Investigating Antibody Access to Adsorbed Protein Nanocapsule Interiors Using the Quartz Crystal Microbalance and Surface Plasmon Resonance

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INTRODUCTION

Vaults are nanoscale, ribonucleoprotein capsules (41 nm × 41 nm × 72.5 nm [1]) comprised primarily of 96 self-assembled copies of one 96 kDa protein, termed MVP (major vault protein). When deposited on polylysine-coated mica and imaged using cryoelectron microscopy, vaults appear to "open" into flower-like structures with eight rectangular 'petals' [2]. Upon closer examination, each 'flower' consists of a central ring with hooks that attach each petal to the center. The flowers are usually seen in pairs, suggesting that each whole vault is composed of two flowers, folded so that the ends of their petals touch. It is likely that vaults in cells open and close in response to cellular signals, reversibly encapsulating and releasing their contents. However, whether vaults open into flower-like structures *in vivo* is unknown.

The biological function for vault nanocapsules, which are ubiquitous intracellular components of eukaryotes, is unknown; yet they may prove useful for drug delivery and for compartmentalized materials encapsulation. Our aim is to design mechanisms for reversible vault assembly/disassembly in order to control the encapsulation and release of materials. Whole vaults self-assemble from MVP subunits with fused peptide tags which have been cloned and overexpressed [3], thereby providing an attractive system for the study of biological self-assembly and a potentially versatile platform for biomaterials design.

Using fluorescence, fluorescence quenching, multi-angle laser light scattering (MALLS), the quartz crystal microbalance (QCM) and transmission electron microscopy (TEM), we have previously shown that vaults disassemble into halves when exposed to pH values below pH 4.0 [4]. Current studies are designed to investigate molecular access to the interiors of vaults that have been adsorbed onto modified quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) substrates, and opened by exposure to low pH. Substrates consisted of a self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid (MUA) on gold. In an effort to demonstrate targeted material encapsulation within vaults, thereby exploiting their use as nanocarriers, we have employed the use of anti-vsvg antibodies which have specific affinity for vsvg peptide tags internalized within the vault waist. In vsvgMVP vaults, each MVP is tagged with the C-terminal 11 amino acid sequence of vesicular stomatitis virus glycoprotein, which is recognized by the anti-vsvg antibody. cpMVP vaults, in which each MVP is tagged at its N-terminus with a 12 amino acid peptide containing 4 cysteine residues which help to stabilize the vaults, should not be recognized by the anti-vsvg antibody and are therefore used as a control.

EXPERIMENTAL

Quartz Crystal Microbalance

The use of the quartz crystal microbalance (QCM) is based on the converse piezoelectric theory whereby applying an oscillating electric potential across the non-conducting quartz crystal surface propagates an acoustic wave within the surface which has a certain resonant frequency. In a liquid environment, the addition of "wet" mass (here, protein plus bound water) to the surface damps the oscillation, resulting in decreasing oscillation frequency.

Surface Plasmon Resonance

Surface plasmon resonance (SPR) is an optical technique where at certain conditions, light that is totally internally reflected at a glass/gold interface leaks an electric field which is absorbed by electrons within the gold layer, causing a "dip" in the intensity of the reflected light. The position of this dip is very sensitive to changes in the optical properties (i.e. refractive index and thickness) of materials within a few hundred nanometers of the interface, and thus is useful for monitoring the "dry" mass of protein adsorbed onto the surface.

Design of Experiments to Investigate Molecular Access to Vault Interiors

Figure 1 shows a schematic of experiments designed to investigate molecular access into adsorbed vault interiors. In the proposed experiments, cpMVP or vsvgMVP vaults are adsorbed onto the QCM or SPR substrate in an approximate monolayer [4], and may be opened by exposure to low pH (*Panel I*). After blocking with milk at pH 6.5, antibody adsorption onto "half" or "whole" vaults is monitored, and the corresponding decrease in QCM oscillation frequency (Hz) or increase in the SPR angle (RU) is recorded. Ideally (*Panel II*), anti-vsvg antibody will adsorb specifically to the exposed vsvg tags of "half" vsvgMVP (*IIa*), but not to "whole" vsvgMVP (*IIb*) or cpMVP (*IIc,d*).

