Proceedings of the 8th International Conference on Theoretical and Applied Nanoscience and Nanotechnology (TANN 2024)
Chestnut Conference Centre - University of Toronto, Toronto, Canada – June 10-12, 2024
Paper No. 112
DOI: 10.11159/tann24.112

Self-Assembly Of Elastin-Suckerin Based Diblock Copolypeptides For Biomedical Applications

Aamna Basheer[1], Gul Karima[2], Lubna Ghani[3]
[1,2]Department of Bionano Engineering and Department of Bionanotechnology, Center for Bionano Intelligence Education and Research, Hanyang University, Ansan 15588, Republic of Korea.
aamna@hanyang.ac.kr; karimaali99@gmail.com
[3]Department of Chemistry, Women University of Azad Jammu and Kashmir, Bagh, Pakistan.
lubnaghani41@gmail.com

Abstract **-** Protein engineering enables the design and synthesis of diverse biomaterials with different functionalities required for advanced drug delivery systems, tissue engineering and regenerative medicine. In this work, we designed and synthesized the recombinant suckerin with different repeating numbers and fused these suckerin blocks with elastin based polypeptides (EBP). EBP-suckerin block copolypeptides self-assembled into nanostructures due to β-sheet structures of suckerin blocks. The EBP block was hydroxylated to form 3, 4-dihydroxyphenylalanine (DOPA) for adhesive properties of self-assembled nanostructures. The modified diblock copolypeptides were successfully purified via inverse transition cycling (ITC) and self-assembled into nanostructures as characterized by dynamic light scattering (DLS). The results showed that particle sizes decreased with increasing suckerin block length as increasing length enhance their intermolecular interaction and reduce the sizes of nanostructures. Morphologies of self-assembled nanostructures were confirmed via TEM and SEM that clearly showed vesicles of EBP-suckerin block copolypeptides before and after modification. Bright field images of nanostructures before and after washing confirmed the adhesive properties of self-assembled nanostructures. These EBP-suckerin diblocks would be promising for advanced drug delivery applications as drug could be encapsulated in hydrophobic part of nanostructures.
Keywords**:** Suckerin, Elastin, Block copolypeptides, Self-assembly, vesicles, DOPA

1. Introduction

Protein based materials have several benefits over synthetic materials because they self-assemble into complex hierarchical structures and have programmable physicochemical properties with improved biocompatibility and biodegradability. Great attention has been given to amphiphilic polypeptides combined with different functional domains because they have potential to generate well-ordered, self-assembled nanostructures for advanced drug delivery applications. Elastin based polypeptides (EBPs) with lower critical solution temperature (LCST) behavior are well-known for their thermal responsiveness and mostly studied as drug delivery carriers [1], [2] as these polypeptides are designed from natural tropoelastin [3], [4]. EBPs are artificially designed pentapeptide repeats of Val-Pro-Gly/Ala-Xaa-Gly, where Xaa can be any amino acid accept proline. EBPs are fully soluble below their LCST and coacervate on heating above their LCST [5] and this thermal responsiveness is completely reversible. EBPs with different proteins have been broadly studied for biomedical applications owing to their programmable design with multifunctional domains, self-assembly into diverse nanostructures such as spherical or cylindrical micelles, vesicles and most importantly their easy purification method [6]. Recently new proteins, suckerins, are examined in the sucker ring teeth (SRT) of humboldt squids (*Dosidicus gigas*) that are amphiphilic in nature and self-assemble into nanoconfined β-sheet structure and impart robust mechanical properties without any chemical cross-linking [7]. Suckerins have repetitive blocks of amorphous and crystal forming domains. Precisely, suckerins consist of alanine and histidine rich modules called M1 block which is intervened by longer Glycise and Tyrosine rich modules known as M2 block. M1 segments self-assemble into nanoscale β-sheet structures surrounded by amorphous matrix of M2 modules [8], [9]. The self-assembly of M1 module with peptide sequence of AATAVSHTTHHA called as "A1H1" has been studied in water and it was reported that these blocks self-assembled into compact β-sheets [10]. These β-sheet structures act as reversible cross-linker due to their hydrogen bonding, imparting dynamic and programmable mechanical properties of nanomaterials.

