Spherical Protein Shell Formation from an 11S Subunit of Bacteriophage f_2

Abstract. *An 11S protein component obtained from specific dissociation of bacteriophage f_2 underwent a change in the state of association when dialyzed to pH 4. At low temperatures a dissociation to 5.5S subunits was observed, while at 24° to 34°C a 37S particle was formed. The latter particles have properties which closely resemble those expected for the RNA-free viral capsid.*

Ribonucleic acid–mediated self-assembly in vitro of the protein shells of small RNA-containing bacteriophages has been described (*1*). The self-assembly of phagelike particles from protein in the absence of RNA has also been reported (*2*, *3*). However, these particles have properties (for example, sedimentation rate and penetrability by negative stain) which differ from those expected for the viral protein shell. Hohn (*3*) discusses several possible alternatives which account for these discrepancies but favors the hypothesis that the 68*S* particles that he obtained consist of an outer shell of 180 subunits arranged in a manner identical to that of the virus, but contain additional subunits inside, possibly arranged as a small 60-subunit icosahedron with a triangulation number (*T*) of 1.

We report here the self-assembly of spherical protein shells from an 11*S* protein component derived from bacteriophage f_2 (*4*). The properties of these shells are consistent with those expected for RNA-free f_2 capsids. In addition, the 11*S* protein starting material is quite soluble in a variety of solvents, is stable, and is easily manipulated; therefore the 11*S* protein may lead to a more convenient way to study self-assembly in terms of defining intermediates in the process, of obtaining thermodynamic parameters, and in studying the effect of various conditions on reconstitution.

The preparation and characterization of 11*S* protein subunits from f_2 has been described previously (*4*). For our study, the 11*S* component was further purified from the small amount of contaminating RNA by ultrafiltration in 4*M* guanidine hydrochloride buffers. A 280/260 nm absorbancy ratio of 1.6 or greater was obtained for all 11*S* protein subunits used for the self-assembly experiments.

The 11*S* subunits were dialyzed at 4° to 6°C against a buffer (0.03*M* sodium acetate and 0.1*M* KCl titrated to *p*H 4.0 with acetic acid at 4°C). Examination of the protein by sedimentation velocity with the ultraviolet scanning system at 280 nm showed that up to 85 percent of the protein was present in a boundary with an $s_{20,w}$ value of 5.5*S*. Most of the remaining protein sedimented at 11*S* with some protein sedimenting between 5.5 and 11*S*. Reversal of the 11*S* to 5.5*S* conversion was confirmed by redialyzing to *p*H 7.0 [0.04*M* tris(hydroxymethyl)-aminomethane, 0.10*M* KCl, and 0.005*M* $CaCl_2$, titrated to *p*H 7.0 at 4°C with HCl]. The 5.5*S* material was reconverted to 11*S* subunits (70 percent 11S; 30 percent specific aggregates) as demonstrated by identical sedimentation rates in a multiplex scanner run, and by the same predominantly elliptical appearance with central stain penetration when observed in the electron microscope (*4*).

Protein (about 0.6 mg/ml) dialyzed to *p*H 4.0 and incubated at 24° to 34°C was converted in 80 percent or higher yield to a product which sediments as a sharp symmetrical 37*S* boundary. The remainder of the protein sedimented more rapidly and was heterogeneous. At 31°C, the conversion was complete in 1 hour; at temperatures above 34°C some precipitation occurred. The reaction has not been studied below 24°C or at *p*H values below 4. We attempted reconstitution at *p*H 5.0 but obtained a more heterogeneous product with a higher average sedimentation rate. Both the higher sedimentation rates (55 to 70*S*) at this *p*H and the different morphological appearance in the electron microscope suggest a closer though not identical relation of these particles to those obtained by Herrmann *et al.* (*2*) and by Hohn (*3*).

The 37*S* particle was further characterized after dialysis at 23°C to *p*H 7. The sedimentation velocity pattern shows (Fig. 1) that, except for about 15 percent aggregation, all sedimenting material is represented by the 37*S* boundary. A single band was obtained in gel electrophoresis at *p*H 7.7 with mobility indistinguishable from f_2 or "artificial top component" (empty shells) prepared as described by Kaesberg (*5*) (that is, mixtures of the three particles taken two at a time gave only a single band in the 4 percent gel after moving 2.4 cm). The aggregates evidently were either too heterogeneous to be visualized or did not enter the gel because of molecular exclusion. We compared electron micrographs of the reconstituted incubation mixture stained at *p*H 6.8 with uranyl oxalate to identically stained f_2 (Fig. 2).

