THE SELF-ASSEMBLY OF RNA FREE PROTEIN SUBUNITS FROM BACTERIOPHAGE MS-2

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SUMMARY

Coat protein subunits of the RNA phage MS-2 are capable of self-assembly into particles which are antigenically related to capsids but possess a higher S value. There appear to be at least two intermediates present during this assembly which are antigenically unrelated to the capsid.

INTRODUCTION

The assembly of viral subunits into higher ordered configurations has been studied for a variety of viruses. The first studies initiated on tobacco mosaic virus demonstrated that RNA free preparations could aggregate to form viral coats under proper conditions (1). More recently the studies have focused on the self-assembly of icosahedral viruses.

Bancroft (2) demonstrated that the protein subunits of cowpea chlorotic mottle virus aggregated, in the absence of nucleic acid, to form particles that were antigenically related to the infectious virus. Hermann et al. (3) described the aggregation of protein subunits from the RNA coliphage fr into particles that had a higher sedimentation coefficient than naturally occurring empty capsids. Hohn (4) described the kinetics of self-assembly of RNA free protein subunits of phage fr under various experimental conditions. He suggested that these particles have a higher sedimentation coefficient because they assemble around a protein core.

Evidence is presented in this report that the protein subunits of the icosahedral RNA coliphage MS-2 will aggregate in the absence of RNA to form particles that are antigenically related to empty virus capsid particles. In the process of aggregation and prior to the formation of the self-assembled

particle, two intermediates were observed which were antigenically unrelated to the assembled particle.

MATERIALS AND METHODS

Phage. Purified suspensions of phage MS-2 were prepared by a modification (5) of the method of Strauss and Sinsheimer (6).

Labeled Phage. Phage MS-2, labeled with H^3-uridine (6.97 c/mM, New England Nuclear Corp., Boston, Mass.) was prepared as described by Vinuela (7). Escherichia coli AB261 was grown in MTPA medium at 37° C with moderate aeration to approximately 2 x 10^8 bacteria/ml, at which time 5 µg/ml of unlabeled uridine was added and the culture incubated for an additional 10 minutes. Phage MS-2 at a multiplicity of approximately 10 and 1 µc/ml H^3-uridine were added and the culture was incubated for 4 hours. The resulting lysate was purified as described above.

Phage Protein. Protein was prepared according to the method of Hohn (4). One volume of phage (approximately 10 mg), suspended in buffer A (0.1 M NaCl, 0.01 M Tris, pH 7.6), was added to 2 volumes of precooled glacial acetic acid. After 15 minutes the precipitated RNA was removed by centrifugation at 12,000 x g for 10 minutes. The supernatant was dialyzed against 3 changes of distilled water for 24 hours and brought to pH 8.5 with 0.02 M NaOH. The ionic strength was then adjusted to 0.015 M NaCl and the remaining precipitate was removed by centrifugation at 12,000 x g for 10 minutes. Phage protein was determined by the method of Lowry et al. (8).

Capsids. Virus capsids were prepared by rapidly freezing and thawing 10 mg of infectious phage in buffer A 10 times and incubation with 10 µg/ml of RNAse at 37° C for 30 minutes. Viral capsids were purified by centrifugation in cesium chloride density gradients (refractive index of 1.363) at 70,000 x g for 72 hours in the No. 40 rotor of the Spinco L2-65B ultracentrifuge. The tubes were pierced from the bottom and the capsid band was collected and dialyzed against 3 changes of buffer A for 24 hours.

Antisera. Antiserum to capsids was prepared by immunizing San Juan rabbits (9) with weekly intramuscular injections of 1 mg of capsid protein in complete

Freund's adjuvant for 4 weeks. Four weeks after the final injection the animals were injected intravenously with 1 mg of capsid protein. Five days later the animals were bled and the serum was stored at -20° C.

Gel Diffusion Analysis. Double gel diffusion analysis was carried out on standard microscope slides using 0.75% agarose (Marine Colloid, Springfield, N. J.) dissolved in 0.015 M NaCl and 0.01% merthiolate. A micro immunodiffusion matrix (Mann Research Laboratories, New York, N.Y.) was used. The slides were incubated in a water saturated atmosphere at room temperature for 24 hours.

Molecular Weight and S Value Determination. All physical measurements were done on a Spinco Model E Analytical Ultracentrifuge. Subunit molecular weights were calculated by the miniscus depletion method (10) using absorption optics (11, 12, 13). The S values of viral capsids and reaggregated particles were calculated by measuring sedimentation using schlieren optics.

Analytical Acrylamide Electrophoresis. Solutions of protein subunits and viral capsids were analyzed by polyacrylamide Disc electrophoresis as described by Davis (14). Samples were mixed in the upper gel before polymerization and 7% separating gels, pH 9.5 were used. Electrophoresis was carried out for 1 hour at 5 mA/gel. The gels were stained with aniline black for 1 hour and destained electrophoretically. The gels were photographed using Polaroid transparency film (Type 46-L) and the transparencies were traced in a Joyce-Loebl microdensitometer.

