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Natural Supramolecular Building Blocks: Wild-Type Cowpea Mosaic Virus

Qian Wang,[1] Eiton Kaltgrad,[2] Tianwei Lin,[2] John E. Johnson,[2,3] and M.G. Finn[1,3]
[1]Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
[2]Department of Molecular Biology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, California 92037

Summary

Cowpea mosaic virus (CPMV) can be isolated in gram quantities, possesses a structure that is known to atomic resolution, and is quite stable. It is therefore of potential use as a *molecular* entity in synthesis, particularly as a building block on the nanochemical scale. CPMV was found to possess a lysine residue with enhanced reactivity in each asymmetric unit, and thus 60 such lysines per virus particle. The identity of this residue was established by a combination of acylation, protein digestion, and mass spectrometry. Under forcing conditions, up to four lysine residues per asymmetric unit can be addressed. In combination with engineered cysteine reactivity described in the accompanying paper, this provides a powerful platform for the alteration of the chemical and physical properties of CPMV particles.

Introduction

Viruses straddle the boundaries between living and nonliving matter and are the object of important investigations in biology, biochemistry, evolution, and medicine. The application of modern structural methods, principally X-ray crystallography and electron microscopy, have also revealed viruses to be fascinating chemical assemblies [1]—among the most impressive examples of molecular recognition, polyvalent interactions, and self-assembly to be examined at near-atomic resolution. Because certain types of viruses can be made in large quantities and manipulated at the genetic level, they afford a unique opportunity for chemists to expand the repertoire of natural starting materials for synthetic and catalytic applications. We report here the results of studies designed to enable the icosahedral Cowpea mosaic virus (CPMV) to be employed in this fashion. Precedents for the exciting possibilities enabled by such efforts are provided by the work of Douglas, Young, and coworkers on the construction of hybrid inorganic materials [2, 3] and organic-soluble ferritin particles [4].

CPMV is a nonenveloped virus of approximately 300 Å diameter. It can be isolated from infected plants in yields of 1–2 g/kg of leaves via a simple and convenient procedure [5–7]. It is noninfectious toward other organisms and presents no biological hazard. Its structure, determined to 2.8 Å resolution [8], shows a *Picorna*-like $T = 1$ ($P = 3$) protein shell (capsid) with an asymmetric unit containing three jelly-roll β barrel sandwich folds formed by two polypeptides (Figure 1) [9, 10]. Sixty copies of the 2 protein asymmetric unit (composed of a "small" subunit, the A domain, and a "large" subunit, the B + C domains in Figure 1) are assembled in an icosahedral surface lattice around the single-stranded viral genomic RNA to form the virus particle. Three components are isolated from the typical virus preparation and are distinguished by their differing densities in sucrose gradients. The top component is obtained in small amounts and contains no RNA ("empty" capsids, molecular weight = 3.94×10^6 Da). The two dominant components contain the two different RNA molecules required for infection; these are packaged by identical capsid structures, giving virions of differing densities and molecular weights (5.16×10^6 and 5.98×10^6 Da) [11]. The assembly displays sufficient stability and chemical reactivity to be regarded as a "molecule"—akin in topology and potential application to a very large dendrimer [12, 13]—with a molar mass of approximately 5.6×10^6 Da.

The crystal structure of CPMV displays no free cysteine residues accessible to the solvent on the exterior surface of the capsid. We have reported elsewhere the selective addressing of cysteine residues on the interior capsid surface with thiol-selective reagents and the installation of a reactive cysteine on an exposed loop of mutant CPMV [14]. Here we describe the chemical reactivity of wild-type CPMV toward lysine-selective species and demonstrate that the native particle is a viable starting material for diverse applications. In the accompanying paper, we report expanded studies on new cysteine mutants with high reactivity.

Results and Discussion

Lysine Reactivity

CPMV reacts with fluorescein N-hydroxysuccinimide ester **1** and isothiocyanate **2**, which are selective for reactions with available lysine residues (equation 1 in Figure 2); Figure 3 shows the results of such reactions. Stoichiometries were determined by absorption measurements on solutions of labeled particles by comparison of the intensities of dyes (494 nm for fluorescein) to that of protein (260 nm), each having nonoverlapping bands with well-established molar absorbtivities. Covalently modified virions were separated from excess dye reagents by multiple passages through size exclusion filters until dye was undetectable in the wash solution and the relevant absorbance ratios for the virus samples were constant. The intact nature of the derived particles was substantiated by sucrose gradient ultracentrifugation and size exclusion FPLC chromatography, and nonspecific dye adsorption to the virus was ruled out by appropriate control experiments.

At neutral pH, wild-type CPMV was found to take on

[3]Correspondence: jackj@scripps.edu (J.E.J.)
mgfinn@scripps.edu (M.G.F.)

