THE ASSEMBLY OF PROTEIN PARTICLES OF THE RNA BACTERIOPHAGE fr IN ABSENCE OF RNA

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Summary

In absence of RNA the selfassembly of fr phage-like particles proceeds at a lower rate and a lower ionic strength than in presence of RNA. The resulting particles are similar in diameter to the empty capsids obtained by phage degradation, however, they have a higher sedimentation constant and are less penetrated by negative stain. A hypothesis for the structure of these particles is offered.

Three ways can in theory be visualized leading to a successful formation of a viral nucleocapsid: (1) The nucleic acid first forms a core with or without the aid of minor protein components, around which the capsid is assembled in a second step. (2) The nucleic acid is incorporated into an empty preformed capsid. (3) Condensation of the nucleic acid and formation of the capsid occur simultaneously and catalyze each other.

Attempts were made to distinguish between these possibilities in the case of the icosahedral RNA bacteriophage fr by studying its reconstitution in vitro. Two sets of conditions have been described for the reconstitution of the phage capsid, one depending on the presence of RNA (Hohn, 1967; Sugiyama, Hebert and Hartmann, 1967), which does not have to be specific in length and base composition (Hohn, 1969) and one

independent of RNA (Herrmann, Schubert and Rudolph, 1968). While the former assembly system can best be explained by the third hypothesis, i. e., the simultaneous condensation of RNA and assembly of the capsid, the latter assembly system could favor an explanation according to the second hypothesis, i. e., the incorporation of the RNA into the preformed capsid. The first hypothesis, which would predict a specific RNA being a necessary component in capsid formation is made unlikely by both assembly systems. In this study a comparison was made of the rates and ranges of conditions of the RNA dependent and the RNA independent capsid reconstitution. The product of the RNA independent assembly system is discussed in comparison with the empty shell, which can be obtained by degradation of the phage. These studies were made possible by using a method of adjusting the ionic strength of the aqueous protein solution by addition of concentrated buffer rather than by a dialysis method.

Materials and Methods

fr, S^{35} labelled fr, P^{32} labelled fr and fr RNA were isolated as described and cited elsewhere (Hohn, 1967). Unlabelled and labelled phage were mixed for each experiment to give the appropriate concentrations and radioactivities. Polyuridylic acid was purchased from Calbiochem.

fr protein was isolated in reference to the method of Herrmann, Schubert and Rudolph (1968): 2 mg fr, labelled with 10^5 cpm S^{35} and 10^5 cpm P^{32} and dissolved in 200 µl TSE-buffer (0.02 M trischlorid, pH 7.2, 0.15 M NaCl, 0.001 M EDTA) was added to 400 µl precooled glacial acetic acid. The mixture was kept in the ice bath for 15 min. and the precipitated RNA removed by low speed centrifugation. The protein solution was dialyzed for 24 hours against 3 changes of distilled water, brought to

pH 8.5 by addition of NaOH and centrifuged at 30,000 rpm for 20 min. The supernatant contained 90% of the phage protein and was contaminated with less than 3% of the RNA originally present.

Empty shells were obtained by freezing and thawing fr prior to RNAse digestion and separation by glucose gradient centrifugation (Hohn, 1967). Other degradation methods (Kaesberg, 1966; Argetsinger-Steitz, 1968) gave similar results. The assembly of fr protein in presence of polyuridylic acid (Hohn, 1969) was also used to obtain empty shells.

Results

Fig. 1 shows sucrose gradients of fr protein samples which were kept with and without RNA in buffered NaCl-solutions of different molarities. In absence of RNA the protein, which was kept in 0.15 M NaCl remained nearly quantitatively at the meniscus (Fig. 1b), while the protein kept at 0.015 M NaCl sedimented nearly quantitatively at 68S (Fig. 1d) as compared to the sedimentation constant of the viable phage (80S). If RNA (60 μg, labelled with 5×10^3 cpm P^{32}) was added to the protein prior to adjustment of the ionic strength, phage like particles (Hohn, 1967) consisting of RNA and protein and sedimenting at 70 S with shoulders at 80S and 55S, and a small amount of complex I (Sugiyama, Hebert and Hartman, 1967) were formed at both ionic strengths (Fig. 1a and Fig. 1c).

The 68S product of the RNA free assembly mixture was compared with the empty shells obtained by phage degradation and by the assembly process in presence of polyuridylic acid. The empty shells sedimented slower than the 68S particles, i.e., at 50S (Fig. 2a), whereas the buoyant density in CsCl was similar for all samples, i.e., 1.29 g/cc (Fig. 2b).

