SYNTHESIS OF CROTAMINE GOLD NANOPARTICLES AND CHARACTERIZATION OF THEIR BINDING ACTIVITY

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BACKGROUND

Crotamine is a protein found in the venom of the rattlesnake Crotalus durissus terrificus, and has been shown to bind DNA and negatively-charged cell membranes. The biological activity of crotamine has potential as an anticancer agent as previous studies have demonstrated its selective preference for binding cancer cells compared to healthy cells.

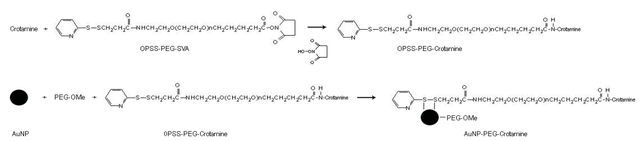
An effort to make use of this targeting action involves linking crotamine to gold nanoparticles. The central gold particle can serve as a hub to attach additional targeting proteins or other medical compounds into a single combined unit of delivery, forming a multifunctional drug.

RESEARCH GOALS

- Synthesis of gold nano-particle linked crotamine
- Evaluation of the binding characteristics of gold linked crotamine

METHODOLOGY

Figure 1 Process for the formation of gold linked crotamine, performed in two steps.



Synthesis of Crotamine- PEG intermediates

- Polyethyleneglycol (PEG) used as the link between protein and gold
- Modified version of PEG with orthopyridyldisulfide end (OPSS) and succinimidy lvalerate end (SVA)
- Sodium bicarbonate used to remove succinimidyl valerate (SVA) from PEG
- Mixed with crotamine in PBS buffer
- Ratios of 1:1 , 1:6 and 1:4 crotamine:PEG were used to determine the ideal way to efficiently drive the reaction

SDS polyacrylamide gel electrophoresis

- Yields of first reaction were compared with electrophoresis
- 12% hand made and 15% premade acrylamide gels

Azure A dye competition titration

- The dye azure A absorbs light at 632nm.
- This absorbance is decreased upon binding of the protein heparin
- Crotamine delays the interaction of heparin and azure A
- Aliquots of heparin were added to azure A until the peak is extinguished
- In the presence or absence of either free crotamine or crotamine bound to peg

Formation of Gold Nano-particles

- $HAuCl_4$ and sodium citrate solutions mixed while heated
- Vary the ratio of reactants to influence the size of the particles
- Quality of products was assessed with dynamic light scattering

INTERMEDIATE PRODUCTS

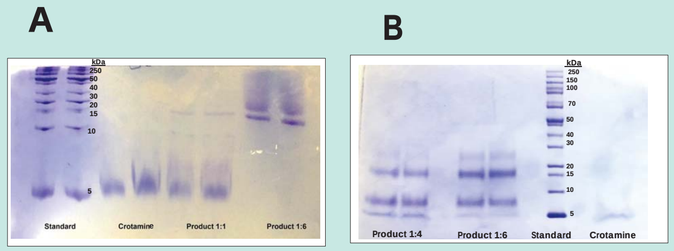


Figure 3

A) The 1:1 ratio left a strong band representing free unreacted crotamine, while the 1:6 ratio indicates a greater amount of heavier products and little to no free crotamine.
B) Both 1:4 and 1:6 show a presence of heavy products but the 1:6 ratio shows a less visible band at the weight of crotamine, than the 1:4 ratio.

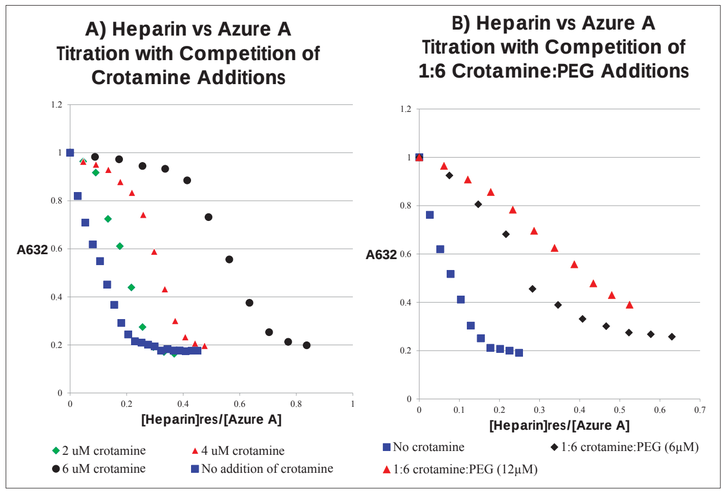


Figure 4

A) In the presence of pure crotamine, the interaction between heparin and azure A is slowed. The more crotamine added, the more heparin needed to effect the absorbance of azure A, indicating a competing interaction between the crotamine and the heparin.
B) The same titration was performed with the synthesized crotamine-PEG products. The presence of crotamine-PEG has a similar inhibiting effect on the reaction between azure A and heparin, as crotamine-PEG is also able to competitively interact with heparin. This shows that while bound to PEG, crotamine retains a portion of its binding ability .

SYNTHESIS OF GOLD PARTICLES

Dynamic Light Scattering

	PDI	Size by number	Size by volume
Gold Nano-Particles	0.049	15.2 nm	17.5 nm
Crotamine-PEG	0.556	3.3 nm	4.2 nm
Gold linked crotamine	0.243	24.1 nm	31.8 nm

DISCUSSION AND CONCLUSIONS

Crotamine PEG reactions

- Multiple adducts are formed as can be seen by multiple bands in the SDS-PAGE gels in figure 3
- Possibly a variable number of PEG molecules can bind to a single protein
- The band sizes were consistent through out the reactions indicating the production of similar products each time
- Varying amounts of product based on the ratio of reactants
- Large ratios of PEG to crotamine were required to obtain an efficient yield of product

Absorbance titrations with heparin and azure A

- As shown in figure 4 the presence of both pure crotamine and crotamine-PEG interferes with the heparin-azure A interaction
- Crotamine and crotamine-PEG are both able to interact with heparin

Gold Linked Crotamine

- Gold nano-particles were successfully synthesized with the target size of 15 nanometers and were consistently uniform
- Crotamine PEG intermediates show inconsistent sizes in both PAGE and DLS
- Combination of the Gold nano-particles with the crotamine-PEG adducts results in larger particles that correspond to a gold particle surrounded by crotamine-PEG, as expected

FUTURE EXPERIMENTS

Experiments to assess the characteristics of the gold linked crotamine are ongoing. Future experiments will involve repeating the dye competition assay as well as utilizing chromatography columns to measure the binding abilities of the product. Also of interest are new, methods of synthesis and purification that may prove more efficient.

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