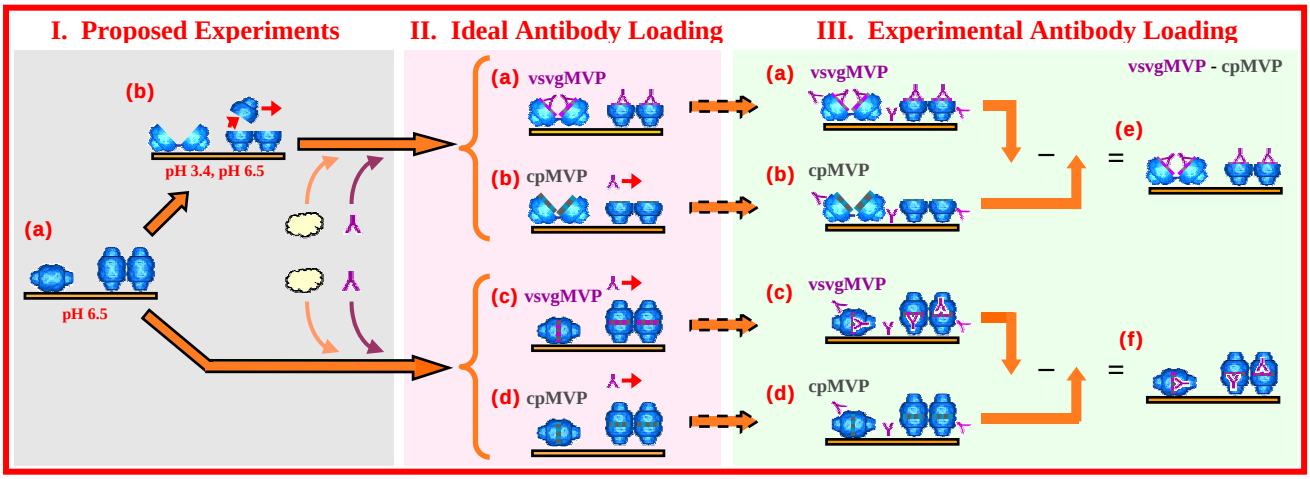

Figure 1: *Panel I*: Proposed experiments investigate the access of anti-vsvg antibody (人) to the interiors of adsorbed intact vaults at pH 6.5 (*Ia*), or vaults which have been "opened" by exposure to low pH (*Ib*), after blocking with 1% milk (☁). vsvgMVP vaults contained specific antibody binding sites, and cpMVP vaults were used as a control. *Panel II*: Ideally, the vsvg antibody will attach to "half" vsvgMVP (*IIa*), and not to "whole" vsvgMVP (*IIc*) or to cpMVP (*IIb,d*). *Panel III*: Experimentally, nonspecific binding of antibody to vault protein is observed (*IIIa-d*) in addition to specific binding onto both "half" (*IIIa*) and "whole" vsvgMVP (*IIIc*). For analysis, specific binding of anti-vsvg onto vsvgMVP (*IIIe,f*) is obtained by subtracting the non-specific cpMVP signals (*IIIa* minus *b*, *c* minus *d*). Note: substantial binding of antibody to "whole" vsvgMVP interiors is observed.

RESULTS AND DISCUSSION

QCM Surface Studies

The average QCM frequency decreases due to vsvg-antibody adsorption onto "half" and "whole" vaults were found to be 18.1 and 24.3 Hz (vsvgMVP) and 5.8 and 7.2 Hz (cpMVP), respectively. For further analysis, the non-specific cpMVP signals were subtracted from their corresponding vsvgMVP signals (Figure 1, *III*) to obtain vaults of 12.3 and 17.1 Hz for the specific binding of anti-vsvg onto "half" and "whole" vsvgMVP (Figure 1, *IIIe,f*), respectively. Using the sensitivity factor of the QCM crystal (56.6 Hz cm^2 μg^{-1} [5]) in the Sauerbrey equation [6], we *estimated* that "wet" (protein plus bound water) mass per unit area of antibody absorbed specifically onto "half" and "whole" vsvgMVP is 0.22 and 0.30 μg cm^{-2}, respectively. With general knowledge of the antibody dimensions, we calculated that the mass fraction of protein in the antibody adlayer is ~0.40. When the "wet" masses were multiplied by this value, the "dry" masses of antibody (protein only) adsorbed onto "half" and "whole" vaults were found to be 0.0870 ± 0.0009 and 0.121 ± 0.002 μg cm^{-2}, respectively, which could then be compared to results for antibody adsorption obtained using SPR.