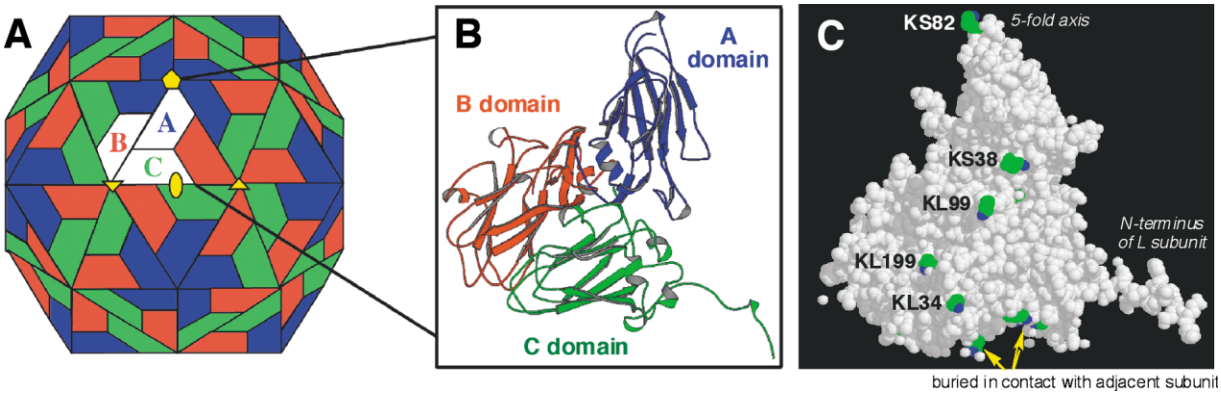

Figure 1. CPMV Structure

(A) Subunit organization, (B) subunit ribbon diagram, and (C) space-filling model of the coat protein. The latter shows the exterior surface of the asymmetric unit with lysine side chain carbons in green and side chain nitrogen atoms in blue. Two lysine residues of the small subunit (S82, S38) appear to be exposed to solvent, whereas three in the large subunit (L99, L199, and L34) are similarly visible. The atomic coordinates of CPMV are available from the VIPER web site (http://mmtsb.scripps.edu/viper).

NHS ester at room temperature, up to a ratio of between 60 and 70 dye molecules per virion, in a dose-dependent fashion through a reagent ratio (dye:viral protein) of 200:1 (Figure 3B). This suggests that, under these conditions, one lysine residue in each capsid protein asymmetric unit may be uniquely reactive. As expected, somewhat greater reactivity was observed with the NHS ester at pH 8.3, resulting in the attachment of approximately 1.5 dye molecules per asymmetric unit, or about 90 per virus particle (Figure 3B). Pushing the reaction with greater amounts of **1** (up to dye:protein = 4,000) gave a maximum loading at both pH 7.0 and 8.3 of 240 dyes per virion, indicating that four lysine residues per asymmetric unit are accessible under forcing conditions (Figure 3A). Proving to be less reactive and more selective, the isothiocyanate reagent required approximately 1000 equivalents to deposit 60 dyes per virus particle (Figure 3C). Further increasing the amount of dye-isothiocyanate by a factor of 5 resulted in only a modest increase in the number of dye molecules attached per virion. Electrophoresis of the resulting particles under denaturing conditions showed predominant lysine reactivity at lower loading (50 dyes per particle with isothiocyanate **2**) to be associated with the small subunit (Figure 3D). Analysis of fluorescein-decorated CPMV made with NHS ester **1** revealed a small amount of labeling of the large subunit as well (our unpublished data), giving further evidence of the greater selectivity obtained with isothiocyanate.

