Monitored Dynamics of the Self-Assembled Peptide RADA16l Nanofiber Scaffold

Alicia Calero, Department of Chemical-Biomedical Engineering at Florida State University, Tallahassee, FL
Mentored by Dr. Ogiri Enuklere at the FAMU-FSU College of Engineering, Department of Mechanical Engineering, Tallahassee, FL

ABSTRACT

Extending nanotechnology into the biomedical field is on the frontier of science. As such, employing proteins for this use is an innovative approach as micromotors and microelectronic devices, along with materials fabrication can be applied to important advancements of many scientific fields of study. Notably, utilizing proteins in this manner has led to medical discoveries. Proteins can be fashioned in a variety of shapes and sizes, ranging from the smallest unit of protein, the amino acid, to large macromolecules with many repeating units. This suggests that proteins have various properties which are dependent on their surrounding environment. Despite being sensitive to their own and to some environmental factors (e.g., heat), proteins are abundant. They can be found especially so in humans. Regardless, some proteins are known to exhibit characteristic properties which, if harnessed could lead to potential life-saving applications. In particular, RADA16l belongs to a growing family of proteins which can self-assemble and form nanofibers. These nanofibers have potential applications in tissue engineering and drug delivery systems. In this study, we aim to further understand the self-assembly process of RADA16l and analyze its nanofibers using different imaging techniques. This research provide insights into the dynamics of this system as well as imaging the peptide growth of RADA16l.

INTRODUCTION

To begin, RADA16l is composed of four repeating units of four peptides to make up a sixteen amino acid chain. Arginine, alanine, serine, and alamine (R-A-D-A) make up the repeating amino acid, respectively. A molecular model and conformation of this molecule is given by Fig. 1. On the other hand, Fig. 2 depicts a model of many RADA16l molecules upon self-assembly. Not much is understood on the dynamics of the spontaneous self-assembly of RADA16l. If studied, this protein could be engineered to form nanofiber scaffolds which have actually been shown to support growth of healthy cells and inhibit the growth of tumor cells. As a result, a study has been done where the application of RADA16l to a severed optic neuron in a hamster actually promoted healing. Once harnessed, RADA16l could lead to potentially life-saving applications. As such, Dr. Enuklere's lab is continuing to understand the self-assembly process of RADA16l and use this knowledge as a nanomaterial to control the rate of the peptide's self-assembly along with how many fibrils actually form and align with each other. This engineered peptide could then be applied to microchips, or nanoscale apparatus, as potential delivery devices.

OBJECTIVES

- Understand dynamics of peptide self-assembly and some of its self-assembling peptide applications
- Control and guided self-assembly (engineering) as per application to microchip...
- Other applications: regeneration (e.g., regeneration of severed optic neuron in a hamster)
- Develop protein nanofibers before, during, and after self-assembly mechanical brushing has been applied

METHODS

Scanning Electron Microscope Imaging – (SEM)

SEM was used because of its versatility in being able to image RADA16l in such small overall quantities (the smallest volume used about 30 µL) and because of its high image quality. The RADA16l used throughout this investigation was not synthesized by Dr. Paravastu, but instead supplied by BD Biosciences as one of their extracellular matrix molecules (ECMs). As such, the protein used throughout is the BD™ Puramatrix™ Peptide Hydrogel which is RADA16l kept in a tri buffer solution. RADA16l has been employed as a means to discern whether or not sample preparation methods are effective in disrupting the RADA16l’s extracellular matrix (ECM). Different preparation methods were employed in order to determine what applied perturbations would disrupt the hydrogel and then initiate self-assembly. Fig. 3 is a mixture of the RADA16l hydrogel in DMGS (commonly used solvent) and water. This mixture was made by pre-dissolving RADA16l in DMGS and then diluting that mixture 100-fold to produce a rather dilute mixture of still mostly water and little water. One should note that Fig. 1 depicts a RADA16l molecule with an average length of 6 nm while Fig. 3 shows 100 nmol of about 1 µm. Fig. 4 depicts a RADA16l sample prepared by sonication, filtering, then sonication again. Fracturing through sonication breaks weak chemical bonds (i.e., hydrogen and ionic bonds, and hydrophobic interactions), but not the covalent peptide bonds it is comprised of. Thus, sonication should allow reassembly of the mechanically dissociated individual peptides. By breaking up the hydrogel and then monitoring the nanofiber growth of RADA16l, the peptide’s fibril growth should be seen to be about 20-120 nm in length. Interestingly enough, through our preparation methods we did not observe similar lengths, in actuality, we saw larger lengths, 100 µm, as observed in Fig. 4. One should note that TEM images gave comparable results of small fibril lengths which are closer to nanoscaffold lengths as observed by Yoko and Zhang. On the other hand, Fig. 3 depicts a mixture of RADA16l in water and DMGS. This sample was prepared with hopes being able to disrupt the hydrogel. The dense nanofiber network seen here is similar to Fig. 5 (lengths of 6 nm) and is indicative of undissolved peptide which leads to the formation of a dense nanofiber network. The solution should be employed in further experiments such as HIF, a commonly used organic solvent. TEM is the best imaging method, the drying process in which the protein is subjected to also seems to affect fibril lengths but further investigation is necessary.

Transmission Electron Microscope Imaging – (TEM)

TEM imaging has been employed in order to see the fibrillar compaction of RADA16l as comparable results to SEM images of the same samples. TEM images a somewhat different form from SEM in that the images are taken in a different manner. Due to the way in which TEM images are acquired, the pictures received are “fatter” than SEM images. TEM images should support SEM images, but in this case fibrils of different lengths were observed. Fig. 6 depicts a 10-fold dilution of BD™ Puramatrix™ Peptide Hydrogel in water. The lengths in this image are on the same scale as those observed by Yoko and Zhang, while Fig. 5 depicts larger fibrillar lengths. These samples differ in their preparation by Fig. 6 is a dilute RADA16l samples while Fig. 5 is a sonicated sample. This alone is suspect and should be further investigated because more TEM images should be acquired before concrete conclusions are made.

CONCLUSIONS

Continued investigation and study on the peptide RADA16l are necessary in order to discern the dynamics of its self-assembly. As was stated previously, the benefits of introducing RADA16l as a potential inhibitor in regeneration of certain injured biological structures are invaluable and could potentially lead to engineered healing.

As future work, solid state NMR will be employed in order to discern what RADA16l’s molecular structure and conformation is in a given sample. By incorporating the NMR’s unique magnetics, state-of-the-art data will be acquired. Much solid-state work has yet to be done on this peptide, but could potentially lead to breakthroughs. As aforementioned, the conformation of this protein, as in many others, is important for designing and implementing the desired function in RADA16l. In other works, by determining what RADA16l’s internal structures conform to in a given environment (such as the acidity of the solution and the temperature of the protein) then being able to design this protein to self-heal and to form nanofiber networks is not far behind. Also, separate experiments with different solvates will be performed in order to determine what will dissolve RADA16l in order to facilitate the protein’s synthesis.

At this time, Dr. Paravastu’s laboratory is attempting to synthesize RADA16l so that pure protein may be employed for future experiments. This will enable the production of RADA16l as a hydrogel (for SEM, TEM, and implementation into microchips), a solid (for solid state NMR which requires solid protein), and a liquid (for absorbance studies to quantitatively monitor dynamic reassembly). Thus, the future of RADA16l holds promise and requires more study because of its far reaching benefits for the medical and nanomaterials community.

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REFERENCES