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Correlation of chemical reactivity of *Nudaurelia capensis ω* virus with a pH-induced conformational change

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***Nudaurelia capensis ω* virus, which undergoes one of the largest known structural changes of icosahedral viruses in response to its environment, exhibits chemical reactivity which depends on its conformational state.**

The structural features of icosahedral viral particles make them attractive scaffolds for the development of functional nanomaterials.[1] The outer protein shell (capsid) is a readily accessible platform for the polyvalent presentation of functional groups, which are useful for many biomedical applications.[1a,2] In this respect, such virions can be regarded as naturally-occurring analogs of synthetic dendrimers. Selective chemical modification of such viral capsids is essential to the use of these building blocks. We systematically studied the chemical reactivities of cowpea mosaic virus (CPMV)[1a,2a–c] and flock house virus (FHV),[3] and found that icosahedral viral cages can be employed as robust platforms for versatile chemical reactions.[4] Here we expand these studies to a virus species unique in its dynamic structural properties, and show that its virus-like particles (VLPs) can be addressed by chemical reagents in well-defined fashion. Most importantly, the chemical reactivity of the system is shown to be dictated by the conformational changes of the viral capsid.

Nudaurelia capensis ω virus (NωV) is a single-stranded RNA, $T = 4$, icosahedral virus that belongs to the tetravirus family infecting Lepidoptera insects.[5] The viral capsid is composed of 240 copies of the 70 kDa (α) coat protein that, after assembly, undergoes an autocatalytic cleavage resulting in 62 kDa (β) and 8 kDa (γ) products (Fig. 1).[6] The 2.8 Å crystal structure of authentic NωV showed that each asymmetric unit of the $T = 4$ polyhedral capsid consists of four quasi-equivalent subunits.[7] Since a cell culture capable of supporting authentic NωV has not been identified, VLPs of NωV are readily produced using a recombinant baculovirus system.[8] At pH 7.6 the coat protein assembles into a stable ("procapsid") particle, which is 480 Å in diameter and quite porous. Upon lowering the pH to 5.0, a concerted reorganization into a 410 Å-diameter mature capsid structure occurs, accompanied by a slow autoproteolysis corresponding to the aforementioned assembly-dependent cleavage reaction of the authentic virion. The resulting capsid VLP is structurally indistinguishable from the infectious particle, and has no obvious pores (Fig. 1).[9] This conformational rearrangement (maturation) is initially reversible until about 15% of the cleavage events have occurred, at which point the particles are locked into the capsid conformation, regardless of pH.[9b,10] The pH-induced contraction of NωV is one of the largest relative changes known to occur in virus particles. Since both the procapsid and capsid forms of NωV VLPs are stable at pH 7.6, this system offers a rare opportunity to study the chemical reactivities of such large assemblies in two dramatically different states.[11]

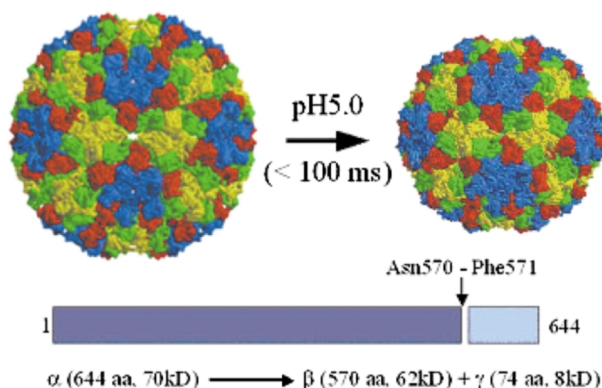

Fig. 1 The pH induced conformational change of NωV procapsid VLP (left) to capsid VLP (right) and subsequent autoproteolysis.

Incubation of capsid and procapsid forms of NωV VLPs with 20 mM fluorescein carboxylic acid **1**, which has no functional groups for covalent attachment, gives no dye association with the particles following purification by size-exclusion chromatography. This demonstrates that fluorescein neither intercalates into RNA nor engages in strong noncovalent interactions with the viral protein. The particles so treated remain intact, as shown by electron microscopy and sucrose gradient sedimentation, as long as the pH (7.6) and ionic strength (250 mM NaCl) are maintained.

Reactive fluorescein derivatives were used to study the reactivities of lysine (using **2** and **3**) and cysteine (**4** and **5**) residues of the coat proteins.† The results are shown in Fig. 2. The use of **2** at 2 mM concentration (representing a ratio of dye reagent to asymmetric unit of approximately 70 : 1) results in the attachment of approximately 240 dye units per VLP in the mature capsid form. Isothiocyanate **3** is less reactive than **2**, but with higher concentrations (20 mM), nearly the same level of functionalization (*ca.* 200 dyes per particle) was achieved. Surprisingly, cysteine reactivity with reagents **4** and **5** was

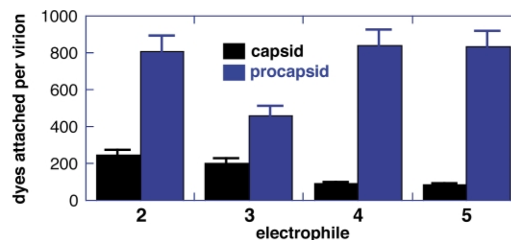

Fig. 2 Reactivity comparison between capsid and procapsid NωV VLPs. The reactions were performed in a pH 7.6 buffer (100 mM potassium phosphate and 250 mM NaCl) with 20% DMSO as co-solvent. The starting concentrations of VLPs were 2 mg mL^{-1}; 2.0 mM of **2** and 20 mM of **3–5** were used.

