FORMATION OF DEFECTIVE BACTERIOPHAGE PARTICLES BY fr AMBER MUTANTS (II)

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Received March 2, 1967

A group of amber mutants of the bacteriophage fr, when grown in a non-permissive, ribonuclease I-deficient host produces so-called "defective particles" (previously called "heavy defective particles"). These particles do not adsorb to host bacteria and also differ from viable phage in their sensitivity to ribonuclease and their hydrodynamic properties. The direct function of the gene in which these mutations occur is unknown, but it has been proposed that it either might code for an adsorption protein which is lacking in the defective particles or might be necessary to give the viral RNA a conformation which allows complete encapsulation (Lodish, Horiuchi & Zinder, 1965; Heisenberg, 1966; Argetsinger & Gussin, in press). In this paper two experiments are described which support the latter assumption.

Previous experiments had suggested that in the "defective particles", which have the same buoyant density and amount of RNA as wild-type phage, portions of the RNA are at the surface of the protein shell (Heisenberg, 1966). These experiments demonstrated that 30% of the RNA of the "defective particles" is sensitive to

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ribonuclease and that the frictional coefficient of these particles is higher than that of viable phage, but can be reduced by ribonuclease treatment. To test this suggestion the reaction of the defective particles with anti-RNA-antiserum was studied. The serum was a kind gift of Professor J. Panijel who has shown that it reacts very effectively with single-stranded RNA but does not react with infectious RNA phage (Cayeux, Panijel & Souleil, 1965).

From the results of this experiment which are shown in the Table it may be seen that normal preparations of phage grown in ribonuclease-deficient cells contain a considerable amount of RNA accessible to anti-RNA-antiserum. In order to reduce this free RNA, both phage and defective particles were purified by sucrose gradient centrifugation of crude lysates. This method leaves the defective particles still completely precipitable. A ribonuclease treatment which reduces the amount of RNA in the purified defective particles to about 70% now completely inhibits the precipitation with anti-RNA-antiserum, showing that the RNA which is sensitive to ribonuclease is also accessible to the anti-RNA-antibodies. Either the protein shell has several large holes which permit the antibodies to reach the RNA and cause precipitation or parts of the RNA are exposed at the surface of the viral coat. The first alternative seems unlikely since the defective particles contain about the same amount of coat protein and appear identical to viable phage in the electron microscope (Heisenberg, 1966).

The second experiment describes a new kind of defective, fast-sedimenting particle or complex (FSM*) which was briefly mentioned previously (Heisenberg, 1966). The FSM is found in lysates of ribonuclease-deficient non-permissive cells after infection with the

*FSM: fast-sedimenting material

Table

Precipitation of heavy defective particles with anti-RNA-antiserum

	% precipitation
1. Heavy defective particles, purified by sucrose gradient centrifugation	96
2. fr phage, purified by sucrose gradient centrifugation	8
3. fr phage, purified by density gradient centrifugation and stored at -15°C	38
4. Heavy defective particles, after treatment with ribonuclease at 37°C for 10 min.	3
5. fr-RNA	101
6. fr-RNA, no anti-RNA-antiserum	2
7. Heavy defective particles, no anti-RNA-antiserum	4

The reaction mixture contained 10 μmoles of tris buffer (pH 7.2), 140 μmoles of NaCl, 0.1 μg of non-labeled fr-RNA, less than 0.01 μg of the material indicated in the table, containing between 1000 to 5000 cts/min, and 0.025 ml. anti-RNA-antiserum. The total volume was 1 ml. The reaction mixture was allowed to stand for 30 min at room temperature. The precipitate was collected on Whatman glass filters and washed with the same buffer. The radioactivity was determined as described before (Heisenberg, 1966).

amber mutant su_m, together with normal defective particles. It does not occur in lysates of wild-type infected cells. It contains fr-antigen but seems to be inhomogeneous with respect to its sedimentation velocity and buoyant density (Figs. 1 and 2). Fig. 1 shows FSM in a sucrose density gradient. The radioactivity at the top of the gradient represents s-RNA and smaller compounds. Both a 27-30s peak of phage-RNA and 30s ribosomal subunits, which are not separated, and a 50s peak of ribosomal subunits is observed in lysates of wild-type and mutant-infected cells. In the lower part of the gradient the peaks of defective particles (71s) and fr-phage (79s) may be seen. The FSM sediments as a shoulder

