PEPTIDE CONJUGATION OF BRANCHED AMPHIPHILIC PEPTIDE CAPSULES

Baltazar Claro-Martinez

PEPTIDE CONJUGATION OF BRANCHED AMPHIPHILIC PEPTIDE CAPSULES

Baltazar Claro-Martinez, Susan Whitaker, John Tomich
Department of Biochemistry and Molecular Biophysics
Kansas State University

ABSTRACT

In recent years, nanocarrier cellular therapy has been a rapidly growing area for research in the treatment of malignant and infectious diseases – most notably cancer. Conventional cancer treatment has consisted of highly toxic, highly insoluble, untargeted delivery of drugs that kill both cancerous and healthy cells. Research in the Tomich lab consists of the synthesis of **B**ranched **A**mphiphilic **P**eptide **C**apsules (BAPCs), which are self-assembling peptide nanospheres composed of one or both of these branched peptide sequences: h_5 and h_9. These peptides possess similar molecular characteristics of phosphoglycerides but are synthesized chemically within the lab. Previous publications by the Tomich group have demonstrated that BAPCs are stabilized by hydrophobic interactions and hydrogen bonds. Here, we further explore the ability of BAPCs to retain their nanosphere shape and encapsulated solutes at varying DMSO (dimethyl-sulfoxide) concentrations (0%, 10%, and 25%) and pH (3.5, 7.5, 8.5) levels. Using this framework, we will attach a short peptide to the outside of the BAPCs using reversible disulfide linkages. This coupling will be followed using Ellman's reagent as a quantitative measurement for reduced versus oxidized cysteines and MALDI-TOF mass spectrometry. Data presented here will show which conditions most favorably contribute to the disulfide formation between the BAPC and the peptide in the presence of DMSO. Upon cellular uptake, the reducing environment of the cell's interior will release the peptide. With the changing face of diseases and medicine, the ability to attach and encapsulate molecules of interest onto and within BAPCs opens the door to many other therapeutic possibilities. This suggests that BAPCs can be an attractive biocompatible carrier for the delivery of various cancer therapy drugs.

BACKGROUND

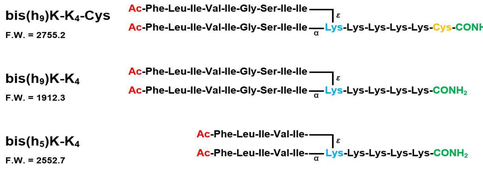


Fig. 1 Branched amphiphilic peptide sequences

BAPCs have demonstrated great stability while having fine tune-availability. These amphipathic particles self assemble and form a functional membrane like bilayer; Fig. 2, 3 – composed of a hydrophobic outer membrane and a hydrophilic inner membrane. Their formation occurs when two (15-20 residue) poly-cationic peptides are mixed. Fig. 1 shows the chemical structure of three usable peptides. The 'blue' labeled lysine on all represents a 90° segment common in diacyl phospholipid.

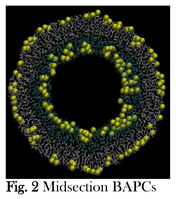


Fig. 2 Midsection BAPCs

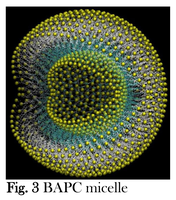
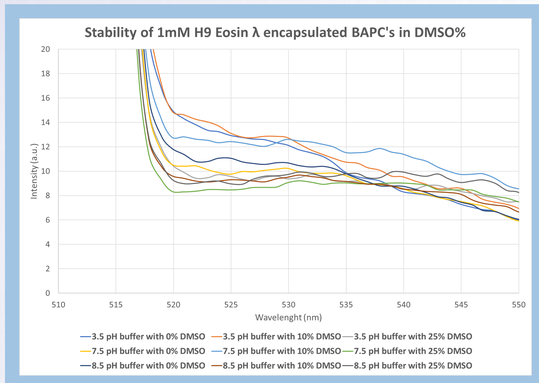
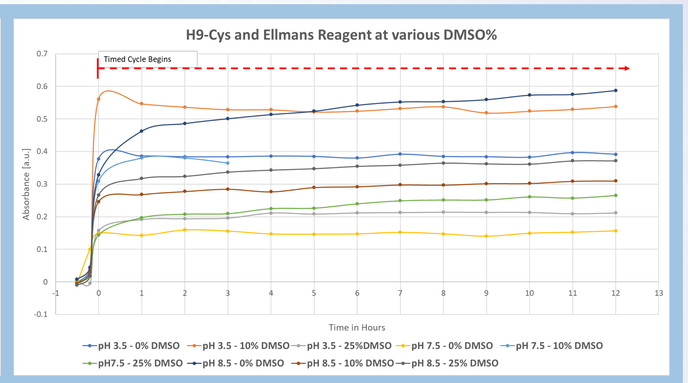