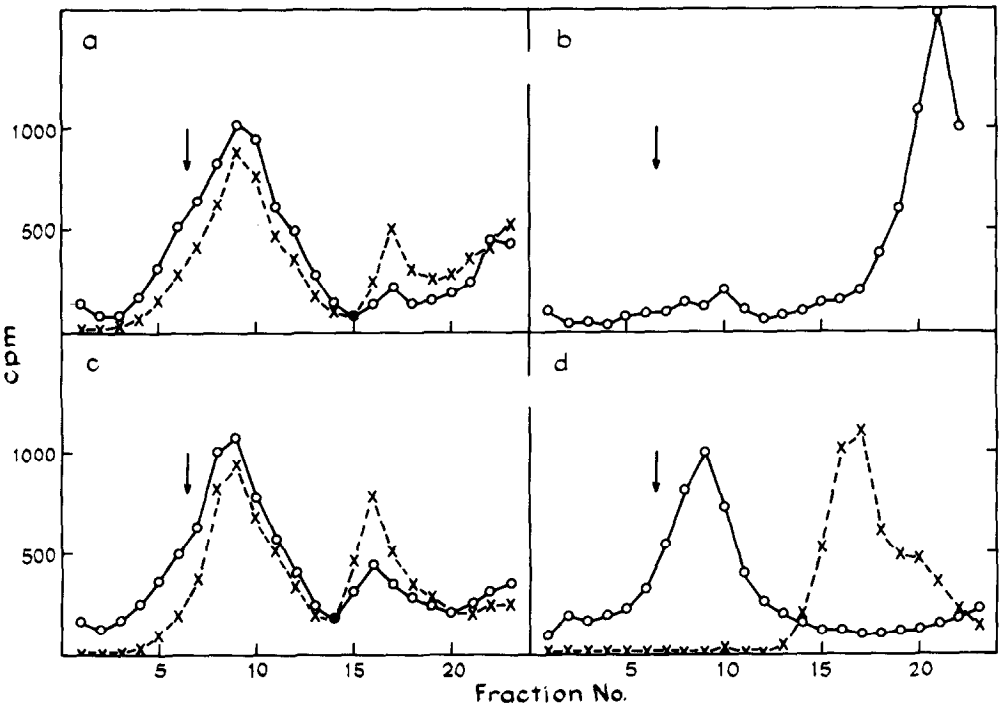


Fig. 1 Sucrose gradient centrifugation of different assembly mixtures.

Samples of 100 µg coat protein labelled with 8.10^3 cpm S^{35} and dissolved in 200 µl H_2O were mixed with

a) 40 µg fr RNA labelled with 5.10^3 cpm P^{32} and dissolved in 50 µl H_2O, and 25 µl 10 fold TSE buffer
b) 20 µl 10 fold TSE buffer
c) 40 µg fr RNA labelled with 5.10^3 cpm P^{32} and dissolved in 5 µl H_2O, and 2.5 µl 10 fold TSE buffer
d) 2 µl 10 fold TSE buffer

The samples were kept for 12 hr at 4°C. To one of the samples (d) 40 µg fr RNA labelled with 5.10^3 cpm P^{32} in 50 µl TSE buffer was added, centrifugation was performed in a linear sucrose gradient (5 to 20%) in TSE buffer for 1 hr at 4°C and 50.000 rev/min in the SW 50 rotor of a Spinco L2 centrifuge. Protein as radioactivity of S^{35} (o —— o), RNA as radioactivity of P^{32} (x —— x). Arrows indicate position of viable phage.

Electronmicroscopy of a negative stained assembly mixture in absence of RNA revealed particles with a diameter of 260 ± 10 Å (Plate 1a). These

particles were penetrated by the stain even less than the phage (Plate 1c) and appeared very distinct from empty shells (Plate 1b). About 2% of the particles have a diameter of only 160 $\pm$ 15 Å (insert in Plate 1a). This smaller diameter fits well with a T=1 icosahedral surface lattice, whose theoretical diameter would be 152 Å (Anderer, Schlumberger, Koch and Eggers, 1967). One of the small particles has a halo of additional protein, that one could speculate to be the remainder of a disrupted outer shell.

For the assembly processes in 0.015M NaCl with and without RNA the rate of particle formation was compared at 4°C, 25°C and 37°C. (Fig. 3). While the RNA containing phage-like particles were formed immediately after adjustment of the ionic strength, the assembly of 68S protein particles took a longer time: the half-optimal yield was obtained after 3 hours, 1.5 hours and 1 hour at 4°C, 25°C and 37°C, respectively.