In this study, we propose the fusion of suckerin with EBPs due to its amphiphilic nature and repetitive block structures which self-assemble into nanoconfined β-sheets and provide the enhance stability in resulting biomaterials. No chemical cross-linking is involved in native suckerins for their structural stability, which is an attractive feature to select them for this work. As compared to silk, suckerins are highly expressed in recombinant systems and do not require harsh conditions for their solubilization [11]. A new EBP library was designed to introduce 3,4 hydroxyphenyalanine (DOPA) in polypeptides via hydroxylation of tyrosine residues for their adhesive strength. DOPA is receiving significant interest in scientific couminty due to its strong adhesive strength with substrate. The fusion of suckerins with adhesive EBPs provide a new class of polypeptides with unique characteristics and functionality where EBPs provide thermal responsiveness along with adhesive properties and repetitive sequences of suckerins form well-ordered β-sheets. Due to its hydrophobic/hydrophilic nature, elevated histidine content, higher water solubility parallele to silk, suckerin offers great potential as a nanoscale drug carrier [12]. The attractive adhesive properties of EBP-suckerin block copolypeptides will make these materials potential candidates for underwater applications.

2. Result and discussion

Suckerin blocks with Pro-M1-Pro-M2 design were selected in this work for self-assembly of polypeptides. The suckerin was mutimerized to get control over its self-assembly that would subsequently effect the structure of diblock copolypeptides. The EBP block, EBP$[Y_2K_4]_6$, was fused to the "N" terminal of suckerin block. Tyrosine residues, which were modified into DOPA for adhesive properties and lysine was introduced as it was cited to have potential contributions to adhesion strength. Tyrosine residues of EBP blocks were converted into DOPA using mushroom tyrosinase. These EBP-suckerin diblock copolypeptides self-assembled into nanostructures due to β-sheet structures of suckerin blocks below the LCST of EBPs. Three types of diblock copolypeptides, E6S1, E6S3 and E6S12 were synthesized in this work to study the suckerin block effect on self-assembly of nanostructures. The E6 and E6Sn were purified by ITC using their thermally induced aggregation and re-solubilization in 5 % acetic acid supplemented with 8 M urea to solubilize suckerin below their LCST. All block copolypeptides were successfully purified by 3-4 rounds of ITC. The yield of purified blocks were > 300 mg per liter of culture.

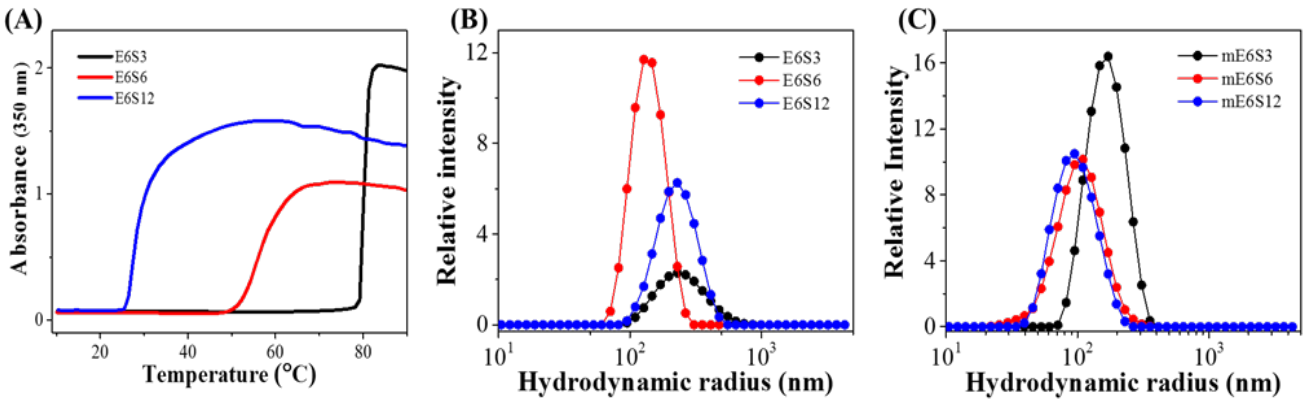

Fig. 1: Characterization of E6Sn diblock copolypeptides. (A) Turbidity profile of diblock copolypeptides with 0.5 M NaCl from 10°C to 90°C at the heating rate of 1°C /min. Hydrodynamic radius (Rh) of diblock copolypeptides via DLS, (B) before modification (C) after modification.

