilic in nature[2-4]. The hydrophilic polar head may consist of mono/oligopolysaccharide or a peptide/protein or glycolipid or lipopeptide or polymer and the hydrophobic moiety consists of a saturated or unsaturated fatty acid or fatty alcohol (Fig. 1). Biosurfactants increase the surface area of hydrophobic, water insoluble substances. Biosurfactants are also excellent emulsifiers, foaming and dispersing agents like conventional surfactants. Further, they possess many advantages, for example, they are environment friendly, biodegradable, less toxic and non hazardous[5-7]. They have better foaming properties and higher selectivity. Biosurfactants are active at extreme temperatures, pH[8] and salinity. An important advantage of biosurfactants is that they can be produced from industrial wastes and from by-products[9]. Some biosurfactants can also self aggregate forming a hydrophobic core that helps solubilize and internalize hydrophobic impurities like oil, grease *etc*. (Fig. 2). This helps in the fast removal of impurities from polar solvents. Thus, these biosurfactants found suitable applications in bioremediation of polluted soil/water and in oil recovery from oil slick in oceans/sea shores[1]. However, this proposal was not tremendously successful as these biosurfactants cannot be genetically engineered and cannot be bioproduced at reasonable cost.

A similar class of synthetic amphiphilic peptide molecules has recently emerged as a solution to above problems[10]. Peptide amphiphilic molecules also known as peptide surfactants/ pepfactants have the capability to self assemble at fluid interface to give cohesive films, stabilizing foams and emulsions. Peptide amphiphiles exert their surfactant property by lowering interfacial surface tension or by stabilizing oil-in-water emulsions[11]. The properties of these compounds at liquid-liquid interface have not been studied at the molecular level. A detailed understanding of properties and mode of action of biosurfactant/pepfactant are required in order to design compounds with low synthetic cost and good surfactant property. This study is directed towards understanding the mode of action of biosurfactant Iturin A at the molecular level.

EXPERIMENTAL

ab initio Hartree Fock molecular orbital calculations with complete geometry optimizations[12,13] have been performed on

