Toward Controlled Conformational Change of Self-Assembled Vault Nanocapsules in Solution

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INTRODUCTION

Vaults are self-assembled ribonucleoprotein nanocapsules found in nearly all eukaryotic cells that consist of multiple copies of an untranslated RNA and 3 proteins (major vault protein, VPARP, and TEP1) [1, 2]. The 96 copies of major vault protein (MVP) account for about 70% by mass of the entire vault particle, and stable vault particles can self-assemble from MVP alone in solution [3]. Vault particles have approximate dimensions of 72.5 by 41 nm, and an apparent molecular weight of 13 x 10^6 daltons [1, 3]. The hollow interior volume is about 5 x 10^4 nm^3 and is large enough to accommodate two ribosomes [4]. Previous studies have shown that vaults dissociate into halves that open further into two flower-like structures on polylysine-coated mica surfaces [3]. Our goal is to discover methods for reversible vault assembly/disassembly thereby enabling application of these nanocapsules in drug delivery and encapsulated materials synthesis.

METHODS

Recombinant vaults were overexpressed in Sf9 insect cells using a baculovirus system [5]. All the experiments described below use a CP-MVP recombinant vault, which contains 96 copies of major vault protein modified at the N-terminus with a 12 amino acid peptide from the metal binding protein, metallothionine, which contains 4 cysteine residues. CP-MVP appears to be the most stable recombinant vault yet produced with minimal variations in size, shape, and conformation [1].

Multi-angle laser light scattering (MALLS) was used to investigate the conformational changes of recombinant vaults in solution. MALLS can be used to determine the size, conformation, and molar mass of macromolecules and macromolecular assemblies in solution without the use of size standards [6-8]. A 5 mW He-Ne laser at 632.8 nm is impinged on the macromolecule solution, and time-averaged scattered light intensity is measured using 18 photodiodes at different angles around the sample compartment. Using the light scattering theory derived by Zimm and Debye, molecular weight and radius of gyration (information about shape) are estimated from Zimm plots [6-9]. Figure 1 is an illustration of the MALLS instrument setup.

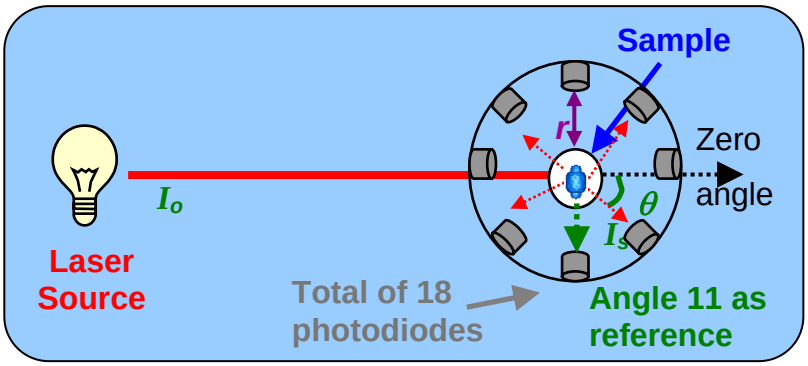

Figure 1. An illustration of the MALLS instrumental setup.

RESULTS AND DISCUSSION

Conformational Changes of CP-MVP with pH Observed by MALLS

The first set of studies was conducted with vaults in 20 mM MES at pH 6.5. Light scattering data was gathered and a Zimm plot (representative of several studies) such as that below (Figure 2) was constructed. The molecular weight calculated from the plot was (9.1 ± 0.3) x 10^6 g/mol, while the radius of gyration was 28 ± 2 nm. The expected M_W of CP-MVP is approximately 9.3 x 10^6 daltons calculated from the weight of individual MVP with the addition of CP-tag, which agreed with our MALLS result. Based on the theoretical models of radius of gyration for intact vaults, the calculated R_G should be in the range between 25 to 30 nm. Therefore, the radius of gyration calculated by this particular run was within the range of estimations, confirming the presence of intact vaults in the pH 6.5 sample.

