

Molecular Dynamics Simulation of the Aggregation of Extracellular Polymeric Substance

Yingjie Liu^{1*}, Xia Liu¹ and Lu Liu¹

¹ College of Environmental Science and Engineering, Ocean University of China, 266100 Qingdao, China

Abstract. Extracellular polymeric substance (EPS) play an important role in the transport and transformation of pollutants. At present, some scholars have conducted basic experimental characterization of EPS, but the molecular level interactions among the various components of EPS still need to be fully characterized. Here, we use molecular dynamics (MD) simulation to explore the structural properties of EPS systems in atomic detail. The process and mechanism of the aggregation of different EPS components (polysaccharide, lipid, nucleic acid and protein) were revealed by the simulation results. EPS aggregation consist of a hydrophobic core and an amphiphilic exterior. Lipid tail, as a hydrophobic core, promoted the aggregation of EPS. But strong hydrophilic nucleic acid and protein components inhibited the aggregation of EPS in water and were located outside the aggregation body. The details of the structure of EPS aggregation are revealed here, which provides a micro molecular mechanism for understanding the interaction between EPS.

1 Introduction

Natural organic matter (NOM) widely exists in the water environment, and is composed of organic compounds with different molecular weights, components, and isomers [1]. According to different sources of NOM, it can be divided into autochthonous and allochthonous compounds. Autochthonous compound is an organic compound directly released by various microorganisms in water, accounting for a large proportion of organic macromolecules in aquatic ecosystems [2]. The most important component of in situ NOM is the highly viscous EPS secreted by microorganisms into the surrounding environment. EPS is an important component of biofilms, which is a by-product of metabolism secreted by microorganisms in response to pressure or adaptation to habitats, its secretion greatly improves the survival rate of microorganisms in water [3]. EPS is generally composed of four components: high molecular weight polysaccharides, proteins, lipids, and nucleic acids [4]. These components not only contain a variety of isomers, but also contain rich chemical groups, such as carboxyl, amino, and carbonyl groups [5]. At the same time, these EPS can be divided into hydrophilic EPS and hydrophobic EPS according to their hydrophilicity and hydrophobicity [6]. The different hydrophilicity and hydrophobicity of EPS will inevitably have different effects on its own aggregation.

As a heterogeneous mixture composed of various organic compounds, EPS has received widespread attention in the field of ecological environment in recent years. The complexity, heterogeneity, and variability of EPS increase the difficulty of research, making our limited understanding of its interfacial behavior between

microbial cells and the aquatic environment. With the continuous development of molecular characterization and identification technology, the research on the composition of EPS is also deepening. Some studies have confirmed the assembly of humus in water through experiments [7]. However, the self-assembled structure of EPS in water and the molecular mechanism of its formation still need to be studied, and related microscopic issues cannot be verified through experiments, requiring in-depth exploration in combination with MD simulation.

2 Materials and Method

2.1 Model construction

The complex EPS was simulated by adding six polysaccharides (Pol), two lipids, one RNA, and one protein to the simulation system. Among these, four Pol components (Pol 1-4) are neutrally charged (Figure 1A) and two Pol divisions are negatively charged (Pol 5) and positively charged (Pol 6) by deprotonation and protonation of carboxyl and amino groups (Figure 1B). Lipids consist of 1-palmitoyl-2-oleoyl-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho (POPG). POPG has a negative head group (Figure 1C). The short RNA possesses the AUCGAUCG sequence (Figure 1D) and their protein components are represented by Cytochrome type C (Cyt-C) (Figure 1E), which is secreted by many bacteria to aid in extracellular respiration. The molecular structure of EPS components except Cyt-C was constructed by Materials Studio 2017. These structures are imported into an automatic topology generator to generate interaction parameters for MD

* liuyingjie7785@stu.ouc.edu.cn

simulations. The whole atomic molecular structure of Cyt-C was obtained from the Protein database (PDB:1akk).