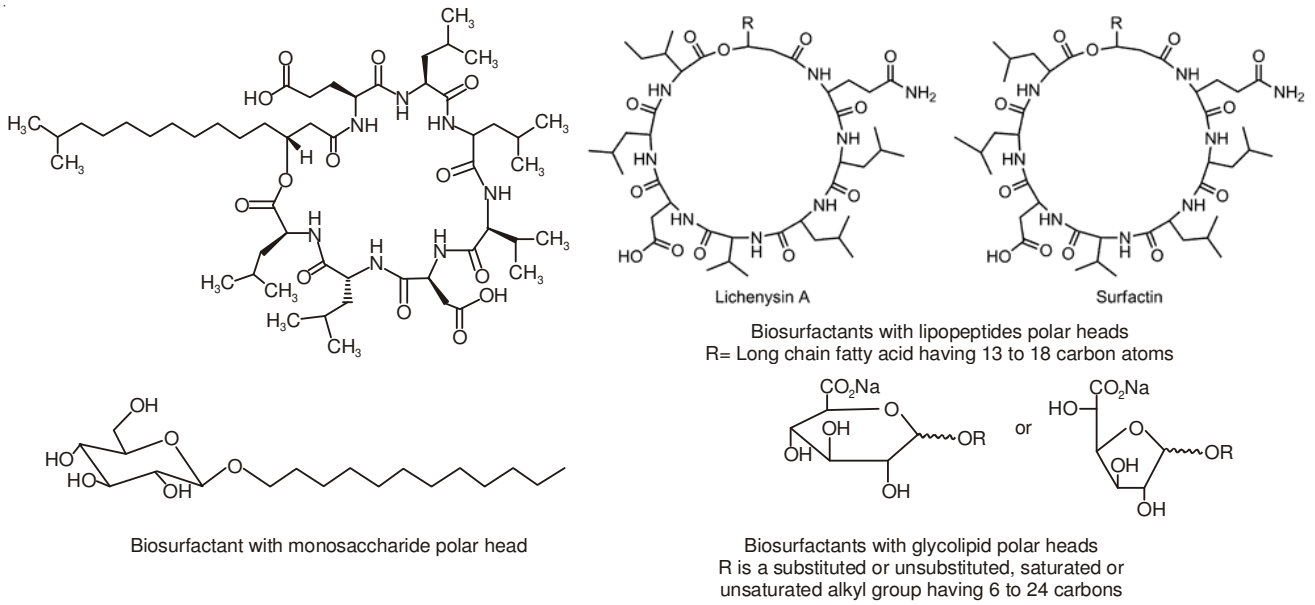

Fig. 1. Different types of biosurfactants

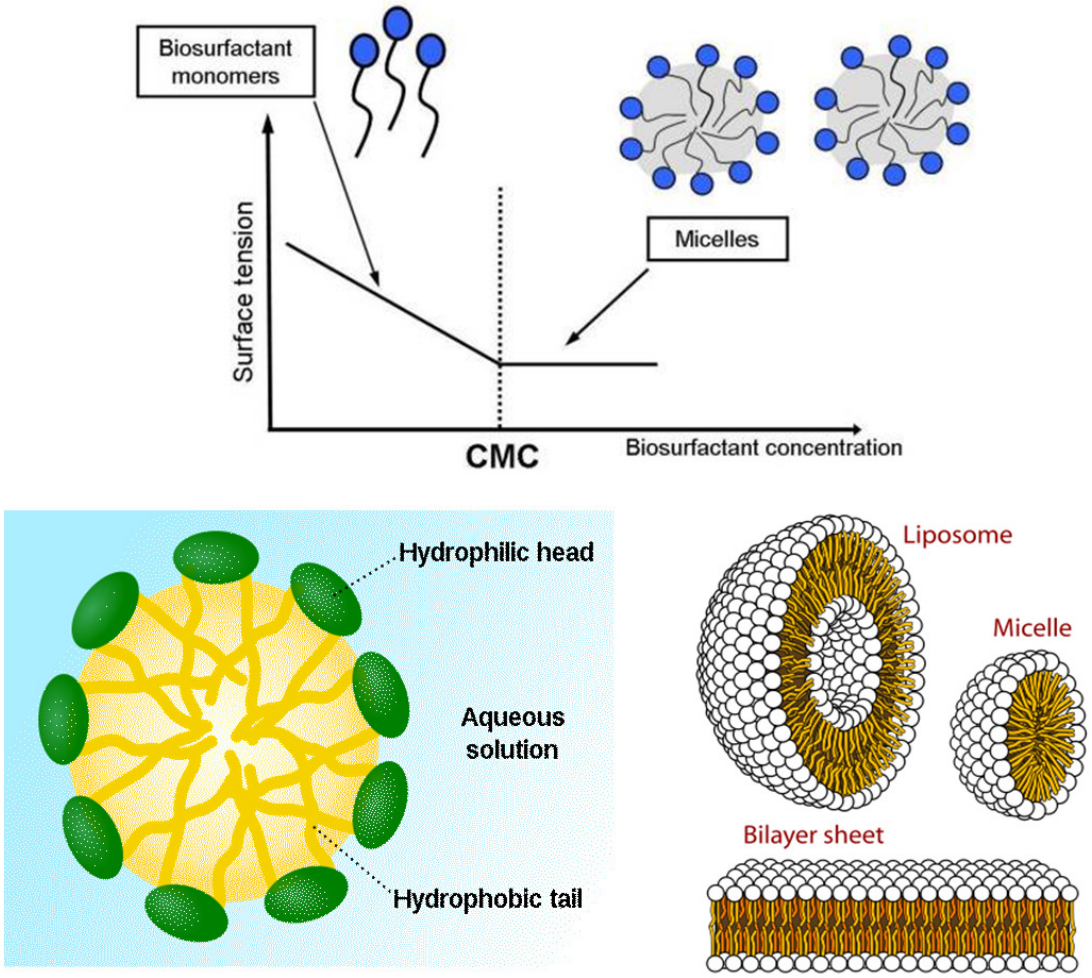

Fig. 2. Different aggregated forms of biosurfactants

monomer of Iturin A utilizing GAUSSIAN '03[14] and GAUSSVIEW[15] softwares. Self aggregation possibility has been explored by intermolecular interaction calculations of supermolecule type. Interaction energy has been calculated as follows:

$$\text{Interaction energy} = E_{\text{supermolecule}} - n \times E_i$$

where n is the number of monomers in supermolecule and E_i is the energy of monomer.