The formation of 68S particles could in no case be observed in 0.15M NaCl, even when higher protein concentrations were used. Below a concentration of about 2 mg protein/ml all protein remained at low molecular weight (Fig. 1b), while above this protein concentration the protein precipitated immediately after the adjustment of the ionic strength.

Reaction between 68S particles and RNA was attempted by incubating 200 particles with 100 µg P^{32} RNA in 250 µl TSE buffer and in 250 µl 1/10 TSE buffer, both with and without 0.01M Mg^{++}, at temperatures both of 4°C and 37°C. Samples were assayed by sucrose gradient sedimentation after 6 hours and after 96 hours. The RNA sedimented in all cases unchanged at 28S (See Fig. 1d as an example); in no case it was found incorporated into protein particles.

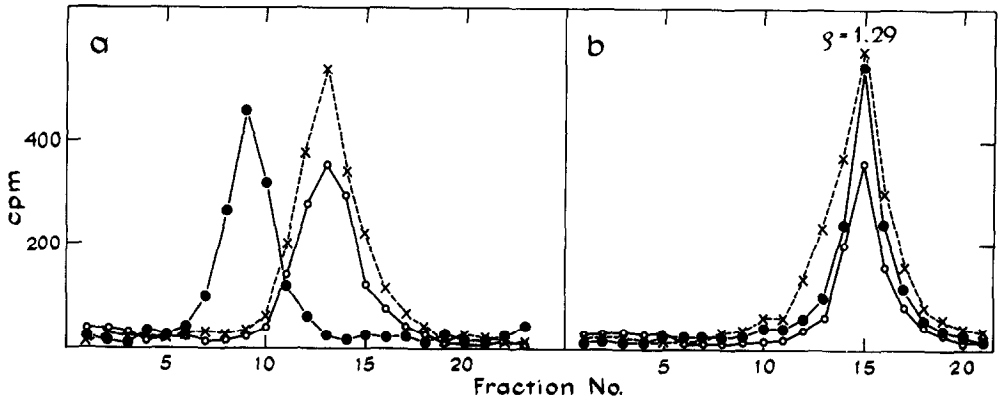


Fig. 2 Comparison of various protein particles in respect to sedimentation and buoyant density.

a) Sucrose gradient centrifugation performed as in Fig. 1

b) CsCl density gradients obtained by centrifugation of the sample in CsCl solution (mean density 1.35 g/cc) for 24 hr at 4°C and 35.000 rev/min in the SW 50 rotor of a Spinco L2 centrifuge.

In Fig. 2a and 2b the following preparations are analyzed and the gradients superimposed.

• — • preparation as in Fig. 1d

o — o 100 µg fr labelled with 10^4 cpm S^{35} was suspended in 100 µl TSE buffer and frozen at -20°C. After 30 min the solution was thawed at room temperature. Freezing and thawing was repeated twice. The sample was digested for 10 min at 37°C with 1µg pancreatic ribonuclease and centrifuged in a CsCl gradient (mean density 1.35 g/cc) for 24 hr at 4°C and 35.000 rev/min in the SW 50 rotos of a Spinco L2 centrifuge. The material banding at 1.29 g/cc was collected and dialyzed against TSE buffer.

x — x 100µg for protein, labelled with 8.10^3 cpm S^{35} and solved in 200 µl H_2O was mixed with 40µg polyuridylic acid solved in 50 µl H_2O and 25 µl 10 fold TSE buffer. The mixture was kept for 12 hr at 4°C, digested with 1 µg pancreatic RNAse for 10 min at 37° and centrifuged in a CsCl gradient as above. Most of the material was banding at 1.29 g/cc. It was dialyzed against TSE buffer.