Fig. 3 BAPC micelle

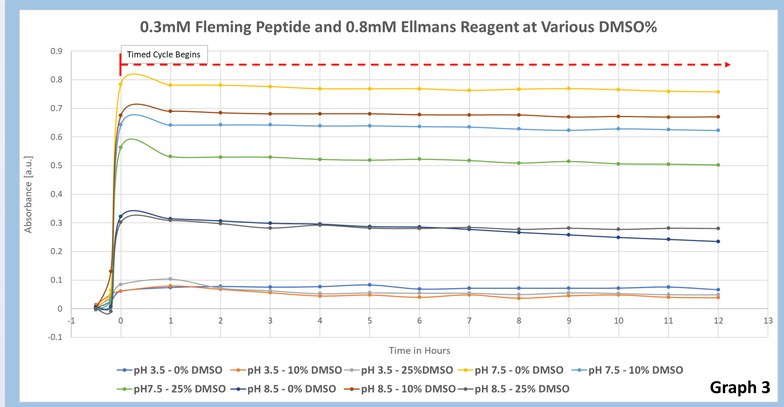
RESEARCH & RESULTS



Graph 1: Details Eosin-BAPCS encapsulation and fluoresce.

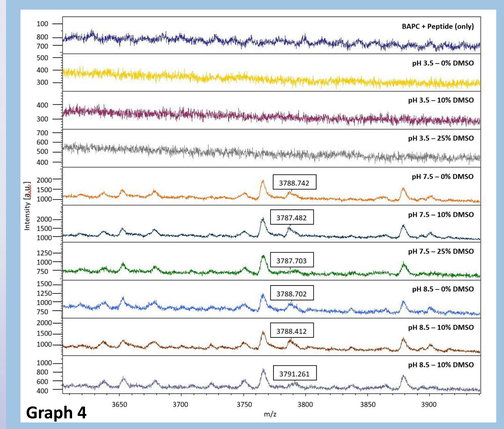


Graph 2: H9-cysteine and Ellmans reagent under varying DMSO concentrations.



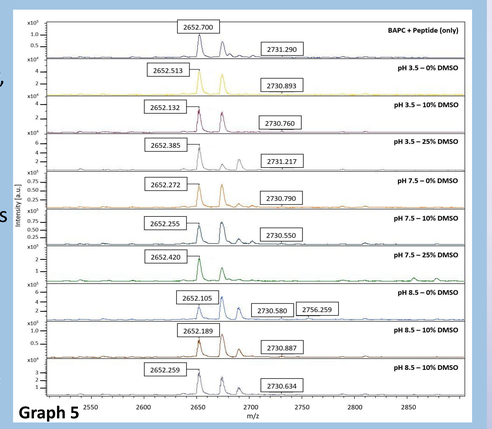
Graph 3

Graph 2 and 3: Demonstrates three different constituents which make the graph. First, the initial points on the graph belong to the blank absorbances at 412nm for all our samples collected.
The next set of data points emphasize the absorbance of our Ellmans reagent with only pH buffer conditions (3.5,7.5,8.5). Lastly, beginning at x=0, the peptides (H9-Cys and Fleming) are introduced to the Ellmans and pH buffer at varying concentrations of DMSO at time intervals of 1hour for 13 cycles.



