"The Isolation from *E. coli* DH5α and Radiolabeling with Indium-111 Of 6His-Annexin V for Use in the Improvement of Apoptosis SPECT Image Resolution in Diseased Tissues"

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BACKGROUND

Apoptosis

A programmed sequence of intricately controlled events that lead to the death and ridding of cells while avoiding the release of harmful substances. Diseased states exhibit apoptosis.

Phosphatidylserine

The cellular membrane is asymmetric. Phosphotidylserine, a phospholipid, is normally found in the intracellular layer of the membrane. An early sign of apoptosis is the inversion of the cellular membrane's symmetry, and therefore an introduction of phosphotodylserine to the extracellular environment. Detection of this phospholipid by use of nuclear imaging can have important implications in the early diagnosis of diseased states.

6His-Annexin V

6His-Annexin V is a human protein that has a strong affinity for phosphatidylserine. Radiolabelling 6His-Annexin V would therefore allow the detection of apoptosis using nuclear imaging technology.

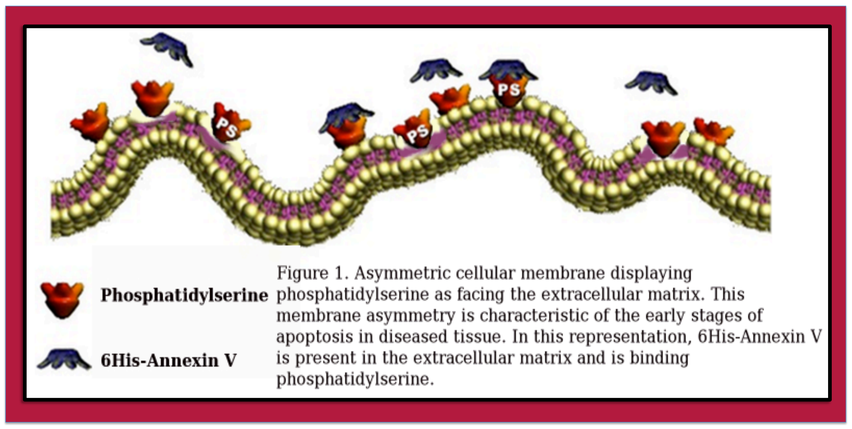
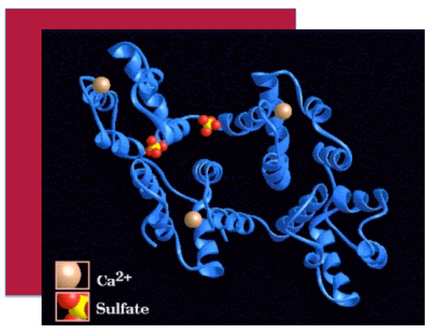


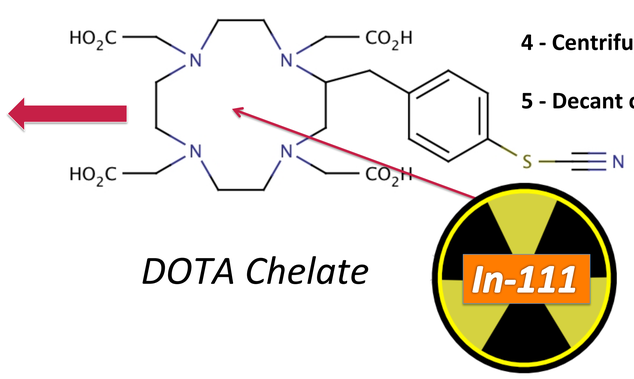
Figure 1. Asymmetric cellular membrane displaying phosphatidylserine as facing the extracellular matrix. This membrane asymmetry is characteristic of the early stages of apoptosis in diseased tissue. In this representation, 6His-Annexin V is present in the extracellular matrix and is binding phosphatidylserine.

Radiochemistry

DOTA is a chelate molecule that can bind lysine residues, as well as form coordination bonds with radioactive indium-111. 6His-Annexin V contains 22 Lysine residues. DOTA can therefore permit the radiolabelling of 6His-Annexin V with indium-111, which will allow for the protein's use in nuclear imaging technology.



Annexin V



DOTA Chelate

SPECT Imaging

Single Photon Emission Commuted Tomography Imaging is a type of nuclear imaging technology. It detects patterns of gamma ray emission and can create an image from the manipulation of these patterns using complex mathematics. Annexin V, a close relative of 6His-Annexin V, is currently used for SPECT imaging of apoptosis in diseased state tissues. Annexin V is the only form of the protein available on the market.

SPECT Scanner

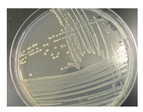
Annexin V

Annexin V is a close relative of 6His-Annexin V. It is the only form of the protein available on the market. Unfortunately, Annexin V is not economical, it does not provide optimal image resolution, and its use in present day nuclear imaging is limited to the diagnosis of only a select number of diseases. We hope the development of 6His-Annexin V will resolve these issues.