The dye-labeled virions were also characterized by

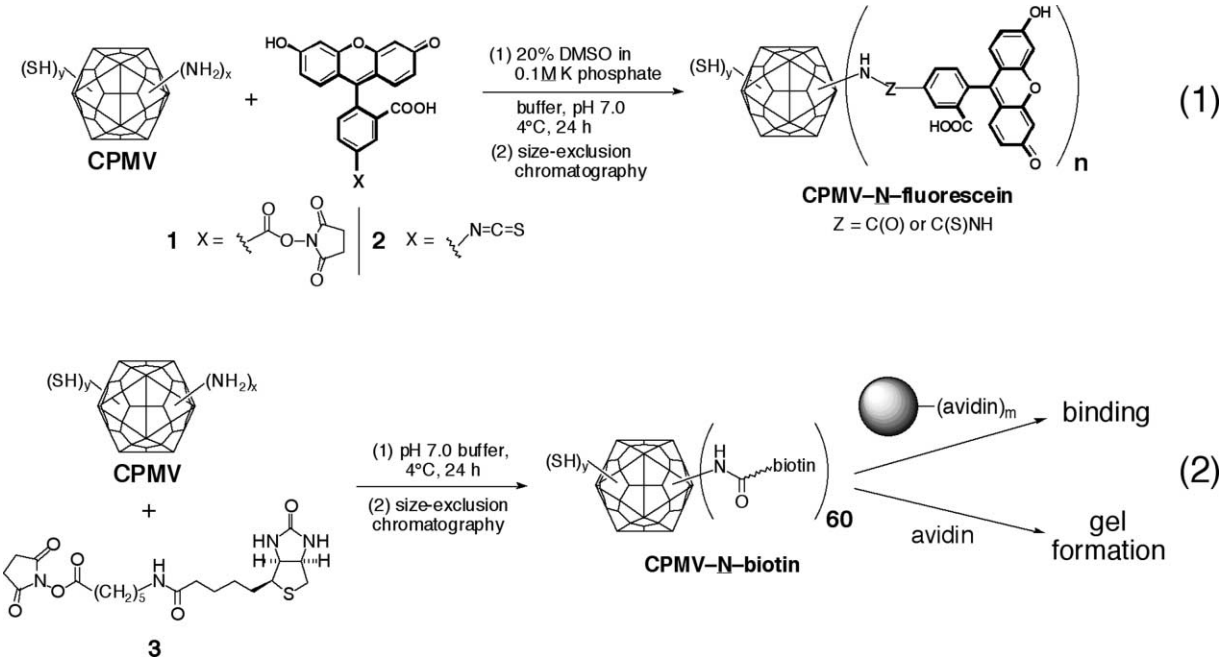

Figure 2. Attachment Reactions to CPMV Particles

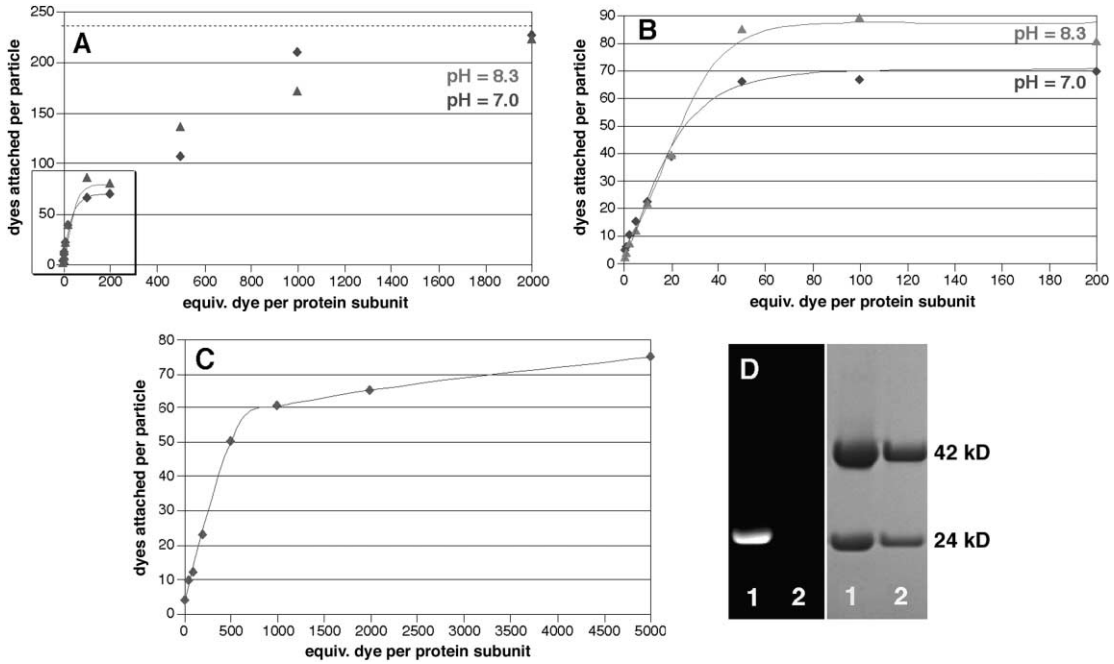

Figure 3. Relationship of the Ratio of Reactants to the Number of Attached Dyes per CPMV Particle

(A) Results of reactions with fluorescein NHS ester **1**. The full range of ratios of the reactants (equation 1 in Figure 2) used is shown.

(B) Expansion of the boxed area in plot (A).

(C) Results of reactions with fluorescein isothiocyanate **2**. All values are the average of three independent determinations; experimental error is ±12% of the average value.

(D) SDS-PAGE: lane 1, CPMV-N-fluorescein prepared using reagent **2**, containing approximately 50 dyes per particle; lane 2, underivatized wild-type CPMV. Left (black background), visualization directly under ultraviolet illumination shows the attachment of fluorescein to the small subunit; right (lighter background), staining with Coumassie blue reveals both subunits.

sucrose gradient sedimentation and ion exchange FPLC, as shown in Figure 4. The former showed that dye-labeled CPMV particles remained intact; they exhibited the same sedimentation velocities as underivatized wild-type particles. The FPLC analysis highlights the capacity of a single substitution per asymmetric unit to dramatically change a property of the particle; in this case that property is interaction with an ion exchange column. Note the excellent peak separation between labeled and unlabeled virions and the appearance of fluorescein absorbance only in association with the second peak.