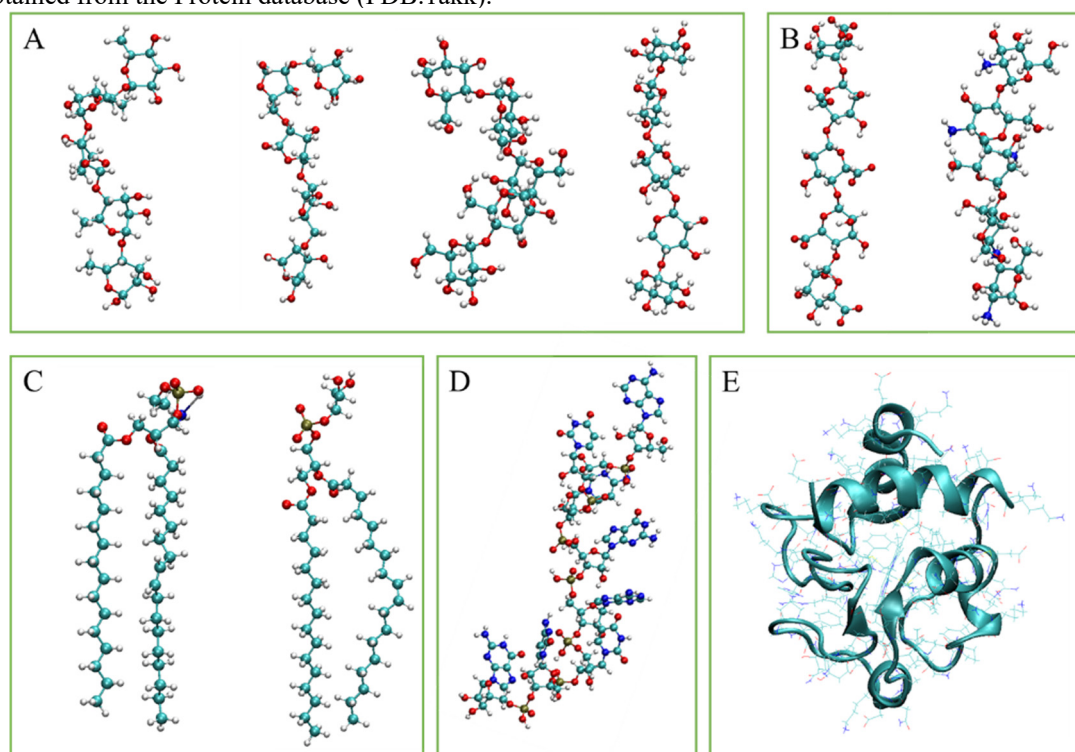


Figure 1. EPS component structure. (A) Four neutral polysaccharide models; (B) Two charged polysaccharide models (C) POPC and POPG models; (D) AUCGAUCG sequence RNA model; (E) All-atomic model of Cyt-C.

2.2 Simulation system

Placing EPS single molecules at $10\text{ nm} \times 10\text{ nm} \times 10\text{ nm}$ in a cubic box and solvated, 10 ns equilibrium was performed to obtain a small molecule after equilibrium. To study EPS assembly, the components of polysaccharides, lipids, nucleic acids, and proteins were sequentially mixed at $15\text{ nm} \times 15\text{ nm} \times 15\text{ nm}$ in a cube box, solvate with water and add Na^+ and Cl^- to neutralize the system charge to prepare four increasingly complex EPS systems. The simulation time was 200 ns.

(1) EPS1 consists of only polysaccharides, with 8 molecules added to each molecule;

(2) In the EPS2 system, 8 POPCs and 8 POPGs were added to the EPS1 system to explore the effect of lipid components on aggregation;

(3) In the EPS3 system, 8 nucleic acids were added to the EPS2 system to explore the effect of nucleic acid components on aggregation;

(4) In the EPS4 system, eight proteins are added to the EPS3 system to explore the effect of protein components on aggregation.

2.3 Simulation detail

All MD simulations were performed using the GROMACS software package version 4.6.7^[8], the Gromos 54a7 force field and TIP3P water model^[9]. The assembly was simulated using a NPT ensemble. Using an isotropic Berendsen constant pressure gauge ($P = 1\text{ bar}$), the coupling constant in all three directions was 0.2 ps,

and the compressibility coefficient was $4.5 \times 10^{-5}\text{ bar}^{-1}$ using a V-rescale thermostat with a coupling constant of 0.2 ps to maintain the temperature at $T = 300\text{ K}$. The truncation radius of both the van der Waals interaction and the Coulomb interaction is 1.2 nm. The Lennard-Jones (LJ) and Coul potentials smoothly shift to zero between 0 nm and 1.2 nm. The particle grid Ewald summation method is used to deal with long-range electrostatic interactions. The time step for all simulations is 1 fs, and the neighbor list is updated every 10 steps. Periodic boundary conditions are considered in all three directions. The molecular conformation is presented through VMD software^[10].

3 Results and Discussions

3.1 Characterization

The Hydrophobicity of EPS components was estimated by solvation free energy (Figure 2). The smaller the free energy of solvation, the stronger the hydrophilicity, and the larger the free energy of solvation, the stronger the hydrophobicity. Because the protein molecule has a hydrophobic core and a hydrophilic surface, the default protein component is the most hydrophilic. The results showed that the order of hydrophobicity of all components was lipid > Pol 1-4 > RNA > Pol 5-6 > Cyt-C.

