ASSOCIATION OF BACTERIOPHAGE PROTEINS AND RNA IN E. COLI INFECTED WITH MS2

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Received November 20, 1967

Infection of E. coli with the RNA bacteriophage MS2 leads to the synthesis of three phage-specific proteins: phage coat protein, "maturation" protein, and RNA synthetase, which can be separated and quantitated by gel electrophoresis (Nathans, Oeschger, Eggen and Shimura, 1966; Vinuela, Algranati and Ochoa, 1967; Eggen, Oeschger and Nathans, 1967). Each of these proteins might be expected to interact with phage RNA in the course of phage development. In the case of coat protein two kinds of in vitro complexes have been described with RNA extracted from purified phage particles. One of these results from the binding of a small number of coat protein molecules (Capecchi and Gussin, 1965; Sugiyama, Hebert and Hartman, 1967), and the other resembles intact virions but is non-infectious (Sugiyama et al., 1967). In the case of MS2 RNA synthetase, the sedimentation rate of this enzyme in lysates of infected cells suggests that it may be bound to RNA or a ribonucleoprotein particle (Weissman, Simon, Borst and Ochoa, 1963). The "maturation" protein (Lodish and Zinder, 1965; Heisenberg, 1966; Argetsinger and Gussin, 1966) has been shown to be present in normal phage particles in small amount (Nathans et al., 1966), and it is possible that a complex between this protein and phage RNA is an intermediate in phage assembly. In this paper we report the detection in extracts of E. coli infected with MS2 of a complex or complexes involving RNA and each of the three phage-specific proteins.

METHODS

For specific labelling of MS2 RNA and protein in infected cells, E. coli was pretreated with actinomycin as described by Oeschger and Nathans (1966) with certain modifications. E. coli strain A19 (RNAse I^-, from W. Gilbert) was grown to a density of 5×10^7 cells per ml, sensitized to actinomycin as described and exposed to 0.5 ug actinomycin per ml. (Although this concentration of actinomycin did not give maximal inhibition of host protein and RNA synthesis, more consistent results were obtained than at higher concentrations.) After 5 minutes the cells were infected with MS2 at a multiplicity of 20, and C^{14}-amino acids and H^3-uridine were then added. At the end of the incorporation period, the culture was quickly chilled in an ice bath and centrifuged. The bacteria were immediately lysed by the procedure of Godson and Sinsheimer (1967), which was slightly modified. After DNAse treatment, EDTA was added to the lysate to give a final concentration of 0.002 M, and the suspension was clarified by centrifugation at 12,000 g for 5 minutes before analysis by sucrose gradient centrifugation as described below.

RESULTS

Sedimentation of protein and RNA in infected cell lysate

Actinomycin-treated E. coli was infected with MS2, and newly synthesized protein and RNA were labelled with five C^{14}-amino acids and H^3-uridine, respectively, from 10 to 45 minutes after infection, as described in Methods. The labelled cells were chilled, centrifuged, and lysed as described. The fresh lysate was layered onto a 5 to 20% sucrose gradient containing 0.01 M Tris HCl pH 7.4 and 0.02 M EDTA and centrifuged at 35,000 rpm for 4 1/2 hours in an SW39 Spinco rotor. The collected fractions were then analysed for radioactive RNA and protein; representative results are shown in Figure 1A. For comparison a similar analysis of uninfected cell lysate is also shown (Figure 1B). As seen in Figure 1A, in infected cell extract newly synthesized RNA was present in three distinct regions: at the bottom

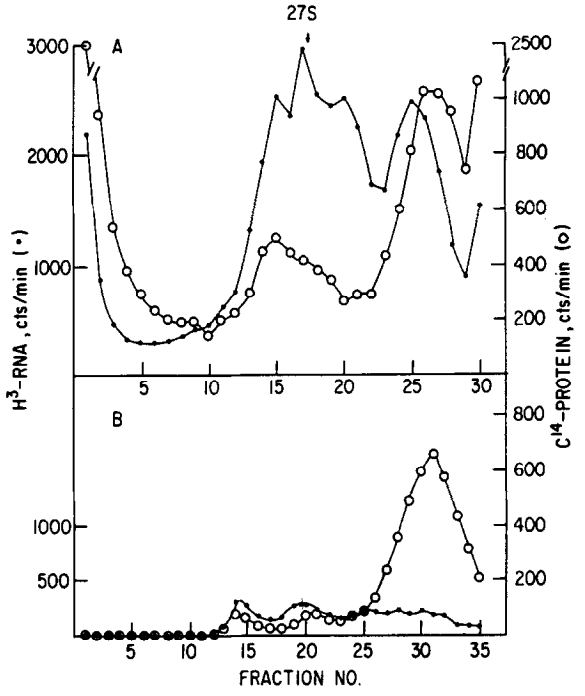

Figure 1. Sucrose gradient centrifugation of E. coli lysates. A. Infected with MS2. B. Uninfected. The procedure is described in the text. The amount of lysate analysed for radioactivity was equivalent to 2 ml of culture. Phage RNA peaked in a separate tube at the position marked 27S.