RESULTS

When MS-2 capsid protein solutions were prepared by dissociating intact virus particles with acetic acid, they were essentially free of RNA. Table I indicates that approximately 1% of the radioactivity from H^3-uridine was associated with a viral protein solution prepared from phage labeled with H^3-uridine.

The protein was shown to be monomeric and to have a molecular weight of approximately 15,000 in 66% acetic acid using the miniscus depletion method with absorption optics for molecular weight determination. When solutions of MS-2 protein were dialyzed against distilled water for 24 hours at 4° C, a

TABLE I*

A comparison of radioactivity from H^3-uridine in MS-2 virus and purified protein.

	cpm/mg protein
Intact Virus Particles	13,250
Viral Protein	180

* Approximately 3 mg of phage, labeled with H^3-uridine, was treated with acetic acid and viral protein was prepared as described in Materials and Methods. Samples were placed in 10 ml of Omnifluor (New England Nuclear Corp., Boston, Mass.) and radioactivity was measured in a Beckman LS-100 liquid scintillation counter.

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precipitate formed that only partially dissolved after the pH and ionic strength of the solution were carefully adjusted to 8.5 and 0.015 M respectively. After removing the precipitate by centrifugation at 12,000 x g for 10 minutes, the supernatants and capsids were examined in the analytical ultracentrifuge (Fig. 1). Under these pH and ionic conditions, the protein subunits aggregated to

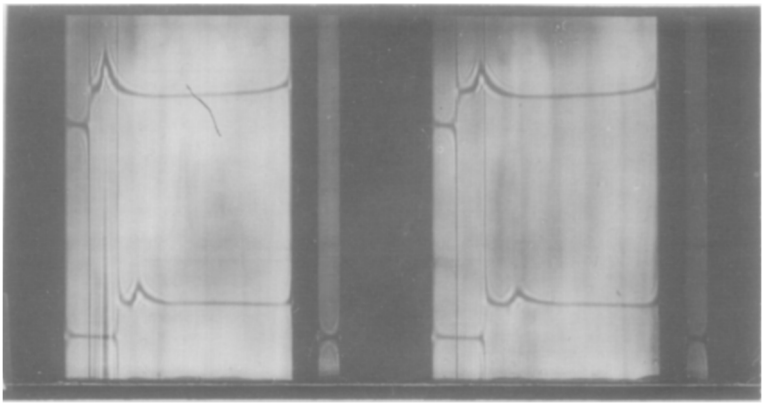

Figure 1: Top: Capsids 0.5 mg/ml in buffer A ($S_{w}20$ = 43).
Bottom: Reaggregated protein 0.1 mg/ml in 0.015 M NaCl, pH 8.5 ($S_{w}20$ = 55). The speed was 29,000 RPM; pictures were taken at 4 minute intervals with a bar angle of 55° in 30 mm cells in an AN-E rotor.

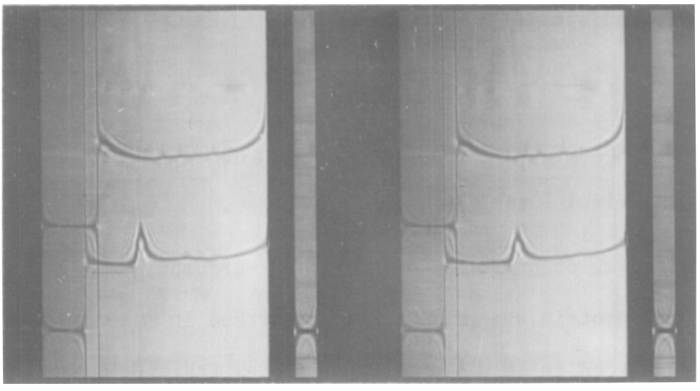

Figure 2: Top cell: Protein 1 mg/ml 0.01 M acetic acid.
Bottom cell: Capsid 1 mg/ml in buffer A ($S_{20,w}$ = 43). The speed was 42,040 RPM; pictures were taken at 4 min intervals with a bar angle of 55° in 12 mm cells in an AN-D rotor.

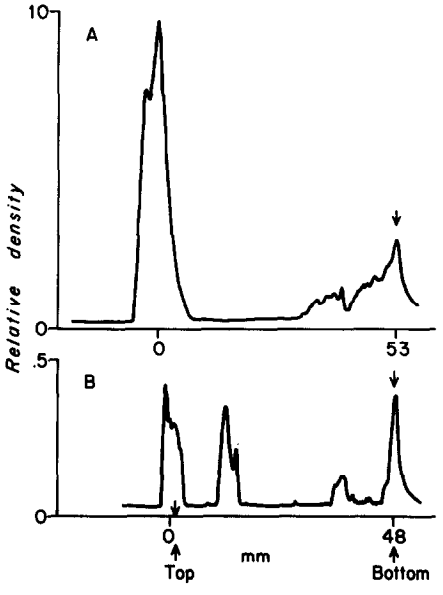

Figure 3: Microdensitometer tracing of (A) viral capsids and (B) viral protein in 0.01 M acetic acid.