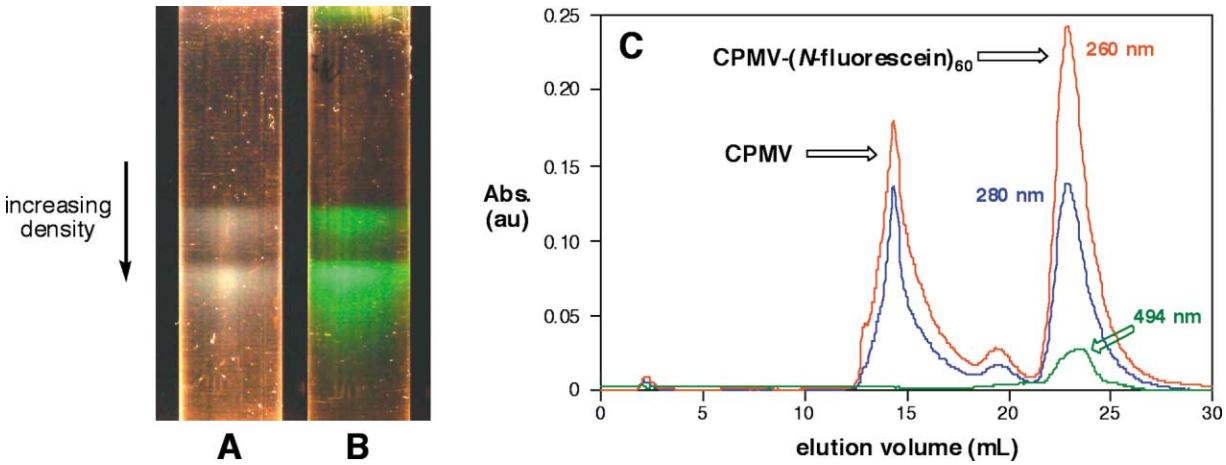

Figure 4. Analysis of Dye-Labeled CPMV

Ultracentrifugation through sucrose gradients of (A) wild-type CPMV and (B) CPMV-(N-fluorescein)$_{60}$ prepared from **1**. The two bands in each sample contain virus particles encapsulating the two different components of the RNA genome. These two components differ in size, and the particles therefore have different densities. A small amount (<5%) of most CPMV preparations comprise assembled capsids without RNA inside; this band is not visible here. (C) Ion exchange FPLC analysis of a mixture of wild-type CPMV and CPMV-(N-fluorescein)$_{60}$.

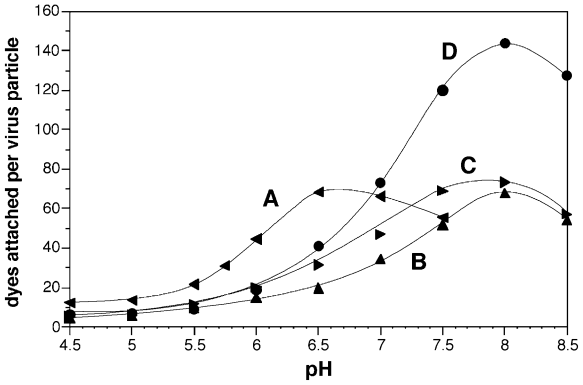

Figure 5. Reaction of CPMV with **1** as a Function of Buffer pH under Otherwise Standard Conditions

(A) CPMV (1 mg/ml) with 100 equivalents of **1** for 24 hr.
(B) CPMV (1 mg/ml) with 200 equivalents of **1** for 6 hr.
(C) CPMV (2 mg/ml) with 1000 equivalents of **1** for 2 hr.
(D) CPMV (2 mg/ml) with 1000 equivalents of **1** for 6 hr.

Identical quantitative reactivity was observed for the attachment of other dyes bearing the same reactive functional groups in buffer alone or DMSO-buffer mixtures up to 50%. We have found CPMV to be stable for more than two weeks in 20% DMSO, and the stability of the particle is inversely proportional to the DMSO concentration past that point. At 50% DMSO, the half-life of the particle is on the order of several hours, and in 80% DMSO the virion is stable for approximately 30 min. This allows chemistry involving relatively hydrophobic organic compounds to be performed on the viruses. Similarly, CPMV can be recovered unchanged from two-phase agitation in mixtures of water with a variety of organic solvents. A full account of the solvent- and temperature-stability of CPMV and selected mutants (chimeras) will be reported elsewhere.

Because lysines of altered basicity are of interest in the mechanisms of many enzymes and catalytic antibodies [15–18], we have performed a preliminary study of lysine reactivity by monitoring the extent of reaction with **1** as a function of pH (Figure 5). In each case, the curves did not reach a stable plateau at higher pH because of an increase in the rate of competitive hydrolysis of NHS ester **1**. Thus, we cannot derive a pK_a value for the reactive lysine from these data. The form of the observed curves were reproduced by a calculation in which the following variables were taken into account: pK_a of the "perturbed," highly reactive lysine; pK_a of the other, "nonperturbed" lysines; the kinetic reactivities of each of these lysines with NHS ester in the unprotonated state (these reactivities are not necessarily the same); and the rate constant for hydrolysis of NHS ester **1**, a process that is assumed to be first order in hydroxide ion concentration. We have insufficient data to come to a unique solution for these interlocking equations, but reasonable approximations for the above values lead to an estimate of 7.0–7.5 for the pK_a of interest (see Supplemental Data). The direct measurement of the lysine basicity will be performed by other methods. Nevertheless, it is apparent that substantial reactivity exists at pH 6.5–7 under "kinetic" conditions (large excess of **1** for shorter reaction times; Figures 5B–5D), and when reactions are allowed to proceed for 24 hr, reactivity is observed at pH values as low as 5.5–6.0 (Figure 5A).

Figure 1C shows a space-filling representation of the outside surface of the CPMV asymmetric unit. Excluding possible effects from dynamic motion of the capsid structure, only five lysine residues appear to be exposed to the solvent on the exterior, whereas at least ten are found on the interior surface. These are presumably involved in electrostatic interactions with the packaged RNA genome and are therefore unreactive with external reagents. Because no more than four lysine residues per asymmetric unit can be chemically addressed with NHS esters under forcing conditions (Figure 3A) and the resulting derivatized particles are quite stable, we suggest that the capsid structure is not sufficiently dynamic to expose additional lysine residues to solvent. The uniquely reactive lysine among the three present in the small subunit was identified as K38 by trypsin digestion and mass spectrometry analysis (Figure 6). Six oligopeptides with strong intensity were observed in the analysis of the wild-type small subunit; these included all three that derive from proteolysis adjacent to lysine. Acylation of the lysine side chain is expected to inhibit enzymatic digestion at that position, and indeed only five of the six peaks were observed for the N-fluorescein conjugate. Missing is the signal corresponding to residues 39–61, which was formerly the most intense observed, providing strong evidence for selective labeling at lysine 38. This was further supported by the observation of the dye-labeled 1–61 fragment as a peak distinct from that of the unlabeled fragment in the digestion of the unmodified native protein (our unpublished data).