The more limited penetration of the negative stain into the virus itself and a greater lability of the 37*S* particle to negative staining characterized the expected differences between the two particles. Micrographs obtained on "artificial top component" were indistinguishable from those shown for the 37*S* particle. A possible exception is the apparently fewer degraded particles observed in "artificial top component" which might indicate some imperfection

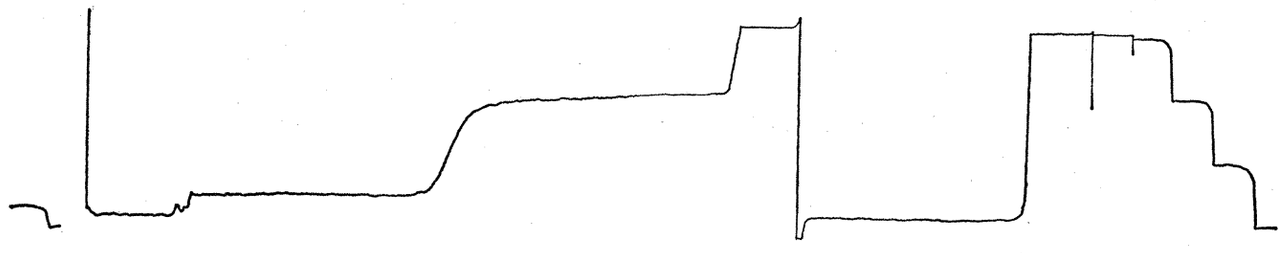

Fig. 1. Sedimentation velocity of reconstituted incubation mixture at *p*H 7.0 and 19.3°C. The photoelectric scanner pattern was taken at 280 nm 40 minutes after a speed of 30,000 rev/min was attained.

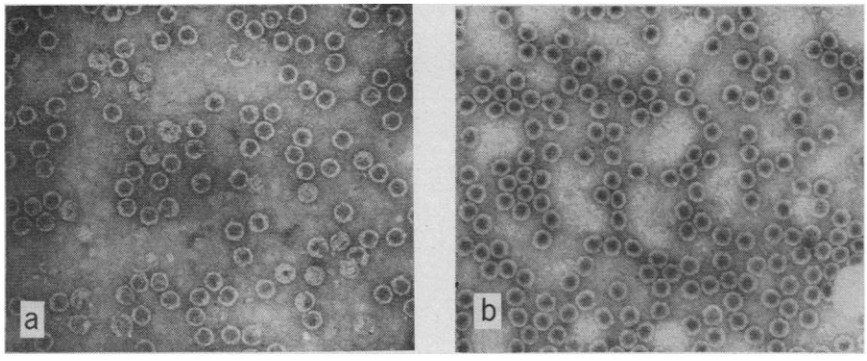

Fig. 2. Electron micrographs negatively contrasted with uranyl oxalate. (a) Reconstituted incubation mixture. (b) Bacteriophage f_2. Photographs were taken on a Philips EM-200 electron microscope at 80 kv with an anticontamination device, double-condenser illumination, and a 35-μm objective aperture. Thin carbon films were used for specimen support.

in the assembly of a few of the 37*S* particles. Objective statistical evaluation of the extent of degradation was complicated by the fact that preservation is increased in areas of thicker stain and that relative stain thickness between two grids is difficult to evaluate. Bacteriophage f_2, "artificial top component," and 37*S* particles were also examined by electron microscopy after staining with 1 percent phosphotungstate (PTA) at *p*H 7. The latter two specimens again appeared identical and differed from the appearance of negatively contrasted f_2 only in that the PTA stain was largely excluded from the virus but extensively penetrated the empty shell structure (*3*). Particle diameters were obtained in each case by internal calibration against the 84.4-Å half-period (*6*) of admixed crystals of beef-liver catalase. "Artificial top component" and 37*S* particles both had a mean diameter of 238 ± 10 Å and f_2 measured 246 ± 7 Å; therefore they have an identical size within experimental error.