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of the gradient, near the middle (about 40S) and near the top (about 7S). In contrast, in uninfected cells little labelled RNA was present due to inhibition of cellular RNA synthesis by actinomycin (Figure 1B). As shown in Figure 1A, radioactive protein from infected cells is also distributed into three classes, two of which appear to be phage specific. The C^{14}-protein at the bottom has been shown to be in bacteriophage particles after centrifugation in CsCl gradients. The C^{14}-protein in the middle of the gradient, which is similar to that described by Haywood and Sinsheimer (1965), overlaps the middle RNA peak, suggesting that this protein fraction might be complexed to phage RNA.

Evidence for phage protein-RNA complexes

In order to test the possibility that the protein which sediments

with phage RNA is bound to the RNA, fractions from the middle peak of a sucrose gradient similar to that shown in Figure 1A were treated with pancreatic ribonuclease and re-sedimented through sucrose. RNAse treatment resulted in the disappearance of the original broad peak of phage protein and RNA and the appearance of more slowly sedimenting radioactive protein and RNA as well as a smaller amount of more rapidly sedimenting protein. Our interpretation of these results is that the radioactive phage protein originally sedimenting with phage RNA was complexed with RNA. When the RNA was digested with RNAse, free protein was liberated, some of which aggregated and sedimented rapidly.

A second test for protein-RNA complex was carried out by determining the extent to which phage RNA was retained on millipore filters (Nirenberg and Leder, 1964; Godson and Sinsheimer, 1967). Sucrose gradient fractions from each region of the gradient shown in Figure 1A were passed through millipore filters and the percentage of H^3-RNA retained on the filter was determined. About 65% of the RNA from the heavier portion of the middle peak (fractions 13-15) was retained and about 25% of the RNA in the lighter portion of the middle peak (fraction 18) was retained, whereas less than 1% of the RNA from the top peak was retained. These results suggest that the phage RNA which co-sediments with phage protein is largely bound to protein.

Electrophoresis of phage proteins

To identify the specific phage proteins which appear to be associated with phage RNA, sucrose gradient fractions were treated with EDTA-mercaptoethanol-sodium dodecyl sulfate as previously described (Eggen et al., 1967), electrophoresed in acrylamide gel, and radioautograms made from the sliced gels. The radioautograms were then traced with a microdensitometer. (As noted in the introduction, this procedure separates the three phage proteins and allows their quantitation.) In Figure 2 are shown the results of electrophoresis of radioactive proteins from the

three regions of the sucrose gradient shown in Figure 1. The radioactive protein at the top of the gradient is a complex mixture (Figure 2A), which includes host proteins and possibly specific phage proteins. The proteins which go to the bottom of the sucrose gradient correspond in mobility to phage coat protein and, to a much smaller extent, with "maturation" protein, respectively (Figure 2C), both of which have been shown to be present in complete phage particles (Nathans et al., 1966). (From the relative amounts