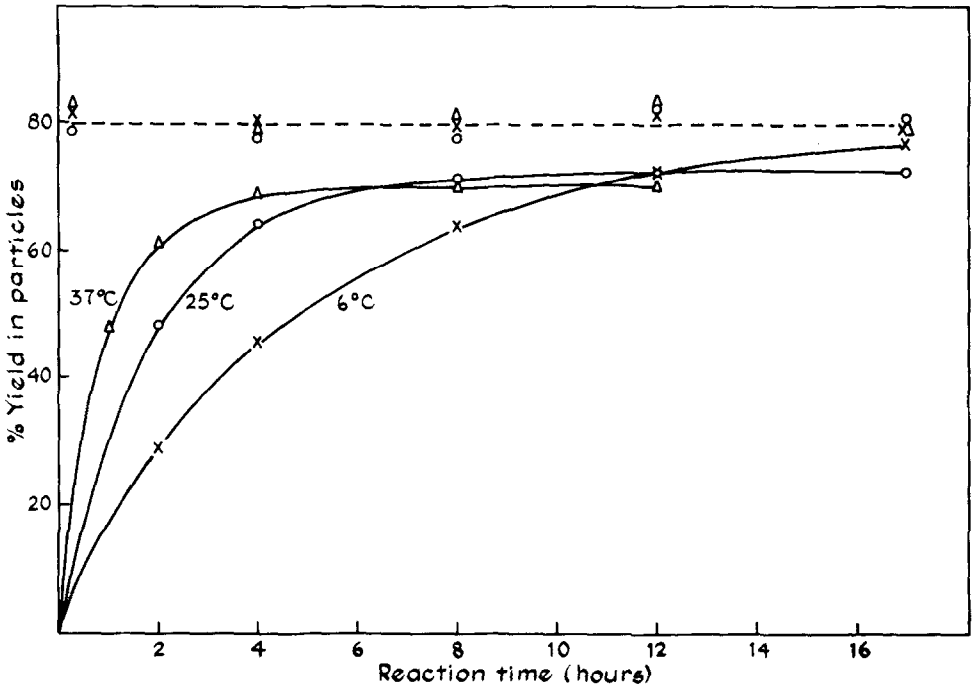


Fig. 3 Kinetics of the self assembly process of phage coat protein. All reactions were carried out in 1/10 TSE buffer. Solid lines (——) correspond to the formation of 68S particles in absence of RNA, broken lines (- - -) to the formation of RNA-containing phage like particles. x, reaction at 4°C; o, reaction at 25°C; Δ, reaction at 37°C. Yield is defined as the percentage of protein in sucrose gradients (Fig. 1) sedimenting between 50 and 80 S.

Discussion

Under certain conditions the coat protein of fr can be assembled in absence of RNA to form phage like protein particles. Obviously the coat protein contains enough information necessary to establish a capsid of normal diameter. On the other hand the RNA has a real catalytic effect on th capsid formation as seen in the wider range of conditions and the faster rate of the assembly process in presence of RNA. The RNA could not be incorporated into the preformed capsid under a variety of conditions studied.

Therefore it is likely that also in vivo the condensation of the RNA and the assembly of the phage capsid are processes that occur simultaneously and catalyze each other.

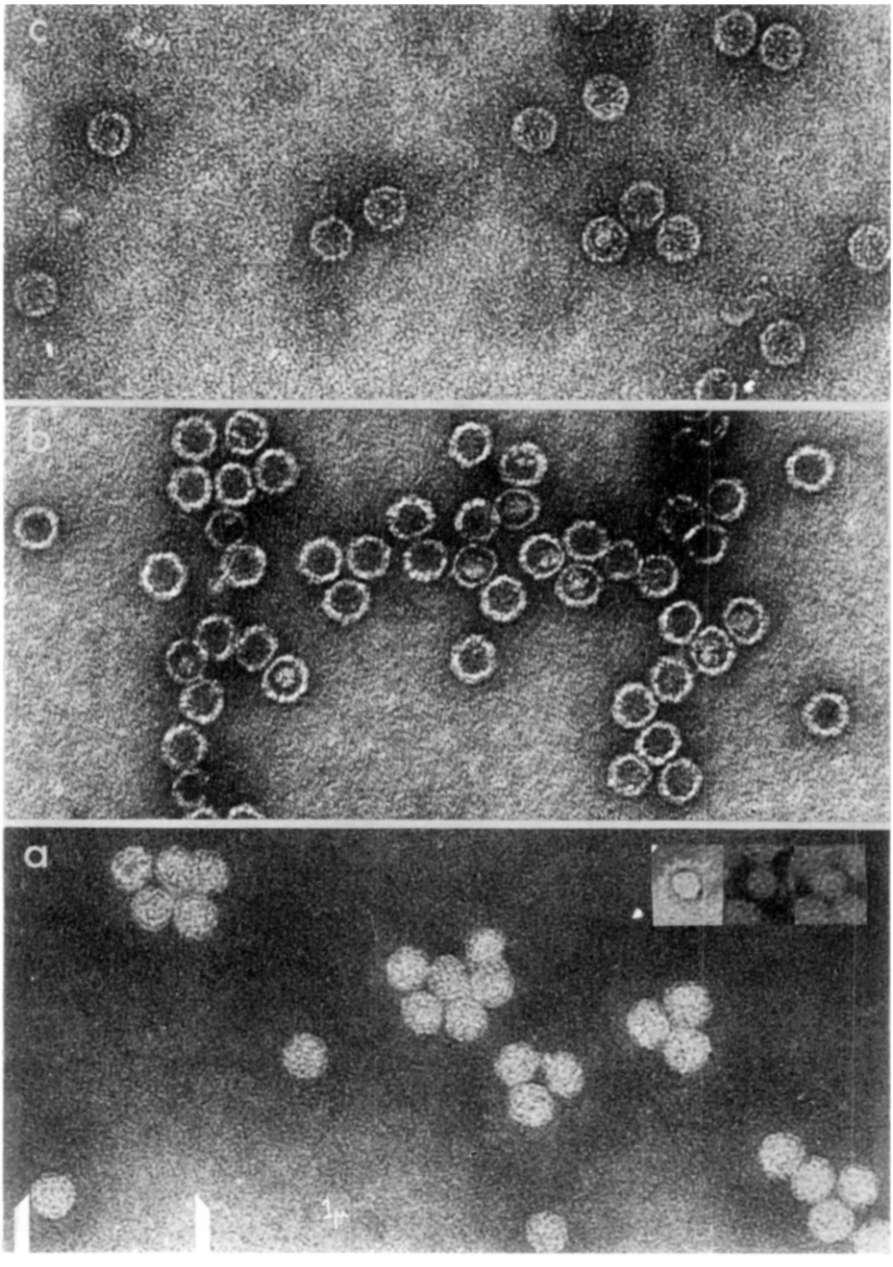
The difference between empty shells and 68S protein particles in sedimentation-constant and in penetration by negative stain can be explained by the following alternatives:

