NANOCER CNSI UCLA

Analysis of Vault Proteins with Engineered Lead-binding

Derrick S. Hsu[1], Benny C. Ng[2], Leonard H. Rome[3] and Sarah H. Tolbert[2]

1. Department of Materials Science and Engineering, University of Pennsylvania
2. Department of Chemistry and Biochemistry, University of California, Los Angeles
3. Department of Biological Chemistry, David Geffen School of Medicine

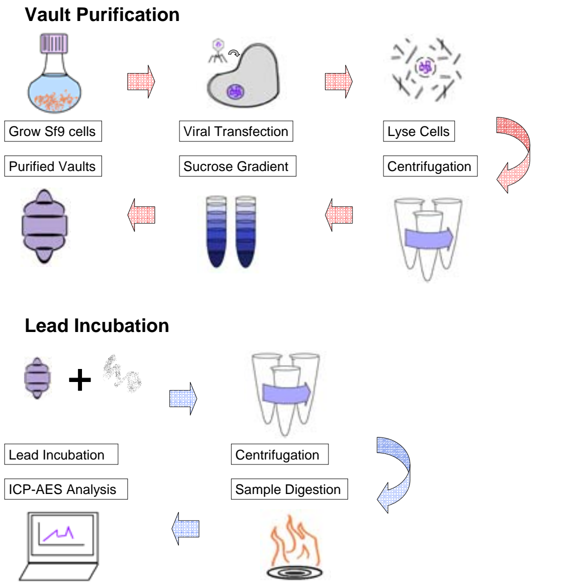
Abstract

Vaults are naturally-forming ribonucleoprotein particles that are present in most eukaryotes, including humans. They consist of a hollow barrel structure composed of major vault proteins (MVP). On the inner surface of the barrel, there is an INT binding domain, which can be engineered to incorporate functional proteins into the vault interior. In this research, *pbrR* and *pbrD*, lead-binding proteins isolated from bacteria *Rastonia metallidruans*, fused with mINT sequence, then attached to the INT domain and incorporated into the vault cages. These fusion vaults were then analyzed for binding efficiency. Along with cysteine-tagged MVP vaults (cpMVP), the lead-binding proteins were expressed in SF9 insect cells using baculovirus transfections. The cpMVP vaults were then incubated with the mINT-pbrR or mINT-pbrD fusion proteins to form the fusion vaults. In order to test binding efficiency, the fusion vaults were incubated in a lead acetate solution. Lead-bound vaults were subsequently extracted using pelleting method. Samples were analyzed for final lead concentration using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) and then back-calculated to determine the amount on lead bound to vaults.

Materials and Methods

Vault Purification

Lead Incubation



Results

Comassi Stain

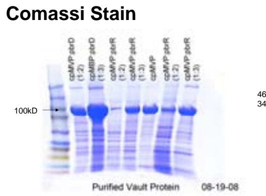


Figure 1. Comassi Staining of cpMVP vaults combined with fusion lead-binding proteins (mINT-pbrD and mINT-pbrR). The gel shows presence of vault particles (~100 kDa) and fusion lead-binding proteins (~34 kDa for mINT-pbrR and 46 kDa for mINT-pbrD).

Western Blot

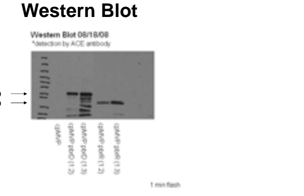


Figure 2. Western Blot of cpMVP and lead-binding protein fusion vaults. ACE antibodies were used to detect mINT sequence on fusion lead-binding proteins. The film shows successful incorporation of fusion lead-binding proteins into cpMVP vaults.

TEM Imaging

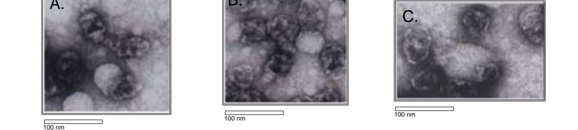
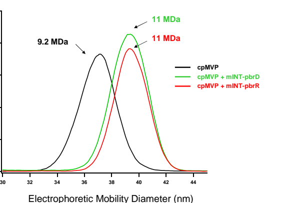


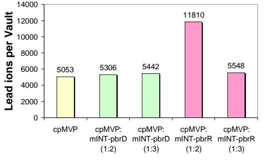
Figure 3. Transmission Electron Microscope images of (A.) cpMVP vaults; (B.) cpMVP vaults with mINT-pbrR; (C.) cpMVP vaults with mINT-pbrD. In (A.) the vaults exhibit a narrow body shape, whereas in (B. and C.) the vault particles are slightly wider to indicate incorporation of fusion lead-binding protein.

ESI-GEMMA Analysis

Graph 1. Comparison of masses measured by Electrospray ionization gas-phase electrophoretic mobility molecular analysis (ESI-GEMMA). The graph shows a shift in mass in the cpMVP samples bound with fusion lead-binding proteins. The analysis determines there are 53 copies of mINT-pbrR and 39 copies of mINT-pbrD incorporated per vault particle.



ICP-AES Analysis



Graph 2. Comparison of amount of lead bound after incubation with lead acetate and pelleting. Detection of lead was conducted using ICP-AES. The data suggests that most of the lead binding is non-specific.

Conclusions

- The vault purification protocol used yields adequately purified vault particles (as shown in the Comassi stains). However, there are still residual amounts of undesired proteins in purified samples.
- There is successful incorporation of fusion lead-binding proteins with the cpMVP vaults using vault purification protocol (as shown in the Western blots).
- ESI-GEMMA mass spectroscopy confirms the incorporation of fusion lead-binding proteins into vaults. In addition, analysis determines approximately 53 copies of mINT-pbrR and 39 copies of mINT-pbrD are bound to each vault particle.
- No concrete conclusion can be established based on ICP-AES lead detection for fusion vaults via pelleting method. Though, there is strong evidence to suggest nonspecific binding to cpMVP vaults.

Future Work

- Repeat vault purification protocol to strive for higher yields and greater purification.
- Attempt stringent washing procedure with EDTA to isolate any specific binding of fusion vaults to lead.
- Experiment with different conditions (lead source, concentration of lead …etc.) for lead incubation.

Acknowledgements

This project was supported by the National Science Foundation REU Site: Nanosystems Chemistry and Engineering Research (NanoCER) program (CHE:0649323) and the California NanoSystems Institute. In addition, I would like to acknowledge the following people for their help and support in this research:

- Professor Robin Garrell
- Professor Leonard Rome
- Professor Sarah Tolbert
- Valerie Kickhoefer
- Shirley Lomeli
- Benny Ng
- Hedi Roseboro
- Laurie Ultan-Thomas