The identical morphological appearance of the 37*S* particle and "artificial top component" supports the hypothesis that both should have a similar frictional coefficient. Sedimentation velocity experiments with the photoelectric scanning optical system provided an $s_{20,w}$ value of 41*S* for "artificial top component" which contained about 3 percent RNA as estimated from the 280/260 nm absorbancy ratio. Using the physical and chemical parameters obtained by other workers as previously referred to by us (*4*), we calculated the sedimentation coefficient of f_2 protein shells to be 39*S*, which is in good agreement with that observed for the 37*S* particles.

The formation of the 37*S* particle seems compatible (within the limitations of the characterization employed) with the hypothesis that it represents the reconstitution of f_2 capsids without the intervention of RNA, core protein, or other assembly-directing influence. We have thus far obtained no indication as to whether either 11 or 5.5*S* subunits are direct intermediates in the assembly process, nor have we as yet unambiguously identified additional intermediates in the reconstitution. Consequently, detailed mechanistic proposals for capsomer interactions and assembly of 37*S* particles are premature at this time (*7*).

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References and Notes

1. T. Sugiyama, R. R. Hebert, K. A. Hartman, *J. Mol. Biol.* **25**, 455 (1967); T. Hohn, *Eur. J. Biochem.* **2**, 152 (1967); J. W. Roberts and J. E. A. Steitz, *Proc. Nat. Acad. Sci. U.S.* **58**, 1416 (1967); P. P. Hung and L. R. Overby, *Biochemistry* **8**, 820 (1969).
2. R. Herrmann, D. Schubert, U. Rudolph, *Biochem. Biophys. Res. Commun.* **30**, 576 (1968).
3. T. Hohn, *ibid.* **36**, 7 (1969).
4. P. Zelazo and R. Haschemeyer, *Biochemistry* **8**, 3587 (1969).
5. P. Kaesberg, in *The Molecular Biology of Viruses*, J. S. Colter and W. Paranchych, Eds. (Academic Press, New York, 1967), p. 241.
6. R. T. Murray, thesis, University of Cambridge (1967).
7. The results described here resemble in some aspects those described by Bancroft and coworkers [J. B. Bancroft, G. W. Wagner, C. E. Bracker, *Virology* **36**, 146 (1968); J. B. Bancroft, C. E. Bracker, G. W. Wagner, *ibid.* **38**, 324 (1969)] for the self-assembly of "pseudotop component" of cowpea chlorotic mottle virus and brome mosaic virus. However, the general significance of similarities and differences between the bacteriophage and plant virus systems is as yet too obscure to justify a detailed comparison.
8. Supported by PHS grant HE 11822 and NSF grant GB-7042. We thank Professor R. Swan and the Department of Anatomy for providing time on their Philips EM-200 electron microscope.

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Australia Antigen: Distribution during Cohn Ethanol Fractionation of Human Plasma

Abstract. *When plasma containing a hepatitis-associated antigen (Au/SH) is fractionated, the antigen is localized in fractions III and IV with none in fraction II and only small amounts in fractions I and V. The amount of antigen found in each of these fractions is probably not predictive of clinical infectivity of Cohn ethanol fractions from normal pooled plasma.*

The fractionation of human plasma by methods 6 and 9 of Cohn and associates (*1*) has been adopted on an industrial scale and yields protein fractions of therapeutic usefulness. However, the risk of transmitting serum hepatitis has been a deterrent to maximum utilization of some plasma protein fractions. With the demonstration that a specific antigen (the "Australia antigen," now often known as hepatitis-associated antigen) was often associated with hepatitis (*2*) and the subsequent confirmation and extension of this association in numerous laboratories (*3–5*), an assay became available which allowed us to follow this antigen (Au/SH) when plasma is fractionated.

For this study, we began with human plasmas containing Au/SH. These plasmas were detected during screening of plasmapheresis donors (*4*), and the Au/SH content of the Cohn fractions was assessed by means of a gel-diffusion procedure (*3*). Two 500-ml portions of different Au/SH-positive units of plasma were fractionated by Cohn methods 6 and 9 (*1*). During these fractionations, samples (0.5 g) of the moist precipitates or of the alcoholic supernatant (5 to 20 ml) were diluted with water and lyophilized to remove alcohol. The dry powders were then dissolved in a measured volume of an appropriate buffer and assayed for Au/SH content (*6*). In all cases the