Tyrosine residues of E6Sn were converted into DOPA by mushroom tyrosinase and purified via ITC. The modification of DOPA was specifically verified by NBT/Glycinate staining by their redox-cycling. Fig. 1 (A) shows the thermal characterization of E6Sn which was carried out by measuring the absorbance of samples at 350 nm at a heating rate of 1 °C/min. The self-assembly of diblock copolypeptides was characterized via DLS measurements before and

after modification of tyrosine residues of EBP block as shown in Fig. 1(B) and 1(C) respectively. Before modification, nanostructures with average Rh of 266.7 ± 47.97 nm, 238.5 ± 41.72 nm and 140.8 ± 14.14 nm were formed for E6S3, E6S6 E6S6 and E6S12 respectively. Previously, self-assembly of suckerin-19 into β-sheets was initiated by salting out and sizes sizes of nanoparticles were controlled using different salts [12]. The average Rh of mE6S3, mE6S6 and mE6S12 are 192.1 192.1 ± 12.00 nm, 109.4 ± 17.54 nm and 100.6 ± 5.18 nm after hydroxylation of tyrosine residues into DOPA.

TEM and SEM measurements were conducted to ensure the nanostructures observed via DLS. TEM samples were prepared at room temperature on carbon coated grids and for better visualization of nanostructures, 2% PTA aqueous solution was used as negative stain. TEM images were taken before and after modification of tyrosine residues of EBP blocks into DOPA that showed all block copolypeptides self-assemble into vesicles.

The nanostructures of E6S3 are shown in Fig. 2. Vesicle formation of E6Sn due to self-assembly of suckerin block was also characterized by SEM imaging before and after modification of tyrosine. All the diblocks self-assembled into vesicles that ensure that E6Sn self-assemble due to β sheet structures of suckerin blocks and modification of tyrosine on EBP blocks did not affect their self-assembly.

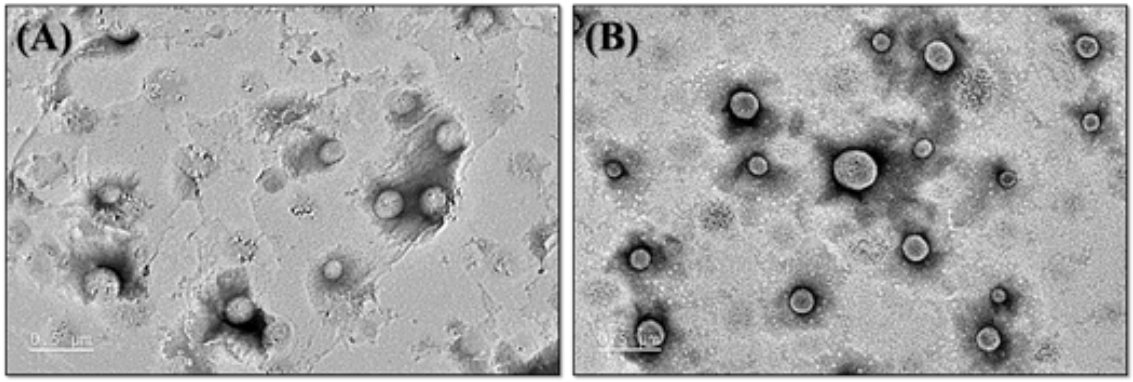

Fig. 2: TEM images of E6S3 at room temperature. (A) Before DOPA modification, (B) after DOPA modification. The scale bars are 0.5 μm.