Graph 4

Graph 4 and 5: Show data from the MALDI-TOF mass spectrometry, peaks of interest are emphasized by being boxed. Here, graph 4 shows that an environment of pH 7.5 is the minimum requirement for formation. Data presented on these graphs did not include the addition of Ellmans reagent.



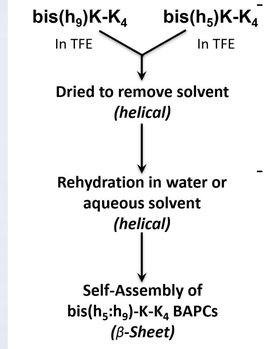
Graph 5

DISCUSSION/INTERPRETATION

The first experimental procedure, regarding BAPCs containing the dye Eosin (2.1mM), was observed over a set temperature where no fluorescence discrepancies were observed. Concluding that these BAPCS did not rapture and release the Eosin dye. Knowing that BAPCS could retain Eosin, we then decided to run Eosin encapsulated BAPCS under 0%, 10%, and 25% DMSO – for all pH values of 3.5, 7.5, and 8.5 respectively. Again, we were able to demonstrate that the BAPC was able to retain its shape even at 25% DMSO. Graph 2 and 3 describe the H9-Cys peptide and Fleming peptide, both under DMSO condition and Ellmans reagent. Here we are able to see how the Ellmans reagent affects the disulfide bonds and interacts with our peptide. Lastly, from data collected on the MALDI-TOF we were able to detect noticeable differences not just from pH, but from DMSO variation at our peak of interest ~3788.

METHODOLOGY

- Peptides were synthesized in lab by solid phase Fmoc chemistry with clear amide being the starting resin.



  - Concentrations were determined by dissolving in 2,2,2-trifluoroethanol and running through the Varian 50 Bio UV-Spectrophotometer (accounting for phenylalanine).
  - The CARY Eclipse fluorescence spectrophotometer was used to measure fluorescence. Beginning with a 500 nm excitation wavelength, followed by the observation from 505 to 698nm using a 0.3 cm quartz cuvette.
- During the hydration of the BAPCs, 1mM of each peptide was combined and Eosin Y was added in a drop-wise manner.
- After hydrating for ~45 minutes, each solution was filtered and centrifuged at 14,000 x g, in a weight cut off Eppendorf filter of 30kDa to removed any external dyes.
- Filters were inverted and centrifuged at 2,000 x g, to collect the BAPCs.
- The use of the Varian 50 Bio, was used for multiple scans.
- Samples were ran again under no Elllmans reagent and were spotted under MALDI-TOF.
- Peaks of interest where further analyzed via mass spectrometry and graphed.

FUTURE STUDIES

Our data reveled that a more basic solution is preferred in order for reduction to take place. This being the case, future studies would consist of testing different ions and their affects on BAPCs stability. Other future work includes the identification of certain unknown peaks in the MALDI-TOF analysis, as some adducts would appear; such as sodium and potassium. Recognizing the ability of water to permeate through the bilayer has also given rise to other possible applications – such as water filtration in developing countries, to the possible improvement of current reverse osmosis techniques. The most important future plan, however, is to successfully modify this delivery vehicle in order to begin clinal trials.

ACKNOLEDGEMENTS

THANK YOU TO:
- Susan Whitaker for running and collecting MALDI Samples.
- Robert Small for preparing and aiding in the analyzing of samples.
- Jennifer Coats for assisting in much of the peptide synthesis.

Sukthankar P, Gudlur S, Avila LA, Whitaker SK, Katz BB, Hiromasa Y, Gao J, Thapa P, Moore D, Iwamoto T, Chen J, Tomich JM. Branched oligopeptides form nanocapsules with lipid vesicle characteristics. Langmuir. 2013; 29(47):14648–14654.

Gudlur S, Sukthankar P, Gao J, Avila LA, Hiromasa Y, Chen J, Iwamoto T, Tomich JM. Peptide nanovesicles formed by the self-assembly of branched amphiphilic peptides. PLoS ONE. 2012; 7(9):e45374.