CPMV-Biotin Conjugate

The chemical reactivity of CPMV, which allows the controlled attachment of 60 chemical units to the exterior of the particle or higher levels of covalent modification under more vigorous conditions, makes the virus an interesting support for functional groups. The CPMV-$(biotin)_n$ conjugate prepared from biotin-NHS ester **3** (equation 2 in Figure 2) provides an example of this utility. In separate reactions, this material and underivatized wild-type CPMV were mixed with avidin-polystyrene beads, followed by extensive washing of the beads with buffer. A suspension of the beads was then subjected to protein denaturation conditions, releasing RNA from virus particles retained with the beads. Analysis by reverse transcriptase polymerase chain reaction (RT-PCR) demonstrated that the biotinylated virus was selectively adsorbed to the avidin-coated beads (Figure 7). Furthermore, biotinylated CPMV participated in rapid gel formation when mixed with solutions of avidin, which possesses four biotin binding sites per protein, as observed in the electron microscopy images shown in Figure 7 and by the formation of a bulk precipitate. Both wild-type CPMV alone and the virus mixed with biotin lacking an NHS ester moiety for covalent attachment failed to show aggregation with avidin. These data provide further evidence that the reactive lysine group is exposed on the exterior of the capsid and highlight the polyvalent nature of the virus as a display platform.

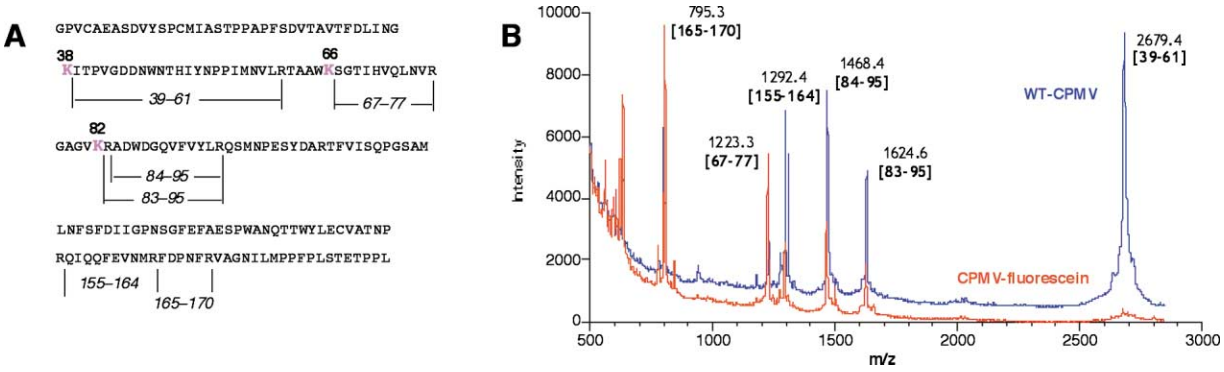

Figure 6. Identification of Reactive Lysine Residue

(A) Protein sequence of the small subunit of CPMV.

(B) Superimposed MALDI mass spectra of trypsin digests of the small subunits of wild-type CPMV (blue) and CPMV-N-fluorescein (red), the latter prepared with isothiocyanate **2**. The samples were obtained by gel electrophoresis, isolation of the small subunit band, and in-gel digestion with trypsin.

Significance

These studies establish CPMV as a robust platform for chemical synthesis and show that it possesses a natural lysine residue of significantly altered basicity. The system can be regarded as a self-assembled "dendrimer" [12, 13] in light of the fact that 60 identically reactive groups are displayed on the periphery of the structure. One can also make an analogy between virions and metallic nanoparticles, which have been used in a variety of applications made possible by the attachment of functional groups via gold-thiol and related interactions [19–22]. Although not as chemically stable as traditional dendrimers, CPMV provides the following advantages to the chemist desiring to display multiple copies of functional groups around a core [23]. (1) Virus synthesis and purification are easy, even on a multigram scale. (2) The virus "dendrimer" is actually a capsule with interior and exterior surfaces that can be differentially addressed and an interior space that can, in principle, be loaded with a variety of materials. (3) The finding, as reported in the accompanying paper, that the reactivity of the virus is adjustable by genetic manipulation provides a powerful complement to the techniques of organic synthesis. (4) Finally, with one remarkable exception [24], synthetic dendrimers are far smaller than viruses because of the steric crowding that occurs with successive attempts to graft layers on the central core. Thus, for the development of catalysts, functional materials, and biologically active structures, viruses should become a valued raw material for the synthetic chemist.