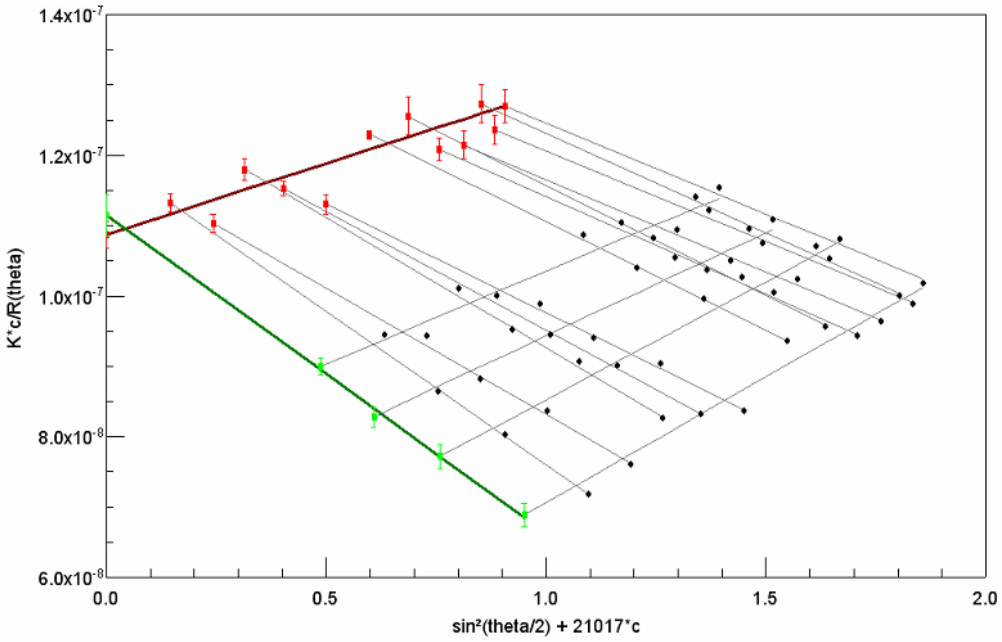

Figure 2. Zimm plot for a representative MALLS experiment for vaults at pH 6.5. The R_G was 28 ± 2 nm and M_W was (9.1 ± 0.3) x 10^6 g/mol. Some of the noisy low angles (1-5, 7) were not included to construct this Zimm plot.

A light scattering study was undertaken to determine if a vault conformational change is induced at lower pH. A CP-MVP sample was introduced into a large reservoir of citrate phosphate buffer at pH 3.4. Light scattering data at four vault concentrations was collected and Zimm plots were constructed. In a representative study as shown in Figure 3, the molecular weight was found to be (6.7 ± 0.6) x 10^6 g/mol, and the radius of gyration was 51 ± 5 nm. Both of these quantities suggest a change in conformation; the decrease in molecular weight may represent the CP-MVP disassembling into two halves, and the increase in radius of gyration may represent the opening up of the vault "petals" into a flower-like structure. Although the trend of increased radius of gyration and reduced apparent molecular weight with reduced pH was observed reproducibly for all vault samples, the absolute values of the radii and molecular weights varied substantially. We believe this is due to many different factors including the heterogeneity in sizes in our vault preparation (e.g., presence of half vaults in pH 6.5 sample), aggregation observed in pH 3.4, and the dynamic structure of vaults at neutral pH [10].