The behaviour of surfactant at liquid-liquid interface has been studied by molecular dynamics simulations. Shaw's

Desmond software[16] has been utilized for the purpose. Oil-water interface has been mimicked by palmitoyl-oleoyl-phosphatidyl choline (POPC) membrane bilayer-SPC water interface. Aggregated form was placed at interface. System was subjected to simulated annealing followed by long simulations at room temperature 300 K. OPLS force field has been used for simulating the system. NPT ensemble has been taken using Martyna-Tobias-Klein barostat method. RESPA integrator has been used with 2 fs time step for near and bonded atoms and 6 fs time step for far atoms. Coulombic interaction cutoff has been set at 9 Å. A 20 ns trajectory was evaluated.

RESULTS AND DISCUSSION

Completely optimized conformation of Iturin A at the Hartree Fock level utilizing 6-31G basis set is shown in Fig. 3. The hydrophobic tail of monomer is almost perpendicular to the lipopeptides head. The polarity of lipopeptide head can be judged by the net atomic charge distribution and molecular electrostatic potential surface also presented in Fig. 3. The electrostatic potential surface indicates that the polar heads of monomers may be oriented in such a way so as to interact with each other giving rise to an aggregated micellar form. Possibility of self aggregation was explored by intermolecular interaction calculations. These results are shown in Fig. 4 which clearly indicate that inverted micelle formation is thermodynamically driven even in gas phase, that is, in absence of any medium.

We have also investigated the behavior of this self aggregated micelle at liquid-liquid interface. Oil-water interface was mimicked by POPC membrane bilayer-water interface. Aggregated micelle was placed at the interface and simulations were run to check its stability at interface. Placement of Iturin A molecules at the interface after simulated annealing can be seen in Fig. 5. All water molecules have now collected near lipopeptide heads of surfactants. Hydrophobic tails remain oriented towards hydrophobic membrane. 20 ns simulation was run which is sufficient to judge the stability of micelle at interface. Snapshot of micelle at interface after 20 ns simulation is shown in Fig. 6. A better judgement of stability of micelle at interface can be made by considering root mean square deviations of all heavy atoms throughout the simulation. Root mean square deviations (RMSDs) are shown in Fig. 7. Root mean square deviation for all atoms are within 4-5 A indicating a successful simulation. Trajectory for entire simulation can be viewed as video still enclosed. Simulation clearly indicates stability of micelle at liquid-liquid interface and the behaviour of self aggregated Iturin A molecules. The movement of hydrophobic tails will facilitate solubilization of hydrophobic impurities.

The results of simulation also indicate that under physiological conditions such biosurfactants will spontaneously self aggregate at lipid interface and facilitate internalization of any polar nuclear targeted drug like lamivudine. The core of self aggregated form is suitable for carriage of polar drug molecule. Simulations to this effect are underway.