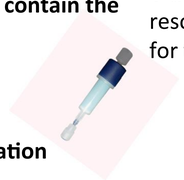
OBJECTIVE

The objective of this project is to domesticate the development of 6His-Annexin V, a derivative of Annexin V. We hope that this will reduce the cost of the protein's use and therefore increase the amount of it that can be used in our research. If 6His-Annexin V becomes more available for our research use, we will be able to determine a way of improving its image resolution, and also find more diseases of which it can be used to diagnose.

METHOD



1 - Purchase genetically engineered *E. coli* DH5α that contain the gene for human 6His-Annexin V



2- Force *E. coli* DH5α to overexpress 6His-Annexin V

3 - Lyse the cells using a lysozyme solution and sonication

4 - Centrifuge solution containing cell debris and protein of interest

5 - Decant crude lysate from cell debris pellets that resulted from centrifugation

6 - Separate 6His-Annexin V from crude lysate solution using ion exchange column chromatography (cationic nickel based column)

7 - Analyze purity of isolated protein using High Performance Liquid Chromatography (HPLC)

RESULTS

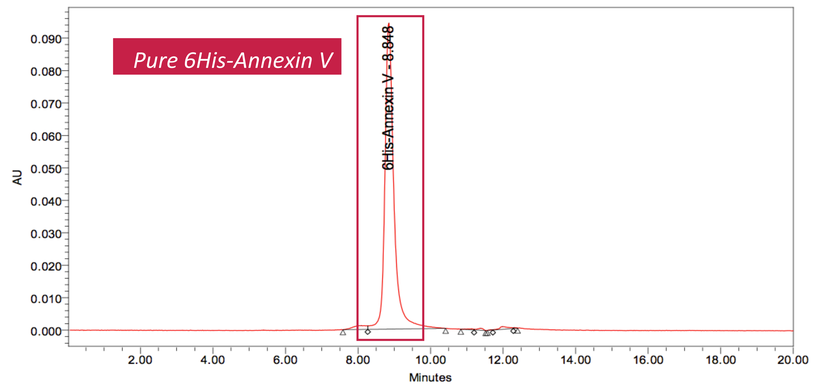


Figure 2. HPLC chromatogram of the 6His-Annexin V solution resultant from the isolation procedure performed in order to obtain the protein. This chromatogram displays a distinct peak at approximately 8 minutes. The characteristic peak of 6His-Annexin V appears at approximately 8 minutes. The product obtained is therefore pure.

A solution of pure 6His-Annexin V was successfully obtained from the isolation procedure performed on *E. coli* DH5α (please see figure above). The concentration of the protein in this solution was calculated to be 3.96mg/mL.

CONCLUSION

The domestic development of 6His-Annexin V was successful. The protein solution obtained was pure and had a large concentration of 3.96mg/mL. The market cost of a similarly concentrated solution of Annexin V is approximately $800.00. It cost only $20.00 to produce our own solution of 6His-Annexin V. We can therefore conclude that our own production of the protein will allow for greater exploitation of its use throughout our research in the future without worry of economical constraints. This will allow us to develop a way to improve the image resolution obtained by the use of the protein in nuclear imaging, and find more diseases for which it can be used to diagnose.

THE FUTURE

The figure displayed below is a SPECT image of a rat heart that underwent a myocardial infarct. Indium-111 radiolabelled Annexin V was used to display the cells of this heart that were not receiving enough blood and therefore beginning to undergo apoptosis. The green represents the Annexin V-labeled cells. This figure is an example of the poor image resolution that Annexin V provides. The labeled areas in the figure are not as distinct as we would like them to be. We hope that the ample amount of 6His-Annexin V that we now have available to us will allow the future improvement of this image resolution. We also hope that it will allow us to discover more diagnostic applications for the protein.

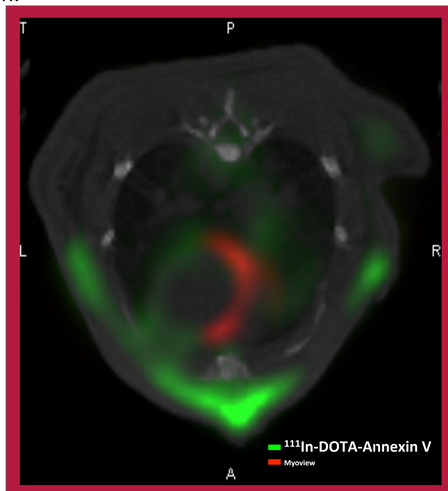


Figure 3. SPECT image of an infarcted rat heart. The green areas represent the cells that are undergoing apoptosis due to a lack of their perfusion. The red area is labeled with a compound called myoview. This area represents the part of the heart that is receiving proper perfusion. This SPECT image was coupled with a CT scan to obtain visualization of the rest of the heart's structure.