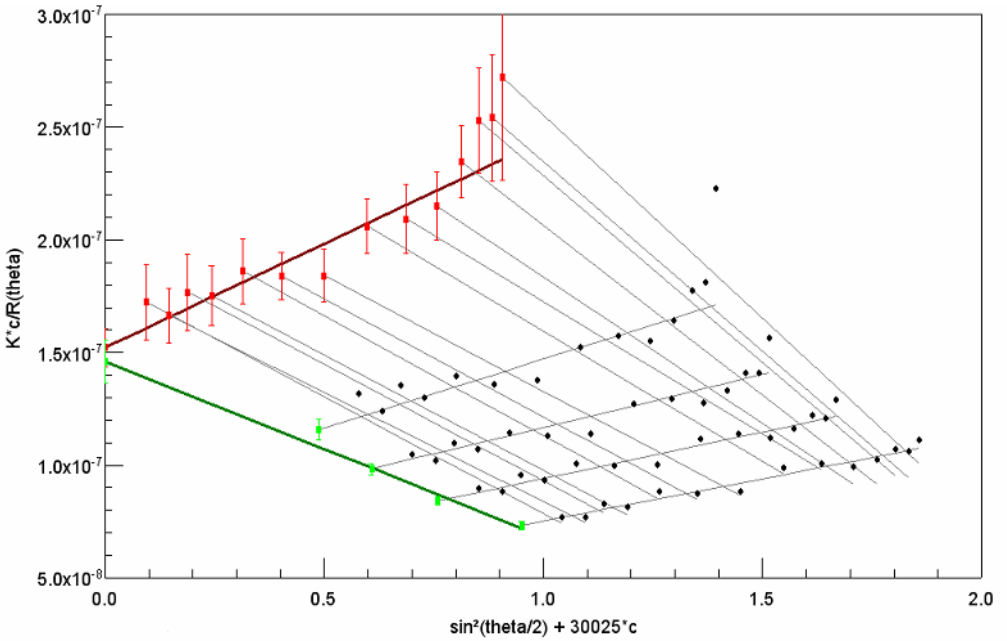

Figure 3. Zimm plot for a representative MALLS experiment for vaults at pH 3.4. The R_G was 51 ± 5 nm and M_W was (6.7 ± 0.6) x 10^6 g/mol. Some of the noisy low angles (1-3, 5) were not included to construct this Zimm plot.

Conformational Changes of CP-MVP with pH Observed by TEM

Negatively stained transmission electron microscopy (TEM) images of the CP-MVP at pH 6.5 and 3.4 were obtained. CP-MVP vaults generally exhibit nearly uniform structure in 20 mM MES at pH 6.5. Figure 4 (a) shows the closed vault structure under neutral pH conditions. A small amount of aggregation and half vaults are observed, which may be responsible for the variation of molecular weights and radii of gyration obtained from MALLS. CP-MVP vaults exposed to pH 3.4 also were examined using TEM as shown in Figure 4 (b). According to the scale in the figure, the particles have the dimensions of half vaults. Although most of them do not reveal the flower-like structures described previously, many half vaults remain connected

as pairs. The pair of half vaults would also give rise to the increased radius of gyration observed by MALLS. From the TEM images, we are confident that the CP-MVP vaults disassemble when treated at pH 3.4. Some opened vaults with flower-like structures are faintly visible in TEM images, although previously published flower-like structure images were prepared using cryoelectron and freeze-etch microscopy.