4. Conclusion

In summary, we designed and synthesized the recombinant suckerin with different repeating numbers and fused these suckerin blocks with EBP. These EBP-Suckerin diblock copolypeptides were successfully over expressed in *E.coli*, purified via ITC and characterized by UV-visible spectrophotometer, DLS, TEM and SEM. EBP-suckerin diblock copolypeptides self-assembled into vesicles due to β-sheet structures of suckerin blocks. The EBP blocks were hydroxylated to form DOPA for adhesive properties of self-assembled naostructures. The modified diblock copolypeptides were also successfully purified via ITC and self-assembled into nanostructures as characterized by DLS. Morphologies of self-assembled nanostructures were confirmed via TEM and SEM that clearly showed vesicles of EBP-suckerin block copolypeptides before and after modification. These EBP-suckerin diblocks would be promising for drug delivery application as drug could be encapsulated in hydrophobic parts of nanostructures.

References

[1] Simnick, A. J.; Lim, D. W.; Chow, D.; Chilkoti, A., "Biomedical and biotechnological applications of elastin-like polypeptides," *J Macromol Sci Polymer Rev.,* vol. 47, no. 1, pp. 121-154, 2007.

[2] MacEwan, S. R.; Chilkoti, A., "Elastin-like polypeptides: biomedical applications of tunable biopolymers," *J. Pept. Sci*., vol. 94, no. 1, pp. 60-77, 2010.

[3] Urry, D. W., "Elastic molecular machines in metabolism and soft-tissue restoration," *Trends Biotechnol*., vol. 17 no. 6, pp. 249-257, 1999.

[4] Urry, D. W.; Pattanaik, A.; Xu, J.; Cooper Woods, T.; McPherson, D. T.; Parker, T. M., "Elastic protein-based polymers in soft tissue augmentation and generation," J. Biomater. Sci. Polym. Ed., vol. 9, no. 10, pp. 1015-1048, 1998.
[5] Urry, D. W., "Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers." *Phys. Chem. B*., vol. 101, no. 51, pp. 11007–11028, 1997.
[6] Lim, D. W.; Trabbic-Carlson, K.; MacKay, J. A.; Chilkoti, A., "Improved Non-Chromatographic Purification of a Recombinant Protein by Cationic Elastin-Like Polypeptides," *Biomacromolecules.*, vol. 8, no. 5, pp. 1417-1424, 2007.
[7] Guerette, P. A.; Hoon, S.; Seow, Y.; Raida, M.; Masic, A.; Wong, F. T.; Ho, V. H.; Kong, K. W.; Demirel, M. C.; Pena-Francesch, A., "Accelerating the design of biomimetic materials by integrating RNA-seq with proteomics and materials science," *Nat. biotechnol*., vol. 31, no. 10, pp. 908-915, 2013.
[8] Guerette, P. A.; Hoon, S.; Ding, D.; Amini, S.; Masic, A.; Ravi, V.; Venkatesh, B.; Weaver, J. C.; Miserez, A., "Nanoconfined β-sheets mechanically reinforce the supra-biomolecular network of robust squid sucker ring teeth." *ACS Nano*., vol. 8 no. 7, pp. 7170-7179, 2014.
[9] Hiew, S. H.; Miserez, A., "Squid sucker ring teeth: Multiscale structure–property relationships, sequencing, and protein engineering of a thermoplastic biopolymer," *ACS Biomater. Sci. Eng*., vol. 3 no. 5, pp. 680-693, 2017.
[10] Sánchez-Ferrer, A.; Adamcik, J.; Handschin, S.; Hiew, S. H.; Miserez, A.; Mezzenga, R., "Controlling supramolecular chiral nanostructures by self-assembly of a biomimetic β-sheet-rich amyloidogenic peptide," *ACS Nano*., vol. 12, no. 9, pp. 9152-9161, 2018.
[11] Ding, D.; Guerette, P. A.; Hoon, S.; Kong, K. W.; Cornvik, T.; Nilsson, M.; Kumar, A.; Lescar, J.; Miserez, A., "Biomimetic production of silk-like recombinant squid sucker ring teeth proteins," *Biomacromolecules*., vol. 15, no. 9, pp. 3278-3289, 2014.
[12] Ping, Y.; Ding, D.; Ramos, R. A.; Mohanram, H.; Deepankumar, K.; Gao, J.; Tang, G.; Miserez, A., "Supramolecular β-sheets stabilized protein nanocarriers for drug delivery and gene transfection," *ACS Nano.*, vol. 11, no. 5, pp. 4528-4541, 2017.