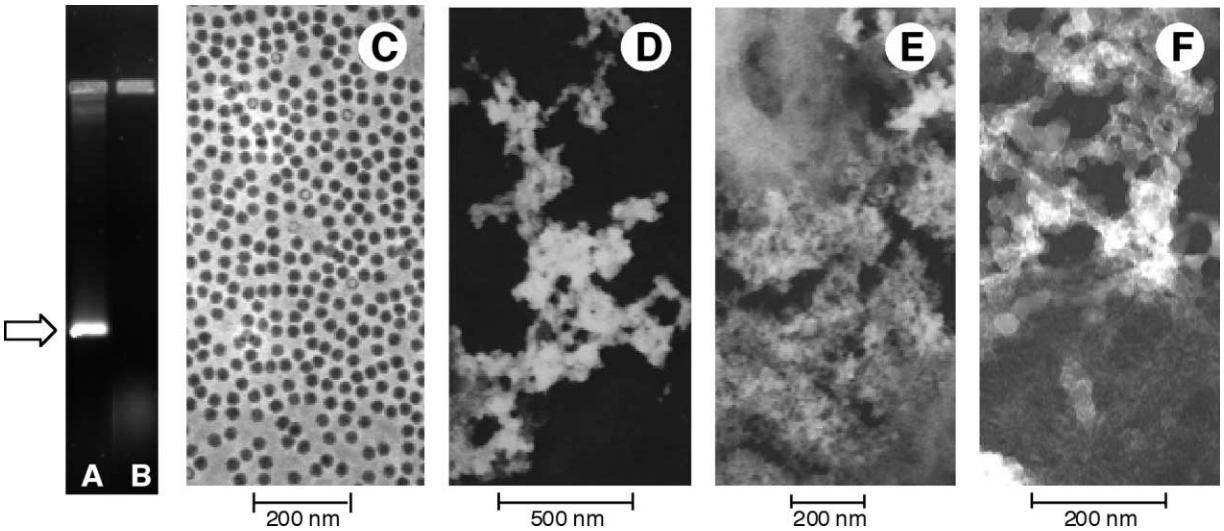

Figure 7. Analysis of Biotinylated CPMV

(A–B) Electrophoresis analysis of RT-PCR products from incubations with avidin-coated beads; lane A is from CPMV-N-$(biotin)_n$, and lane B is from WT-CPMV. Comparisons to a standard ladder and to material obtained from authentic CPMV show the band marked with an arrow to correspond to the intact CPMV genome.

(C–F) TEM pictures (negative staining) show agglutination of mixtures of avidin and CPMV-$(biotin)_{60}$: (C) CPMV-$(biotin)_{60}$ without avidin; (D–F) different samples of gel formed from CPMV-$(biotin)_{60}$ + avidin. See text for more details.

Experimental Procedures

General

Reagents **1** and **2** were purchased from Molecular Probes; **3** was from Sigma; ImmunoPure avidin was from Pierce; and TetraLink Tetrameric avidin Resin, M-MLV reverse transcriptase, and the sequencing grade modified trypsin were from Promega. Wild-type CPMV was prepared by the method described by Siler et al. [25]. The virus was stored in buffer at a concentration of about 33 mg/ml. Unless otherwise indicated, "buffer" refers to 0.1 M potassium phosphate (pH 7.0). NuPage 4%–12% Bis-Tris pre-cast gel was purchased from Invitrogen. Size exclusion columns for the purification of virus-containing reaction mixtures were prepared by preswelling 23 g of Bio-Gel P-100 (BioRad) in 400 ml 0.1 M potassium phosphate buffer (pH 7.0) and loading the gel into Bio-Spin disposable chromatography columns (BioRad). The columns were allowed to drain upon standing and were then further dried by centrifugation (3 min at 800 *g*). For 80 μl of virus solution (1 mg/ml), approximately 1 ml of prepared gel is required. Ultracentrifugation was performed at the indicated rpm values with a Beckman Optima L-90K Ultracentrifuge equipped with either SW41 or 50.2 Ti rotors.

Analytical Methods

For TEM analyses, 20 μl aliquots of each sample were deposited onto 100-mesh carbon-coated copper grids for 2 min. The grids were then stained with 20 μl of 2% uranyl acetate and viewed with a Philips CM100 electron microscope. FPLC analyses were performed on an AKTA Explorer (Amersham Pharmacia Biotech) with Superose-6 size exclusion or Hitrap-Q ion exchange columns. In the former case, 0.05 M potassium phosphate buffer (pH 7.0) was used as eluent; intact virions show retention times of approximately 25 min at an elution rate of 0.4 ml/min, whereas broken particles and individual subunit proteins elute after more than 50 min. Ion exchange FPLC was performed with 0.05 M potassium phosphate as the low-salt buffer and 1.0 M NaCl in 0.05 M potassium phosphate as high-salt eluent. A typical run (Figure 4) employed the following percentages of high-salt eluent for the indicated number of column volumes: 0% (4 column volumes); 36% (14); and 100% (14). Sucrose gradient ultracentrifugation analyses of virus samples up to 500 μg were performed at 38,000 rpm for 2 hr on 12 ml sucrose gradients made by defrosting 20% sucrose solutions in 0.1 M potassium phosphate buffer.

