SELF-ASSEMBLY OF PROTEIN SUBUNITS FROM BACTERIOPHAGE fr

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It is well known that under appropriate conditions protein subunits of tobacco mosaic virus (TMV) can reaggregate to form virus-like rods (Schramm, 1943; Schramm and Zillig, 1955; Fraenkel-Conrat and Singer, 1957). Caspar and Klug (1962) have predicted that such a self-assembly should also be possible for icosahedral viruses. Until now, however, it has only been possible to obtain protein shells of such viruses by degradation of virus solutions (Finch and Klug, 1960) or by reaggregation of protein subunits in the presence of nucleic acid (Sugiyama et al., 1967; Hohn, 1967; Bancroft and Hiebert, 1967).

Using the bacteriophage fr, an icosahedral virus of particle weight 4×10^6 Daltons (Hoffmann-Berling et al., 1963) and diameter of 260 Å (Frank, 1967) we have tried to achieve the self-assembly of icosahedral shells from protein in the absence of nucleic acid. The coat protein used as starting material was in the monomeric form. In this paper it is shown that it is indeed possible to obtain virus-like protein shells in a rather high yield.

Materials and Methods

a) fr-virus : The phage was grown as described by Hoffmann-Berling et al. (1963), using the trypton medium of Knippers and Hoffmann-Berling (1966). Phage containing ^{32}P-RNA was obtained as described by Heisenberg (1966).

b) fr-protein : Isolation of protein was carried out using a modified acetic acid method (Fraenkel-Conrat, 1957). Two volumes of acetic acid were added to one volume of a solution of virus (4%) in water. The mixture was left in an ice bath for about 15 min. By this time most of the RNA had precipitated and it was removed by low speed centrifugation (5 min). After a further centrifugation at 3o,ooo rpm for 2o min, the supernatant was diluted with an equal volume of cold water. The solution was then applied to a column of Sephadex G 5o which had been equilibrated with 33% acetic acid. The fractions containing the protein were collected and dialyzed against water for 24 hours. The solution was concentrated by negative pressure dialysis and was brought to pH 7.5 with o.o2 M NaOH. Most of the precipitate which had formed was dissolved by this adjustment of the pH. After centrifugation at 3o,ooo rpm for 2o min the clear supernatant was dialyzed against o.o2 M phosphate buffer pH 7.6 containing o.1 M NaCl. The final protein concentration was between o.5 and 2 mg/ml.

^{32}P labelled phage ($1x1o^5$ cpm/mg protein) was added to the virus solution to determine the amount of RNA remaining in the protein preparation. Measurement of the radioactivity showed that after column chromatography the counts per mg protein had decreased to less than o.o5% of the value of the original material. Before column chromatography the preparation contained about 5% RNA. The UV absorbance ratio A_{26o}/A_{28o} of the

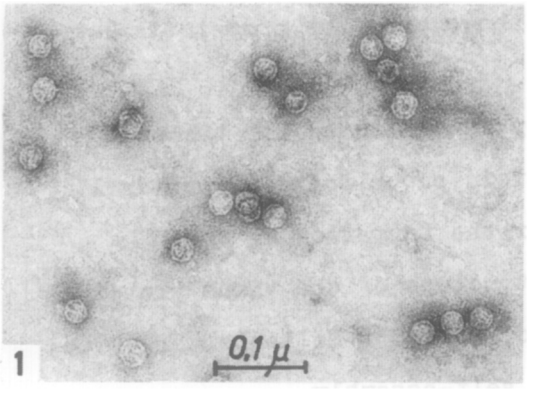
purified preparations measured in 33% acetic acid was o.53-o.59 (without correction for light scattering).
The preparation and purification of the protein was usually carried out in an ice bath, although essentially the same results were obtained at room temperature.
c) Physical measurements : Ultracentrifugation was performed on a Spinco Model E Analytical Ultracentrifuge using schlieren optics. All measurements were made at (2o.o$\pm$ o.1)^{o}C. UV-absorbance was measured on a Zeiss MQ III spectrophotometer. ^{32}p-disintegrations were counted on a Packard liquid scintillation counter. Electronmicrographs of preparations negatively stained with uranyl acetate were kindly made by Dr.H.Frank and Mr.G. Berger on a Siemens-Elmiskop I A.
d) Serological test : Rabbit antiserum against fr was the generous gift of Dr. H.D.Schlumberger. The antigen-antibody precipitates were obtained according to the method of Heidelberger and Mac Pherson (1943).