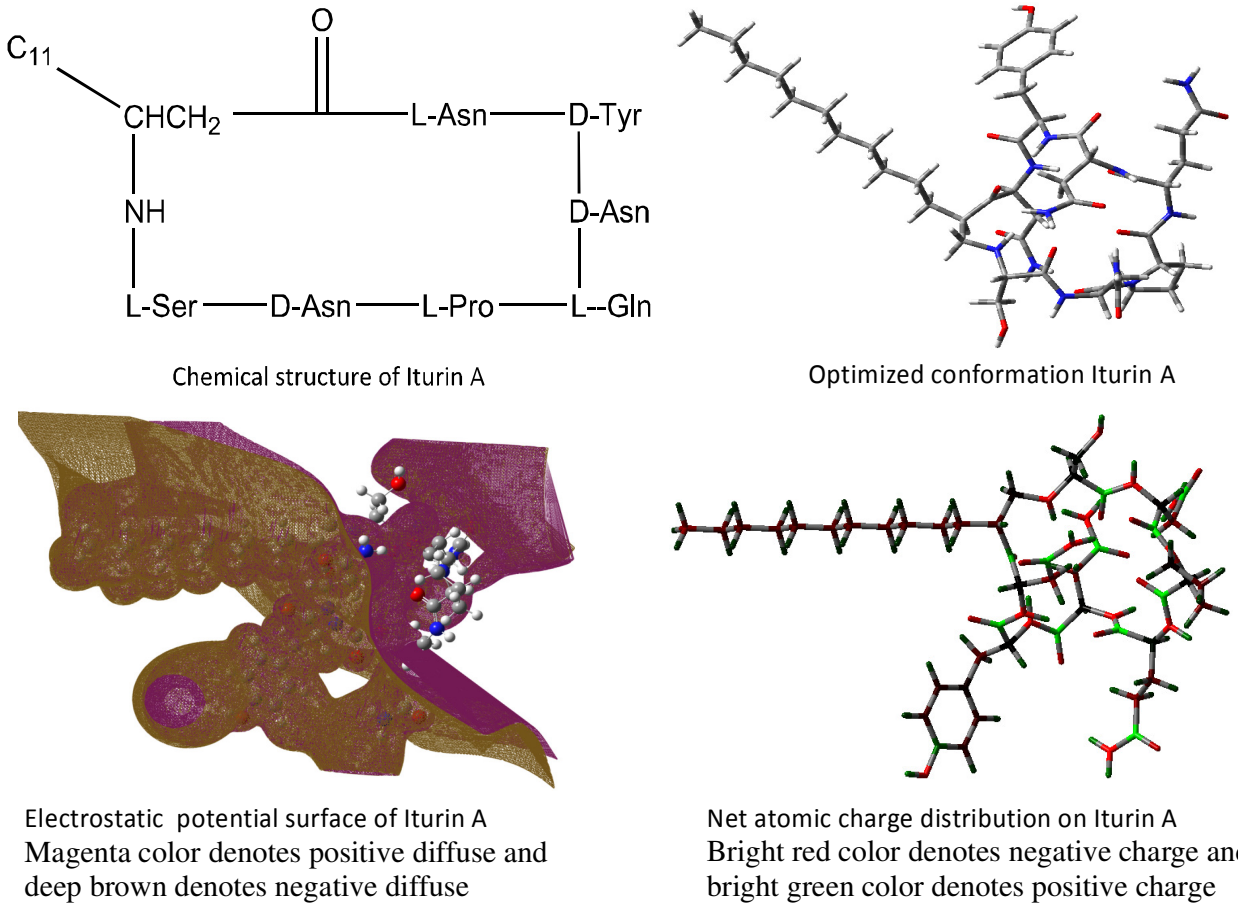

Fig. 3. Optimized conformation and charge environment of Iturin A

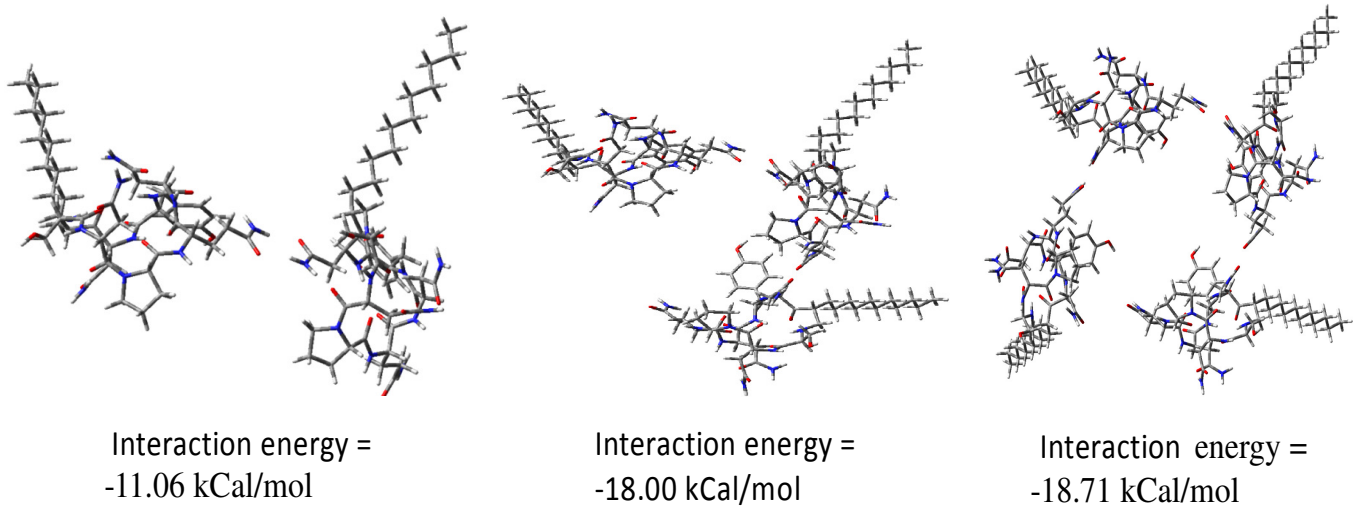

Fig. 4. Self aggregation feasibility of Iturin A in absence of any medium

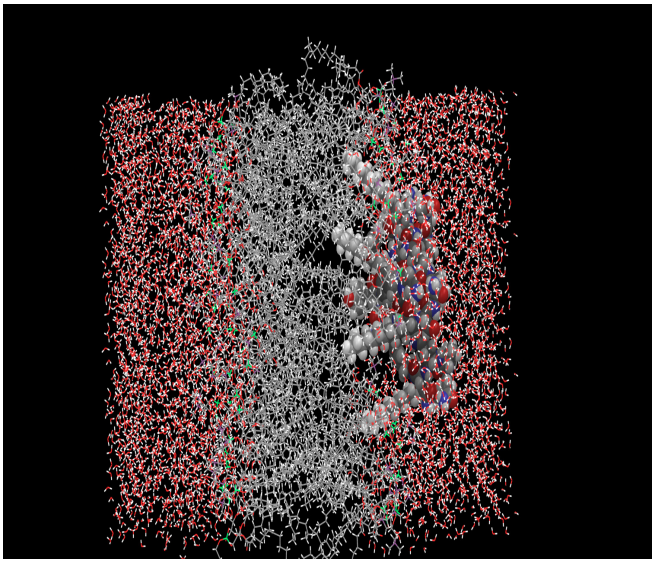

Fig. 5. Self-aggregated Iturin A at hydrophilic (water)-hydrophobic (membrane) interface after simulated annealing

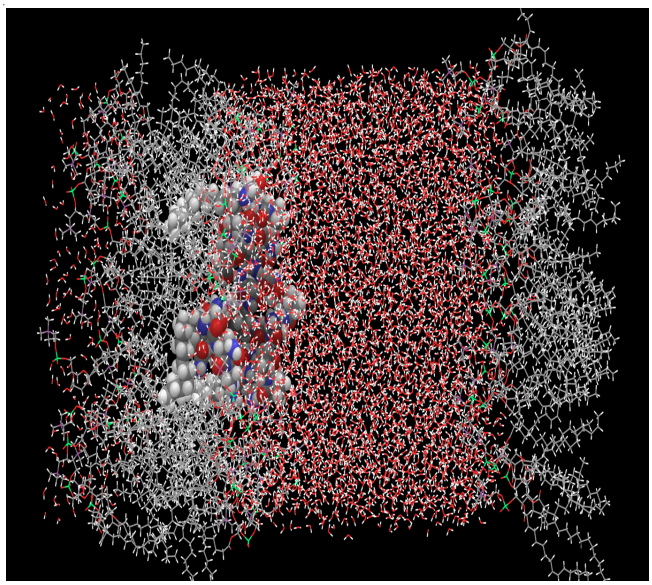

Fig. 6. Snapshot of self-aggregated Iturin A at interface after 20 nanosecond simulation

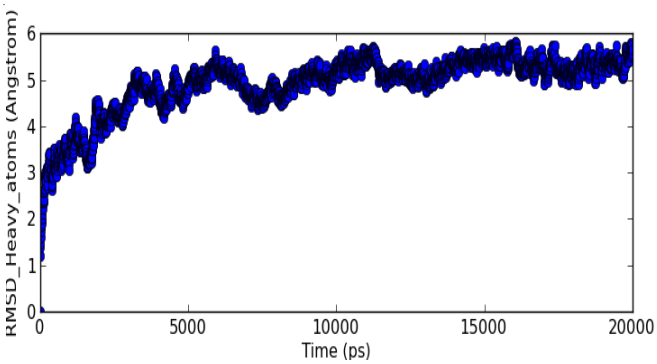

Fig. 7. Root mean square deviations for all heavy atoms from their initial position throughout the simulation

Conclusion

This study has highlighted that lipopeptide biosurfactants have a tendency to self aggregate and undergo inverted micellar formation spontaneously at oil-water interface keeping the polar heads towards water surface. The hydrophobic tails help enhance solubilization of any hydrophobic impurity in water. Ecofriendly pepfactants with low-cost synthetic routes may be designed with the help of this study elucidating the mode of action. Such biosurfactants will also facilitate internalization of any polar nuclear targeted drug. This is the first simulation of its kind where the stability of self aggregated biosurfactant at oil-water interface has been studied.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge infrastructural support from University Institute of Engineering and Technology, Chhatrapati Shahu Ji Maharaj University, Kanpur. One of us (A. Banerjee) is thankful to CSJM University for stipend. D. Bhaskar is thankful to University Institute of Engineering and Technology for infrastructural support for carrying out summer project.

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