Modification of CPMV with Chemical Reagents

Organic reagents were introduced into a solution of virus (1 mg/ml) so that the final solvent mixture was composed of 80% buffer and 20% DMSO. After incubation at 4°C for 24 hr, 80 μl aliquots were purified by passage through a P-100 size exclusion column (centrifugation at 800 *g* for 3 min). This filtration was repeated with fresh columns until all the excess reagents were removed (typically 2–3 times). Larger quantities of derivatized virus (>1 mg) were purified by ultracentrifugation at 42,000 rpm over a 1.0-cm-high sucrose cushion, followed by resuspension in buffer. The recovery of derivatized viruses was typically 60%–80%; all such samples were composed of >95% intact particles as determined by analytical size exclusion FPLC and TEM. Virus concentrations were determined by measuring the absorbance at 260 nm; virus at 0.1 mg/ml gives a standard absorbance of 0.8. The average molecular weight of the CPMV virion is 5.6×10^6. Dye concentrations were obtained by measurement of absorbance at λ_{max} (495 nm for fluorescein), with molar absorbtivity calibrated for each use by mixing known quantities of dye with CPMV (1 mg/ml). This was required to ensure comparable results with different batches of dye, which differed in concentration and/or purity from the manufacturer, and to correct for dye decomposition that may occur in storage.

Studies of the stoichiometry of virus derivatization (Figure 3) were performed at constant virus concentration (1 mg/ml) in buffer with 20% DMSO as cosolvent. After purification, the concentration of viruses and dyes was checked by absorbance UV-vis spectroscopy. Each data point is the average obtained from three independent parallel reactions. The average derivation was 5%, and the maximum derivation was 12%. Most samples were also analyzed by anion exchange FPLC to verify the integrity of the particles and the quantitation of their dye attachments. Biotinylated CPMV was prepared and purified from wild-type CPMV and biotinamidocaproate N-hydroxysuccinimide ester (200-fold excess to the viral subunit) by the above procedures.

Trypsin in-Gel Digestion and MALDI Study

CPMV-(N-fluorescein)$_{60}$ was prepared by the above procedure. Samples (20 μg) of wild-type CPMV and the derivatized virus were each subjected to SDS-PAGE (NuPage 4%–12%, Invitrogen). The bands corresponding to the small subunit were cut out of the gel and treated directly with sequence-grade modified trypsin (0.5 μg in 10 mM ammonium citrate) [26, 27]. The resulting samples were analyzed by MALDI-MS, with α-cyano-4-hydroxycinnamic acid as the matrix. For spectra collection, 250 laser pulse acquisitions were averaged on a Perseptive Voyager STR instrument for each sample.

CPMV-(Biotin)$_n$ Binding with Avidin and RT-PCR Analysis

Tetrameric avidin resin (0.4 nmol) and biotinylated CPMV (50 μg) were mixed in buffer for 2 hr at 4°C. The mixture was then forced through a Millipore filter (0.45 μm) by centrifugation at 200 *g*. The resin was washed with 0.1 M potassium phosphate buffer (pH 7.0, 3×500 μl), PBS buffer (phosphate buffer with saline [10 mM Na_3PO_4, 150 mM NaCl, pH 7.4]) with 0.1% Tween-20 (3×500 μl), and again with 0.1 M potassium phosphate buffer (2×500 μl). The resin was resuspended in 100 μl RNA extraction buffer containing 100 mM glycine, 10 mM EDTA, 0.1 M NaCl, and 2.5 mg/ml bentonite (pH 9.5), followed by phenol/chloroform extraction and ethanol precipitation. The resulting RNA was annealed to primer pBCCC2 (ATA-GTT-CCA-GAT-TTC-CA, which binds to nucleotides 2852–2868 of RNA-2 of CPMV). The first cDNA strand was synthesized with M-MLV reverse transcriptase, followed by PCR with pBCCC1 (TCC-CGC-TTG-CTT-GGA-GC) and pBCCC2 [28]. A control binding test was also performed with unmodified native CPMV. The gel shown in Figure 7 was formed by mixing biotinylated CPMV (17.5 mg/ml in PBS buffer, 20 μl, 350 μg) with ImmunoPure avidin (10 mg/ml in PBS buffer, 6 μl, 60 μg), resulting in the immediate formation of a cloudy precipitate and a white gel after 5 min. PBS buffer (500 μl) was added before sample collection for TEM analysis.

Supplemental Data

The supplemental material contains a description of a simple mathematical model of the pH-dependent processes involved in labeling a protein having multiple reactive lysine sites with an NHS ester derivative. The form of Figure 5 is shown to be reproduced with a reasonable set of assumptions regarding several parameters (lysine basicity, relative lysine reactivities, and rate of competing NHS ester hydrolysis). Please write to chembiol@cell.com for a pdf.

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References

1. Crick, F.H.C., and Watson, J.D. (1956). Structure of small viruses. Nature *177*, 473–475.
2. Douglas, T., and Young, M. (1999). Virus particles as templates for materials synthesis. Adv. Mater. *11*, 679–681.
3. Shenton, W., Douglas, T., Young, M., Stubbs, G., and Mann, S. (1999). Inorganic-organic nanotube composites from template mineralization of tobacco mosaic virus. Adv. Mater. *11*, 253–256.
4. Wong, K.K.W., Whilton, N.T., Cölfen, H., Douglas, T., and Mann, S. (1998). Hydrophobic proteins: synthesis and characterisation of organic-soluble alkylated ferritins. Chem. Commun., 1621–1622.
5. Spall, V.E., Porta, C., Taylor, K.M., Lin, T., Johnson, J.E., and

Lomonossoff, G.P. (1998). Antigen expression on the surface of a plant virus for vaccine production. In Engineering Crops for Industrial End Uses, P.R. Shewry, J.A. Napier, and P. Davis, eds. (London: Portland Press), pp. 35–46.