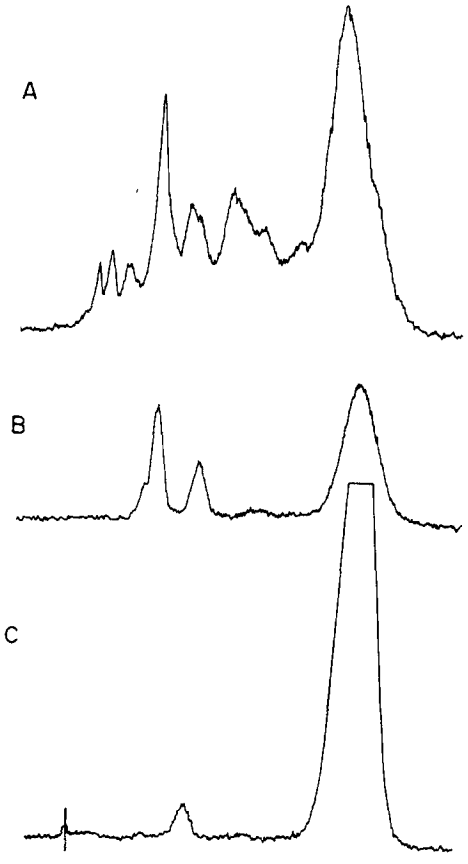

Figure 2. Microdensitometer tracings of radioautograms of electrophoresis gels. A. C^{14}-proteins from the top of the gradient shown in Figure 1 (fractions 25 to 28). B. C^{14}-protein from the middle of the gradient (fractions 14 to 17). C. C^{14}-protein from the bottom of the gradient (fractions 1 and 2). In B the first peak (left) probably represents RNA synthetase; the second peak represents "maturation" protein; and the third peak represents coat protein (Eggen et al., 1967). The origin is at the left.

of radioactive "maturation" protein and coat protein and the known number of coat protein subunits in each phage particle, one can calculate that a single phage particle has about 40,000 daltons of "maturation" protein, which may represent a single molecule.) The finding of immediate interest is that seen in Figure 2B, which shows the results with the radioactive phage protein which bands near the middle of the sucrose gradient tube and has been shown above to be associated with RNA. As seen in Figure 2B, all three phage proteins are present in this fraction. In contrast with the protein at the bottom of the gradient, and with the total phage protein in infected cell lysates (Nathans et al., 1966), the amount of coat protein relative to the other phage proteins is lower. It is also apparent that the ratio of coat protein to phage RNA in the complex is far less than the ratio of coat protein to RNA in the more rapidly sedimenting particle fraction (Figure 1A).

DISCUSSION AND CONCLUSIONS

The experiments reported here show that the three phage proteins detectable in MS2-infected E. coli are found in a rapidly sedimenting form in cell lysates, corresponding with the sedimentation of some of the newly synthesized phage-specific RNA. After ribonuclease treatment the protein has an altered sedimentation rate indicating that it is bound to RNA. Moreover, much of the RNA of this fraction is retained on millipore filters, suggesting that it is also complexed with protein. In this complex less coat protein is present relative to the other two proteins than in whole cell lysates. Based on the radioactivity of RNA and protein in CsCl-purified phage particles and the known ratio of coat protein to RNA in the phage, we estimate that for each phage equivalent of RNA in the 40S region of the sucrose gradient there are about 15 molecules of coat protein. On the assumption that the "maturation" protein is about 40,000 in molecular weight (see above) there would be approximately one molecule per phage equivalent of RNA; if the rest of the phage RNA codes for synthetase, one can estimate that about one molecule of this protein is also present for

each phage equivalent of RNA.

Although the observations reported here suggest that specific phage RNA-protein complexes are formed during virus development, the nature of these complexes, their relation to ribosomes, and in particular the properties of the nucleic acid molecule(s) involved, remain to be clarified. Godson and Sinsheimer (1967) and Hotham-Iglewski and Franklin (1967) have shown that some of the phage RNA present in lysates of infected cells sediments at about 40S at low Mg^{++} concentration and has properties of a replicative intermediate (Fenwick, Erikson and Franklin, 1964) originally present on polyribosomes. The former authors also showed that this RNA (in their case, from infecting phage) has characteristics suggesting that it is bound to protein.

As noted in the introduction, complexes between MS2 RNA and each of the phage proteins could play important roles in virus development. In the case of RNA synthetase, this presumably represents template-bound enzyme. In the case of "maturation" protein, the detection of an RNA complex suggests that this protein attaches to the phage RNA prior to the formation of a particle rather than after all the coat protein molecules assemble. Whether this protein also has a regulatory function remains to be determined. Finally, the detection of a complex between RNA and a small number of coat protein molecules in cell lysates adds further support to the in vitro observations which suggest that phage coat protein by attaching to the phage RNA serves as a specific repressor of the synthesis of phage proteins (Eggen and Nathans, 1967, and manuscript in preparation; Sugiyama and Nakada, 1967).

This research has been supported by a grant from the National Institute of General Medical Sciences, U. S. Public Health Service. E. R. was a recipient of a summer fellowship from The Johns Hopkins University School of Medicine. We are grateful to Drs. R. M. Franklin and B. Hotham-Iglewski for

informing us of their studies with R17 infected cell lysates prior to publication.

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