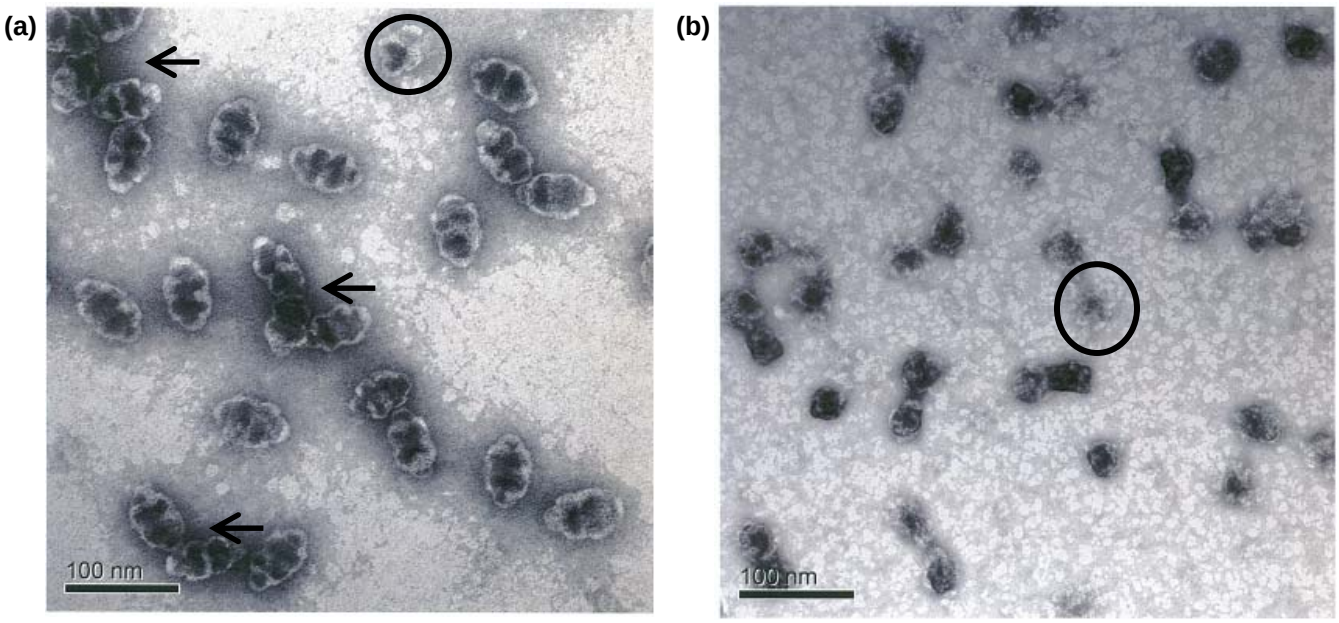

Figure 4. (a) TEM image of intact CP-MVP in 20 mM MES at pH 6.5. The circle indicates a possible half vault. The arrows (→) point at the regions of aggregation. (b) TEM image of CP-MVP half vaults in citrate phosphate buffer at pH 3.4. The circle indicates a possible half vault opening into flower-like structure.

Cross-linking of CP-MVP Using Sulfhydryl Cross-linking Agents

In addition to the dramatic conformational changes of vaults in response to pH, vault structure was found to be dynamic in neutral pH solution as well [10]. In fact, relatively large proteins of 100 kDa or more have ready access to the vault interior. We have proposed the use of sulfhydryl cross-linking agents (e.g., BMH (bismaleimidohexane), BMOE (bis-maleimidoethane), and homobifunctional maleimide-PEG) to link the cysteine residues of CP-MVP vaults to give a more rigid structure. Zimm plots generated for cross-linked CP-MVP seemed less noisy than uncross-linked CP-MVP, implying a more homogenous size in the cross-linked vault sample. TEM images also showed individual half vaults at smaller dimension than uncross-linked half vaults at pH 3.4, suggesting a more rigid half vault structure. Based on the interpretation of MALLS data and TEM image analysis, cross-linked CP-MVP vaults appeared more stable. However, no whole vaults were observed when cross-linked vaults were exposed to pH 3.4 condition, suggesting that cross-linking only occurs within individual halves and not between halves. Additional work is underway to cross-link reversibly the vault halves such that these nanocapsules may be used effectively as drug delivery packages or as compartments for nanomaterial synthesis.

REFERENCES

1. Mikyas, Y., et al., *Cryoelectron Microscopy Imaging of Recombinant and Tissue Derived Vaults: Localization of the MVP N Termini and VPARP.* Journal of Molecular Biology, 2004. **344**(1): p. 91-105.
2. Kedersha, N. L., et al., *Vaults. II. Ribonucleoprotein Structures Are Highly Conserved among Higher and Lower Eukaryotes.* Journal of Cell Biology, 1990. **110**: p. 895-901.
3. Kedersha, N. L., et al., *Vaults. III. Vault Ribonucleoprotein Particles Open into Flower-like Structures with Octagonal Symmetry.* Journal of Cell Biology, 1991. **112**(2): p. 225-235.
4. Kong, L. B., et al., *Structure of the vault, a ubiquitous cellular component.* Structure, 1999. **7**(4): p. 371-379.
5. Stephen, A. G., et al., *Assembly of Vault-like Particles in Insect Cells Expressing Only the Major Vault Protein.* The Journal of Biological Chemistry, 2001. **276**(26): p. 23217-23220.
6. Tanford, C., *Light Scattering*, in *Physical Chemistry of Macromolecules*. 1961, John Wiley & Sons: New York. p. 275-316.
7. Zimm, B. H., *The Scattering of Light and the Radial Distribution Function of High Polymer Solutions.* The Journal of Chemical Physics, 1948. **16**(12): p. 1093-1099.
8. Harding, S. E. and Jumel, K., *Light Scattering*, in *Current Protocols in Protein Science*, J.E. Coligan, et al., Editors. 1998, John Wiley & Sons: New York. p. 7.8.1-7.8.14.
9. Doty, P. M., Zimm, B. H., and Mark, H., *An Investigation of the Determination of Molecular Weights of High Polymers by Light Scattering.* The Journal of Chemical Physics, 1945. **13**(5): p. 159-166.
10. Poderycki, M. J., et al., *The Vault Exterior Shell is a Dynamic Structure that Allows Incorporation of Vault-Associated Proteins into its Interior.* manuscript submitted.