6. Lin, T., Porta, C., Lomonossoff, G., and Johnson, J.E. (1996). Structure-based design of peptide presentation on a viral surface: the crystal structure of a plant/animal virus chimera at 2.8 Å resolution. Fold. Des. *1*, 179–187.
7. Goldbach, R., and van Kammen, A. (1985). Structure replication and expression of the bipartite genome of Cowpea mosaic virus. In Molecular Plant Virology, J. Davies, ed. (Boca Raton, FL: CRC Press), pp. 83–120.
8. Lin, T., Chen, Z., Usha, R., Stauffacher, C.V., Dai, J.-B., Schmidt, T., and Johnson, J.E. (1999). The refined crystal structure of Cowpea mosaic virus at 2.8 Å resolution. Virology *265*, 20–34.
9. Lomonossoff, G., Shanks, M., Holness, C., Maule, A., Evans, D., Chen, Z., Stauffacher, C., and Johnson, J. (1991). Comovirus capsid proteins: synthesis, structure, and evolutionary implications. In Biochemistry and Molecular Biology of Plant-Pathogen Interactions, C.J. Smith, ed. (Oxford: Clarendon Press), pp. 76–91.
10. Lomonossoff, G.P., and Johnson, J.E. (1991). The synthesis and structure of comovirus capsids. Prog. Biophys. Mol. Biol. *55*, 107–137.
11. Lomonossoff, G.P., and Shanks, M. (1983). The nucleotide sequence of Cowpea mosaic virus B RNA. EMBO J. *2*, 2253–2258.
12. Fischer, M., and Vögtle, F. (1999). Dendrimers: from design to application—a progress report. Angew. Chem. Int. Ed. *38*, 884–905.
13. Hecht, S., and Fréchet, J.M.J. (2001). Dendridic encapsulation of function: applying nature's site isolation principle from biomimetics to materials science. Angew. Chem. Int. Ed. *40*, 74–91.
14. Wang, Q., Lin, T., Tang, L., Johnson, J.E., and Finn, M.G. (2002). Icosahedral virus particles as addressable nanoscale building blocks. Angew. Chem. Int. Ed. *41*, 459–462.
15. Barbas, C.F.I., Heine, A., Zhong, G., Hoffmann, T., Gramatikova, S., Bjornestedt, R., List, B., Anderson, J., Stura, E.A., Wilson, I.A., et al. (1997). Immune versus natural selection: antibody aldolases with enzymic rates but broader scope. Science *278*, 2085–2092.
16. Nash, H.M., Lu, R., Lane, W.S., and Verdine, G.L. (1997). The critical active-site amine of the human 8-oxoguanine DNA glycosylase, hOgg1: direct identification, ablation and chemical reconstitution. Chem. Biol. *4*, 693–702.
17. Zhu, L., Kemple, M.D., Yuan, P., and Prendergast, F.G. (1995). N-terminus and lysine side chain pK_a values of melittin in aqueous solutions and micellar dispersions measured by ^{15}N NMR. Biochemistry *34*, 13196–13202.
18. Mason, J.R., Leong, F.C., Plaxco, K.W., and Morton, T.H. (1985). Two-step covalent modification of proteins. Selective labeling of Schiff base-forming sites and selective blockade of the sense of smell *in vivo*. J. Am. Chem. Soc. *107*, 6075–6084.
19. Taton, T.A., Mirkin, C.A., and Letsinger, R.L. (2000). Scanometric DNA array detection with nanoparticle probes. Science *289*, 1757–1760.
20. Elghanian, R., Storhoff, J.J., Mucic, R.C., Letsinger, R.L., and Mirkin, C.A. (1997). Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science *277*, 1078–1081.
21. Mitchell, G.P., Mirkin, C.A., and Letsinger, R.L. (1999). Programmed assembly of DNA functionalized quantum dots. J. Am. Chem. Soc. *121*, 8122–8123.
22. Boal, A.K., Ilhan, F., DeRouchey, J.E., Thurn-Albrecht, T., Russell, T.P., and Rotello, V.M. (2000). Self-assembly of nanoparticles into structured spherical and network aggregates. Nature *404*, 746–748.
23. Mammen, M., Choi, S.-K., and Whitesides, G.M. (1998). Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. Angew. Chem. Int. Ed. *37*, 2755–2794.
24. Olenyuk, B., Levin, M.D., Whiteford, J.A., Shield, J.E., and Stang, P.J. (1999). Self-assembly of nanoscopic dodecahedra from 50 predesigned components. J. Am. Chem. Soc. *121*, 10434–10435.
25. Siler, D.J., Babcock, J., and Bruening, G. (1976). Electrophoretic mobility and enhanced infectivity of a mutant of Cowpea mosaic virus. Virology *71*, 560–567.
26. Helmann, U., Wernstedt, C., Gonez, J., and Heldin, C. (1995). Improvement of an "in-gel" digestion procedure for the micropreparation of internal fragments for amino acid sequencing. Anal. Biochem. *224*, 451–455.
27. Scripps Center for Mass Spectrometry. http://masspec.scripps.edu/information/samprep/digest.html.

28 Taylor, K.M., Lin, T., Porta, C., Mosser, A.G., Giesing, H.A., Lomonossoff, G.P., and Johnson, J.E. (2000). Influence of three-dimensional structure on the immunogenicity of a peptide expressed on the surface of a plant virus. J. Mol. Recognit. *13*, 71–82.