SPR Surface Studies

The average SPR signal increases due to vsvg-antibody adsorption onto "half" and "whole" vaults were found to be 1170 and 1210 RU (vsvgMVP) and 310 and 110 RU (cpMVP), respectively. Subtracting the non-specific cpMVP signals as described above (and shown schematically in (Figure 1, *III*), and using the conversion factor of 1 RU = 10^{-4} μg cm^{-2} [7], we obtained values for the "dry" mass of antibody specifically bound to "half" and "whole" vsvgMVP of 0.09 ± 0.02 and 0.11 ± 0.02 μg cm^{-2}, respectively, which are statistically identical given the experimental uncertainties. These average values were also very similar to those obtained with QCM.

Expected Mass per Unit Area of Adsorbed Antibody

If we assume that each anti-vsvg antibody molecule is capable of binding to two vsvg tags internalized within each vault in a vault monolayer on the surface, we expect that specific antibody adsorption onto "half" vsvgMVP will be ~0.1 μg cm^{-2}, which is slightly higher than what is observed experimentally with QCM and SPR. Although we did not expect that antibody would be able to penetrate inside "whole" vaults, we saw significant specific binding of antibody to vsvg tags internalized within adsorbed vsvgMVP. If we again assume the maximum binding capacity of a "whole" vault where each antibody is capable of binding to two vsvg tags, we expect binding of antibody to a "whole" vault monolayer will be ~0.2 μg cm^{-2}, which is almost twice as much as was observed experimentally. A summary of the surface mass per unit area of adsorbed antibody obtained using QCM and SPR, as well as the expected adsorption if it is assumed each vsvg antibody is capable of binding to two vsvg tags, is shown in Figure 2.

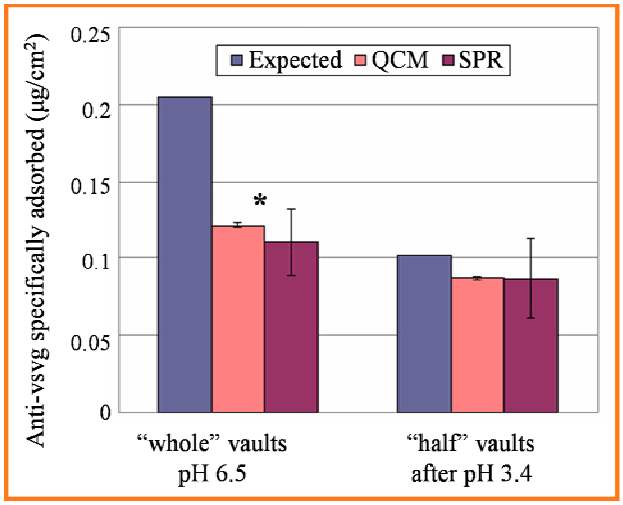

Figure 2: Mass of anti-vsvg antibody adsorbed specifically onto "whole" and "half" vsvgMVP (vsvgMVP – cpMVP) obtained with the QCM and SPR. The "expected" data is the adsorbed mass calculated assuming one vsvg-antibody is capable of binding to two vsvg tags internalized within each vault in a vault monolayer on the surface. *Note that low binding may be due to steric hindrance inside vaults. Bars are the average of three trials; Error bars are the standard errors of the mean.

CONCLUSION

We believe that the results for "whole" vaults indicate that relatively large molecules such as antibodies are able to gain access into the vault interior. Others have suggested this may be possible due to vault "breathing" [8]. The lower than theoretical adsorption onto "whole" vaults, however, suggests that steric limitations may prevent antibodies from being packaged to capacity inside "whole" vaults; such limitations are present to a much lesser extent for "half" vaults. Future studies will focus on how vault "breathing" may be exploited to encapsulate smaller molecules targeted to their interiors, as well as how it may be controlled.

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