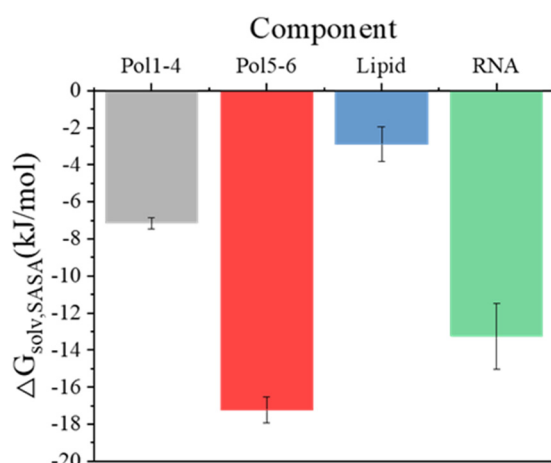


Figure 2. Solvation free energy of each component.

3.2 Effects of each component on EPS aggregation

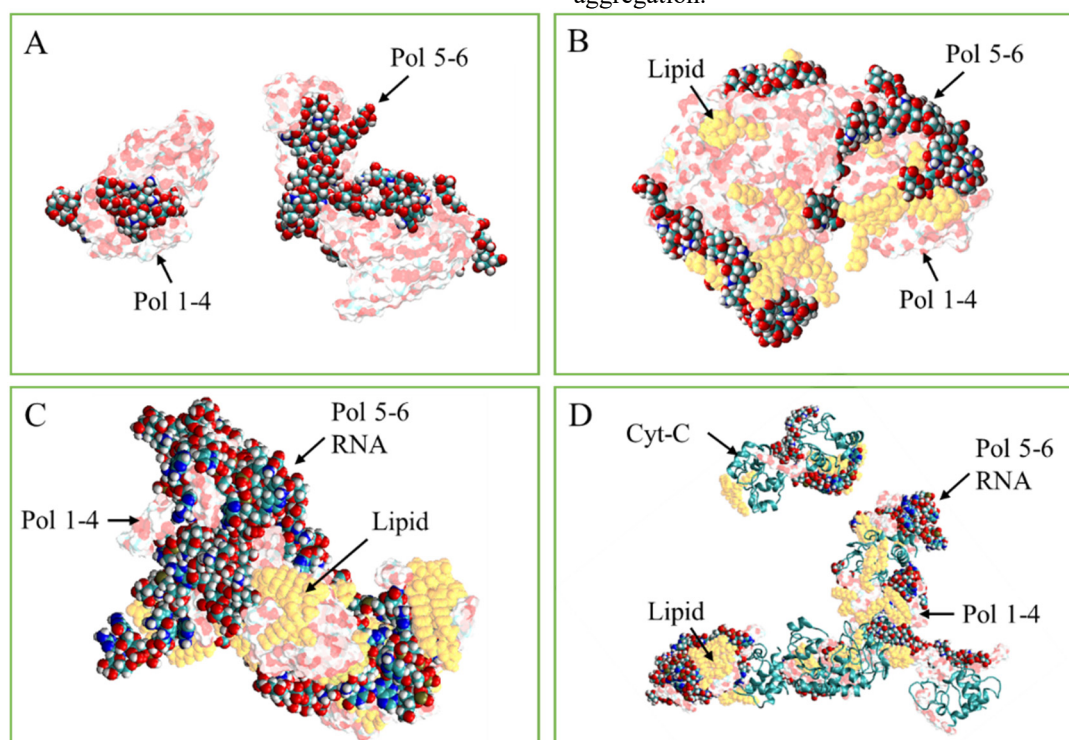


Figure 3. The final configuration of the four systems and the positions of each component in the aggregation were simulated. (A) EPS1; (B) EPS2; (C) EPS3; (D) EPS4

3.3 Surface properties of EPS aggregation

EPS aggregation is affected by the hydrophobicity of components, and the driving force of aggregation is hydrophobic interaction. Hydrophobic components promoted EPS aggregation, while hydrophilic components inhibited EPS aggregation. The hydrophobic component promotes aggregation and is located at the core of the aggregation, while the hydrophilic component is located at the surface of the aggregation. In addition, Pol 5-6 is not only hydrophilic, but also has positive or negative charges, so there are

In order to elucidate the effects of each component on EPS assembly, full-atom MD simulations of EPS1, EPS2, EPS3 and EPS4 were carried out. In EPS1, 48 polysaccharides in the system formed two aggregations (Figure 3A), indicating a weak interaction between polysaccharides. In the EPS2 system with added lipids, all molecules in the system formed an aggregation (Figure 3B), which proved that lipid components promoted the aggregation of EPS in water.