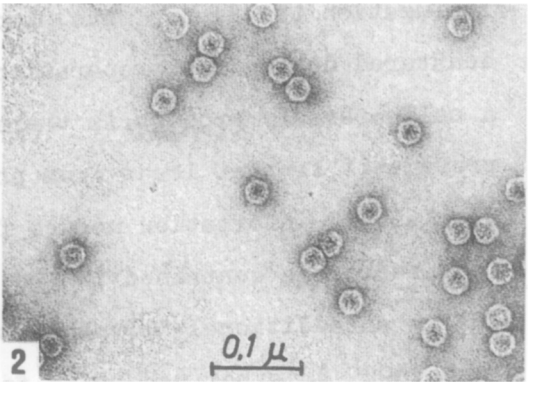
Results and Discussion

To prove that the coat protein of the virus is split to monomeric subunits with acetic acid in the same manner as the protein of TMV (Wittmann, 1959) molecular weight determinations by the sedimentation equilibrium method (Schachman, 1959) were performed. In 66% acetic acid and in 66% acetic acid plus o.1M NaCl we have found a value of 16 ooo, assuming o.75 ml/g for the protein partial specific volume in this solvent (Anderer et al., 1959; Wittmann, 1959). The molecular weight of 16 ooo is in good agreement with the value of 16 6oo obtained by chemical methods for the protein subunit of fr (Wittmann-Liebold, 1966).

It was apparent from the electron micrographs that after dilution to 33% acetic acid the solutions do not contain undegraded virus. Significant impurities of virus or RNA in the preparations are also excluded by the results of experiments with ^{32}P-labelled virus and by the low ratio E_{260}/E_{280}.

Highly opalescent solutions were obtained after the protein had been dialyzed against phosphate buffer pH 7.6 . It was therefore supposed that the solutions contained high molecular weight aggregates. In the electron microscope, these aggregates appeared to be spherical particles (Fig. 1), having diameters, within the limits of error, equal to that of the intact virus (compare with Fig. 2).





Electronmicrographs of reaggregated protein (Fig. 1) and intact virus (Fig. 2).

At concentrations of about 1mg/ml and higher, side to side aggregation of the particles can be observed. The protein sediments homogeneously in the ultracentrifuge with s_o = 68S showing a hypersharp boundary. Under the same contidions the intact virus sediments with s_o = 80S.

Essentially all of the material visible in the electron microscope was present as virus-like particles. There was very

little non aggregated material or material aggregated in other ways. The sedimentation diagrams show that most of the protein sediments with the 68S peak. From the electron micrographs and the ultracentrifugation measurements, it can be concluded that more than 50% of the protein obtained by purification and concentration is reaggregated to virus-like particles.

We had no difficulties in reproducing this apparently specific and extensive reaggregation. The reaggregated material was precipitated by fr-antiserum indicating that its surface structure is at least very similar to that of the intact virus.

In comparison with the method of Hohn (1967) the procedure used here leads to a higher solubility of the protein in neutral buffers. This not only enables the use of normal ultracentrifugation techniques, bus also shows that the protein is less denatured during its preparation.

A self-assembly process is thereby demonstrated for the coat protein of fr that leads from protein subunits to icosahedral shells. All information needed for the formation of the icosahedron is contained in the structure of the virus protein. RNA as an additional "morphopoetic factor" (Kellenberger, 1966; Hohn, 1967) is not necessary for self-assembly.

Experiments will be undertaken to show whether minor differences exist between the structure of the empty protein shell and the protein coat of the intact virus and to elucidate the function of the "A-protein" (Argetsinger-Steitz, 1967) in the process of assembly of the complete virus.

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