1) A different arrangement of subunits of the shell and of the 68S particle results in a lower friction of the latter.

2) The arrangement of subunits occurs in a similar pattern, however, some subunits of the shell are missing. It has holes.

3) The 68S particle consists of more protein subunits than the phage. 180 of these subunits are arranged similarly to the capsid of the phage and additional subunits within this shell are arranged either randomly or regularly.

A calculation of the molecular weight of the empty shell and of the 68S protein particle favors the third solution. In this calculation the friction of the empty shell, the 68S protein particle and the phage were assumed to be similar. The Svedberg equation and the available date for the phage fr were used (molecular weight of protein subunits 14.000 Daltons as calculated from the base sequence (Wittmann-Liebold, 1966; Weber, Notani, Wikler and Konigsberg, 1966); molecular weight of RNA, 1.1×10^6 Daltons (Gesteland and Boedtker, 1964); number of protein subunits, 180, partial specific volume of the phage, 0.69 (Marvin and Hoffmann-Berling, 1963); partial specific volume of protein 0.72 (mean value for proteins); sedimentation constant for the empty shell, 50S; sedimentation constant for the phage, 80S. The resulting molecular weight of 2.5×10^6 Daltons for the empty shell conforms well with the complete structure of 180



Negative stainings (2% phosphotungstate pH 6.8 of 68S particles obtained as in Fig. 1d(a), empty shells obtained by a self assembly of fr protein in presence of polyuridylic acid, as in Fig. 2 (b), fr (c).

subunits; the resulting molecular weight of 3.3×10^6 Daltons for the 68S particle conforms with a phage shell of 180 protein subunits filled with additional 60 protein subunits. These could well be arranged in a small icosahedron of the triangulation T=1, as might be seen in the insert of Plate 1a, the whole structure of the 68S particle thus resembling the structure of the bushy stunt virus (Harrison and Caspar, personal communications).

Such a structure could explain the process of its own formation and would agree with the prediction that a catalyst is necessary for the formation of the T=3 capsid or for capsids of higher order (Kellenberger, 1966). A small number of subunits arranged in a structure which has not necessarily high stability, but which has its negative charges on the surface could replace the negatively charged RNA in the function of a catalyst. As this catalyst is less effective than RNA, the assembly of the 68S protein particles occurs at a lower rate and is more effectively disturbed by counterions than the assembly of RNA containing phage-like particles.

In that connection it is interesting that self assembly experiments of other RNA viruses yield, in the absence of RNA, protein particles that differ from empty capsids: e. g., in absence of RNA protein subunits of the icosahedral cowpea chlorotic mottle virus form pseudo capsids having an electrophoretic mobility different from what would be expected for an empty capsid (Bancroft, Wagner and Bracker, 1968).

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