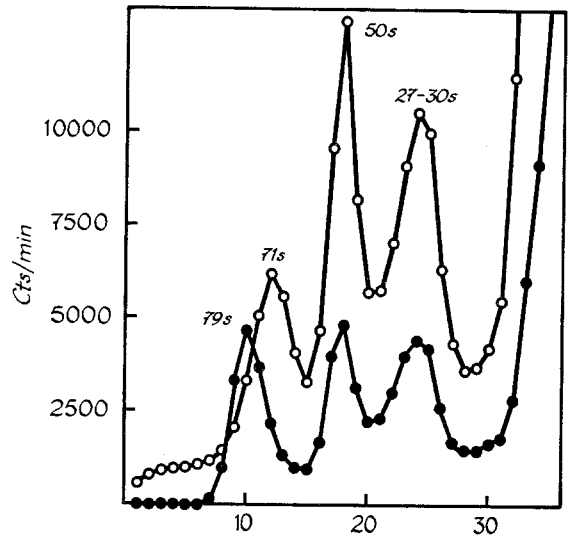

Fig. 1. Sucrose density gradient of lysates of fr- and su_m^+-infected E. coli MRE600 (kindly provided by Dr. S. Brenner). The fr-infected cells were labeled with ^3H-uridine (●——●), the su_m^+-infected cells were labeled with ^{32}P-phosphate (o——o). Label was added 15 min after infection, the cultures were harvested at 45 min after infection, and mixed together; the cells were converted to spheroblasts, centrifuged down and resuspended in a small volume of buffer containing per ml. 10 μmoles of tris buffer pH 7.2, 10 μmoles of $MgCl_2$, and 200 μmoles of NaCl. Lysis was achieved by freezing and thawing and shaking with chloroform. The lysate was centrifuged at 25,000 rev./min for 20 min. About 0.1 ml. of the supernatant were layered on top of a gradient containing 5-20% sucrose in the same buffer as mentioned above. The material was centrifuged for 140 min at 35,000 rev./min. The tritium data are corrected for the quenching of ^{32}P.

(fractions 1-7) in front of the defective particles. Under these conditions very few 70s ribosomes are found, even in uninfected cells. A CsCl density gradient of FSM is shown in Fig. 2a. A considerable part of the FSM has a higher buoyant density (fractions 20-30) than defective particles and viable phage. After treatment with ribonuclease most of the remaining high molecular weight material can be identified as "light defective particles" (Heisenberg, 1966) by its buoyant density (Fig. 2b) and by electron microscopy. (Sometimes a second ribonuclease-resistant component is observed at the bottom of the gradient.)

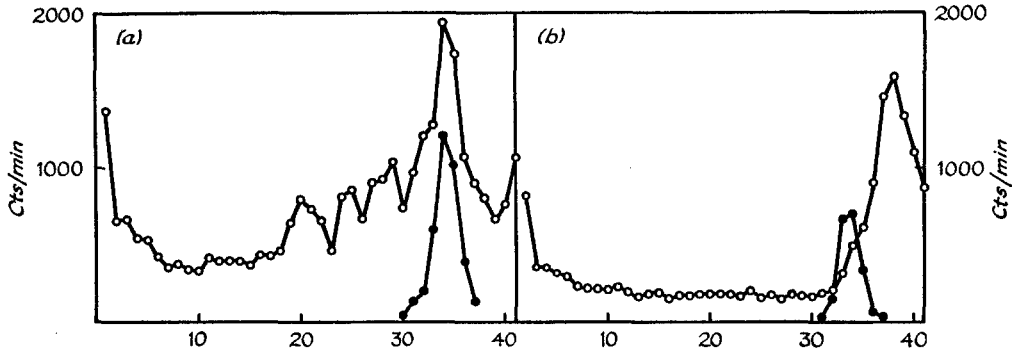

Fig. 2. CsCl density gradients of material taken from the first 7 fractions of the sucrose gradient shown in Fig. 1 (o——o). The buffer was the same as that used in the experiment shown in Fig. 1. ^{3}H-uridine-labeled fr-phage were added as a reference (●——●). The sample was centrifuged for 40 hr at 34,000 rev./min. The mean buoyant density of the CsCl was ρ = 1.49. The material was centrifuged before (a) and after (b) digestion with 20 μg of pancreatic RNase at 37°C for 10 min.

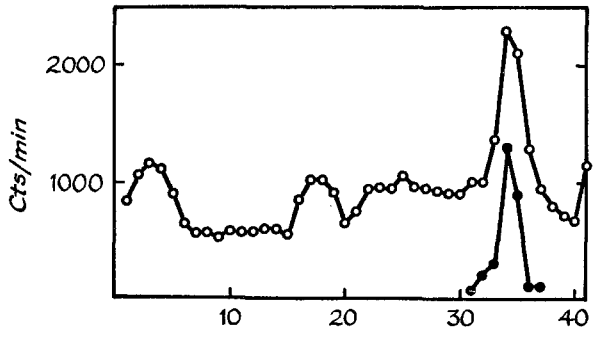

Fig. 3. CsCl density gradient of material taken from Fractions 2 and 3 of the sucrose gradient shown in Fig. 1. Experimental conditions are as described in the legend to Fig. 2.