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form particles with a markedly higher sedimentation constant (55S) as compared to capsid particles (43S). When capsids were exposed to similar pH and ionic conditions they continued to sediment at 43S.

Fig. 3 shows the microdensitometer tracings of polyacrylamide columns of samples of MS-2 protein in 0.01 M acetic acid and capsids in buffer A. The protein solution contained 2 major species: one of low molecular weight that migrated near the bottom of the column and presumably is the monomeric protein and one larger structure that moved a lesser distance (Fig. 3B). The capsid particles could not penetrate the column because of the small pore size of the 7% acrylamide (Fig. 3A). However, the capsid solution contained small amounts of monomeric protein, indicating that perhaps these preparations naturally tend to dissociate. When the sedimentation properties of viral protein in 0.01 M acetic acid were compared with capsids, the intermediate structure(s) and the monomer failed to sediment indicating that under these conditions they are of decidedly lower molecular weight than the capsids (Fig. 2).

Fig. 4 shows a double gel diffusion analysis of MS-2 protein in 0.01 M acetic acid, capsids and reaggregated particles. Antiserum to capsids formed two distinct precipitin bands with MS-2 protein that were antigenically unrelated to either capsids or self-assembled particles. This data also indicates that the protein subunits self assemble to form particles antigenically related to intact capsid particles.

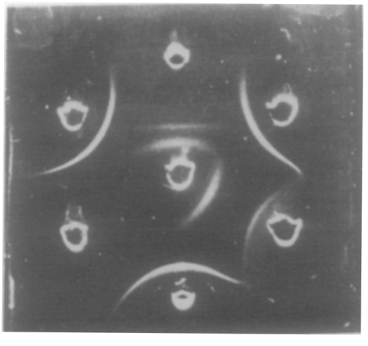

Figure 4: Ten, 2 and 6 o'clock positions: capsid protein 0.5 mg/ml in buffer A. Twelve o'clock protein 1 mg/ml in 0.01 M acetic acid, 4 o'clock reaggregated protein 1 mg/ml.
Note: If the concentration of capsid protein is raised, both of the inner bands seen with the 0.01 M acetic acid preparation appear.

DISCUSSION

This study on MS-2 protein has confirmed the earlier work on fr protein and extended the exploration of the self assembly of icosahedral viruses to the antigenic change occurring during assembly. The assembly process appears to proceed through a series of steps from the monomeric subunits found in 66% acetic acid through a larger aggregate in 0.01 M acetic acid to the 55S particle.[1] Upon forming a higher configuration (55S particle) the structure is altered and loses the antigenic characteristics of the intermediates. This 55S particle is antigenically related to the capsid antigen and the infectious virus (15). This could indicate that it has a similar surface configuration to these two particles. If Hohn's hypothesis (4) that the self-assembled protein contains an inner core of protein is correct, one would expect it to be similar in tertiary structure to the capsid and the infectious virus and therefore to be related. This also implies that although RNA is not required for assembly the protein structure requires something around which to condense. Further studies now underway are focusing upon the molecular weight of the intermediates consistantly found in the 0.01 M acetic acid preparations. If the structures of these particles can be elucidated the process by which monomeric protein subunits aggregate into virus-like particles will be further defined.

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REFERENCES

1. Caspar, D. L. D. and Klug, A. (1962). Symp. Quant. Biol., 27, 1.
2. Bancroft, J. F., Wagner, G. W. and Bracker, C. E. (1968). Virology, 36, 146.
3. Hermann, R., Schubert, D. and Rudolph, U. (1968). Biochem. Biophys. Res. Commun., 30, 576.

[1] It should be noted here that our S values differ significantly from those calculated by Hohn (4) and Hermann et al. (3). This is probably due to our use of the analytical ultracentrifuge rather than sucrose gradients.

4. Hohn, T. (1969). Biochem. Biophys. Res. Commun., 36, 7.
5. Rohrmann, G. F. and Krueger, R. G. J. Immunol, in press.
6. Strauss, J. H. and Sinsheimer, R. L. (1963). J. Molec. Biol., 7, 43.
7. Vinuela, E., Algranati, I. D. and Ochoa, S. (1967). E. J. Biochem., 1, 3.
8. Lowry, O. H., Rosbrough, H. J., Farr, A. L. and Randall, R. N. (1951). J. Biol. Chem., 193, 265.
9. Thompson, J. J. and Evans, C. A. (1964). Lab Animal Care, 14, 155.
10. Yphantis, D. A. (1964). Biochemistry, 3, 297.
11. Hanlan, S., Lamers, K., Lauterback, G., Johnson, R. and Schachman, H. K. (1962). Arch. Biochem. Biophys., 99, 157.
12. Schachman, H. K., Gropper, L., Hanlan, S. and Putney, F. (1962). Arch. Biochem. Biophys., 99, 175.
13. Lamers, K., Putney, F., Steinberg, I. Z. and Schachman, H. K. (1963). Arch Biochem. Biophys., 103, 379.
14. Davis, B. J. (1964). Ann N.Y. Acad. Sci., 121, 321.
15. Rohrmann, G. F. and Krueger, R. G. Manuscript in preparation.