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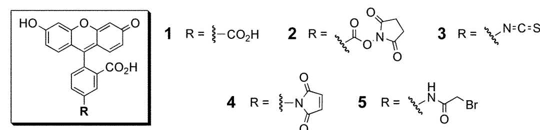
found to be sluggish, even when very high concentrations (20 mM) were used. Under such conditions, fewer than 90 dye units per particle, or less than one thiol residue per individual subunit, were addressed.



As expected, procapsid VLPs were significantly more reactive than their capsid counterparts. As many as 800 lysine residues or 840 cysteine residues were derivatized per particle under identical conditions to the capsid reactions. The overall recoveries of purified particles were > 70%, and size-exclusion FPLC and TEM revealed that even such heavily-derivatized particles remained intact. However, in both cases the use of > 5 mM **2** induced decomposition of the VLP, and the protein isolated from such reactions showed significantly greater levels of dye attachment. This suggests that **2** can modify lysines which participate in protein–protein or protein–polynucleotide contacts that are important to the integrity of the virion.

The labeling of NωV VLPs was dependent on the concentration of reagents until maximum loading values were achieved, as shown in Fig. 3 for compounds **2** and **4**. For the procapsid, a maximum of approximately three lysine residues and three cysteine residues per subunit was addressed, whereas capsid reactivity allowed the addressing of only one lysine residue and less than 0.5 cysteine residues per subunit. Thus, although procapsids and capsids are composed of the same protein, the 16% increase in diameter of the former relative to the latter renders the particle porous and more susceptible to chemical modification.

The product of capsid reaction with thiol-selective reagent **4** was analyzed by trypsin digestion and MALDI-MS analysis to identify the cysteine residue(s) that are chemically derivatized. Peptide fragments incorporating cysteines 135, 507, and 552 showed peak shifts consistent with labeling at each of these sites in roughly equal amounts (data not shown), demonstrating that at least these three positions are comparably, although sluggishly, reactive. The X-ray structure of NωV capsid reveals the cysteine residues at or near the surface of the particle to be largely shielded from solvent by an exterior IgG domain which protrudes from each subunit and which itself is free of cysteines. None of the cysteines appear to be unusually accessible compared to the others,[12] consistent with the experimental results.

We have reported elsewhere that the N570T point mutation provides properly assembled VLPs that do not undergo autoproteolysis in the transition between procapsid and capsid forms, making this conformation change reversible.[10] A preliminary investigation shows its chemical reactivity to be identical to that of the wild-type particle described above. One may therefore envisage using the reversible expansion and contraction of the NωV cage as a mechanism for imparting

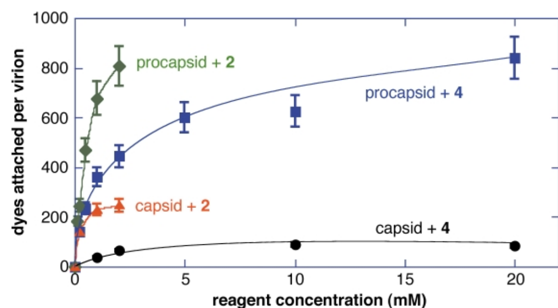


Fig. 3 Plots showing the amount of fluorescein covalently attached to capsid and procapsid NωV VLP as a function of reagent concentrations for **2** and **4**, with VLP concentrations of 2.0 mg mL^{-1}.

environmentally-sensitive function to the particle,[13] and studies along these lines are in progress.

In summary, NωV has been shown to have well-behaved chemical reactivity, affording high yields and controllable levels of functionalization. It is significantly larger than CPMV or FHV, making it an expanded platform (41–48 *vs.* 29 and 32 nm, respectively) with a greater degree of multivalency (240 *vs.* 60 and 180 copies of the capsid protein, respectively) for the display of functional molecules on its outer surface. Most importantly, the pH-induced conversion between procapsid and capsid forms imparts a striking structure-based change in the chemical reactivity of the particle. For these reasons, NωV adds productively to the growing repertoire of biologically-derived scaffolds for chemical, biological, and materials science applications.

Notes and references

† Under standard conditions, different amounts of dye reagents in DMSO were mixed with VLPs in 0.25 M NaCl in 0.1 M potassium phosphate buffer (pH = 7.6), such that the final solvent mixture was composed of 80% buffer and 20% DMSO. The final concentration of VLP in each reaction was 2.0 mg mL^{-1}; and the concentration of the dyes varied from 2 mM to 20 mM. Following incubation at 4 °C for 24 h, the mixture was purified by passage through a P-100 size exclusion column (centrifuge at 800 *g* for 3–5 min). The filtration was repeated (typically three or four times) with fresh columns until all the excess reagents were removed as judged by UV-vis absorbance measurements. The loading of dyes was measured by the absorbance at 496 nm and the concentration of VLPs was determined by the absorbance at 260 nm. The reported values are the average of three independent experiments, with error bars representing the standard deviation of these values.

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