Even without ribonuclease treatment the FSM tends to dissociate into a component of high buoyant density and particles which are indistinguishable in their properties from defective particles. This is demonstrated by the following experiment. The very fastest sedimenting FSM (> 90s) is isolated from a sucrose gradient. If

this material is analyzed in a CsCl density gradient (Fig. 3) significant parts of it band at the position of defective particles. These particles may be sedimented again in a sucrose density gradient. They now sediment with the same velocity as defective particles (71s). The wide distribution of the complexes in the sucrose gradient may therefore be partially due to continuous dissociation during the centrifugation.

The nucleic acid of the FSM consists solely of RNA as judged by uridine incorporation and alkaline degradation. At least two components are found, one sedimenting homogeneously at 27s like fr-RNA, one having sedimentation values between 25 and 10s (Fig. 4a). To exclude the possibility that these smaller RNA-molecules are in vitro degradation products of fr-RNA, the RNA of the defective particles from the same sucrose gradient was extracted using the same experimental conditions and much less small RNA was found (Fig. 4b).

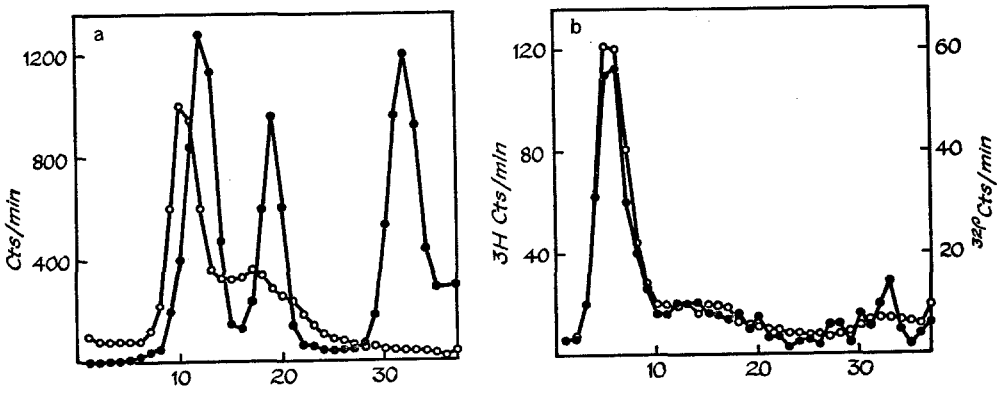

Fig. 4. Sucrose density gradients of RNA extracted from material of parts of the sucrose gradient shown in Fig. 1. The gradients contained 5-20% sucrose in 0.02 M tris buffer (pH 7.2), 0.2 M NaCl and 0.01 M $MgCl_2$. (a) RNA of fractions 1 to 7 (o——o) is sedimented together with ^{3}H-uridine-labeled cellular RNA (●——●) at 49,000 rev./min for 200 min. (b) As control the RNA of fractions 12 and 13 (o——o) is sedimented, using fr-RNA (●——●) as reference, for 220 min at 49,000 rev./min.

This set of experiments indicates that the FSM consists of "defective particles" and some other RNA-rich components. The structure of the defective particles must permit the attachment of these components. A possible site for the attachment would be the RNA hanging out of the protein shell. Since the FSM is unstable and has never been found to form by mixing extracts of su_m-infected cells with defective particles, it may be assumed that it is formed in vivo. A simple explanation of its formation is that during the growth cycle of su_m in the non-permissive host at least some of the RNA-molecules are encapsulated by the coat protein while they are still attached to some phage growth organelles such as ribosomes or the RNA replicating machinery. Further experiments have to be done to verify this interpretation, since it cannot be excluded so far that the FSM is an unspecific aggregation product between defective particles and RNA.

These experiments are mentioned since the results fit very well the concept that the gene function missing in the growth cycle of the amber mutant su_m in the non-permissive host is responsible for establishing the right conformation of the phage RNA so that it can be correctly encapsulated by the coat protein. If so, the FSM might aid in the investigation of the late stages in the growth cycle of RNA phage.

I wish to thank Prof. Dr. G. Schramm for his stimulating discussion and general support. The expert technical assistance of Mrs. B. Krafczyk is gratefully acknowledged. This investigation was supported by the U. S. Public Health Service, Grant 5 ROI CA 04601-07.

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