In the EPS3 system with the addition of nucleic acid, although all molecules in the system formed an aggregation (Figure 3B), the aggregation was more irregular than that of EPS2, indicating that in the EPS3 system, lipid components still promoted the aggregation of EPS in water, but RNA inhibited the formation of aggregation (Figure 3C). When eight protein molecules were added to the system (EPS4), the entire system did not aggregation, and other components formed several smaller clusters and were connected to each other by Cyt-C (Figure 3D). The protein component inhibited EPS aggregation.

charged groups on the surface of the aggregations. As shown in Figure 3, components with strong hydrophilicity are all on the surface of aggregations.

4 Conclusion

This study mainly studied the related mechanism of EPS aggregation in water. As a hydrophobic core, lipid components promoted EPS aggregation through hydrophobic action, while nucleic acid and protein components were highly hydrophilic and inhibited EPS aggregation. After the aggregation of EPS, the surface of the aggregation is hydrophilic and has charge.

Acknowledgments

This work was financially supported by Natural Science Foundation of Shandong Province (ZR2020QD128), China Postdoctoral Science Foundation (2019M660168) and Qingdao Postdoctoral Applied Research Project.

References

1. Wang, R. Q., Gutierrez, L., Choon, N. S., Croue, J. P. (2014) Hydrophilic interaction liquid chromatography method for measuring the composition of aquatic humic substances. *Anal. Chim. Acta*, 853(1): 608-616. 10.1016/j.aca.2014.09.026
2. Wilkinson, K. J., Lead, J. R. (2007) Environmental colloids and particles: behaviour, separation and characterisation. *Angewandte Chemie-International Edition Angew. Chem. Int Edit.*, 146(1): 432-432. 10.1515/ci.2007.29.2.21a
3. Gutierrez, T., Berry, D., Yang, T., Mishamandani, S., McKay, L., Teske, A., Aitken, M. D. (2013) Role of bacterial exopolysaccharides (EPS) in the fate of the oil released during the deepwater horizon oil spill. *PloS One*, 8(6): e67717. 10.1371/journal.pone.0067717
4. Tony, G, Andreas, T., Kai, Z., Uta, P., Antonietta Q. (2018) Microbial exopolymers: sources, chemico-physiological properties, and ecosystem effects in the marine environment. *Front. Microbiol.*, 9: 1822. 10.3389/fmicb.2018.01822
5. Nkoh J.N., Yan J., Hong Z. N., Xu R. K., Kamran M. A., Jun J., Li J. Y., (2019) An electrokinetic perspective into the mechanism of divalent and trivalent cation sorption by extracellular polymeric substances of *Pseudomonas fluorescens*. *Colloids Surf. B Biointerfaces*, 183, 110450. 10.1016/j.colsurfb.2019.110450
6. He S. F., Feng L. K., Zhao W. X., Li J. J., Zhao Q. L. Wei L. L. (2023) Composition and molecular structure analysis of hydrophilic/hydrophobic extracellular polymeric substances (EPS) with impacts on sludge dewaterability, *Chem. Eng. J.*, 462(142234): 1385-8947, <https://doi.org/10.1016/j.cej.2023.142234>.
7. Lan T., Wu P., Liu Z., Stroet M., Liao J., Chai Z., Alan E. M., Liu N., Wang D. (2022) Understanding the effect of pH on the solubility and aggregation extent of humic acid in solution by combining simulation and the experiment. *Environ. Sci. Technol.* 56(2): 917-927. 10.1021/acs.est.1c05938
8. Fernandes H. S., Sousa S. F., Nuno M. F. S. A. C. (2019) VMD Store – a VMD Plugin to Browse, Discover, and Install VMD Extensions. *J. Chem. Inf. Model.* 10.1021/acs.jcim.9b00739
9. Hess, B., Kutzner, C., Spoel, D. V. D., Lindahl, E. (2008) GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J. Chem. Theory Comput.*, 2008(3): 435-447. 10.1021/ct700301q
10. Feng H., Liu Y., Xu Y., Li S., Liu X., Dai Y., Zhao J., Yue T. (2022) Benzo[a]pyrene and heavy metal ion adsorption on nanoplastics regulated by humic acid: cooperation/competition mechanisms revealed by molecular dynamics simulations. *J. Hazard. Mater.*, 424(Pt B), 127431. 10.1016/j.jhazmat.2021.127431