ISOLATION OF AN INFECTIOUS RNA–A-PROTEIN COMPLEX FROM THE BACTERIOPHAGE M12

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Received 11 April 1975
Original figures received 9 May 1975

1. Introduction

Every RNA-coliphage of the f2 group contains one molecule of RNA (mol. wt 1.1×10^6), 180 molecules of coat protein (mol. wt 1.4×10^4), and about one molecule of A-protein (mol. wt 4×10^4) [1–3]. In infected cells, newly-synthesized A-protein is found associated with viral RNA [4,5], suggesting that an RNA–A-protein complex may be a precursor in the assembly of mature virus. Furthermore, treatment of purified R17-phage with acetic acid leads to a precipitate of A-protein and RNA, whereas the major coat protein remains soluble in the supernatant [6]. A possible explanation for the co-precipitation of RNA and A-protein is, that A-protein is bound to RNA even in the mature phage. Here we report the isolation of an RNA–A-protein complex from the acetic acid precipitate of highly purified phage M12. This complex is an important factor in controlling the relative plating efficiency of a phage preparation and is infectious itself for intact *E. coli* cells.

2. Materials and methods

Growth of the virus on *E. coli* AB301 (Hfr, λ^+, RNAse I^-) and purification was essentially as previously described [6]. [^{3}H] histidine-labelled phage was grown on W945 (f^+, λ^-) in a medium described by Steitz [1]. Immediately after infection (m.o.i. = 5) L [2,5-^{3}H] histidine (Radiochemical Centre, Amersham, England) was added to 0.1 mCi/ml. The phage was further concentrated by polyethylene glycol (mol. wt 6000) and purified finally on a single CsCl gradient [7]. ^{32}P-labelled phage was grown on AB301 in Delius medium [8]. 5 min after infection (m.o.i. = 10) $H_3\,^{32}PO_4$ (Büchler and Co., Braunschweig, Germany) was added to a final activity of 0.1 mCi/ml. The phage was further prepared as described above. In the final stocks 10% of the phage particles were infectious. Acetic acid pellets were prepared from 1.2×10^{13} infectious phage particles essentially as described [6]. The pellets were then solubilized in 0.25 ml Tris–EDTA buffer. Plaque forming units (pfu's) were assayed on *E. coli* AB301. For sucrose gradient analysis, solubilized pellets were layered onto 15–30% sucrose in Tris–EDTA buffer. Centrifugation was carried out for 20 hr at 0°C in the SW 41-rotor at 35 000 rev/min. The RNA concentration of each fraction was measured by absorption at 260 nm. A-protein was determined by 5% TCA-precipitable [^{3}H] histidine radioactivity (The samples were first digested with 10 μg RNAse A/ml (from bovine pancreas, Sigma Chemical Company) for 15 min at 37°C in order to remove radioactive label deriving from RNA [9]). The total ^{32}P-labelled-RNA of each fraction was measured by 5% TCA-precipitable radioactivity. The RNA bound to protein was determined by the Nirenberg filtration assay [10,11].

3. Results and discussion

For the identification of an RNA–A-protein complex, we isolated an acetic acid pellet from M12-phage, whose A-protein was specifically labelled with [^{3}H]histidine, an amino acid, which is not present in the coat protein [1] (see Materials and methods). When the dissolved pellet was tested for surviving phage, we

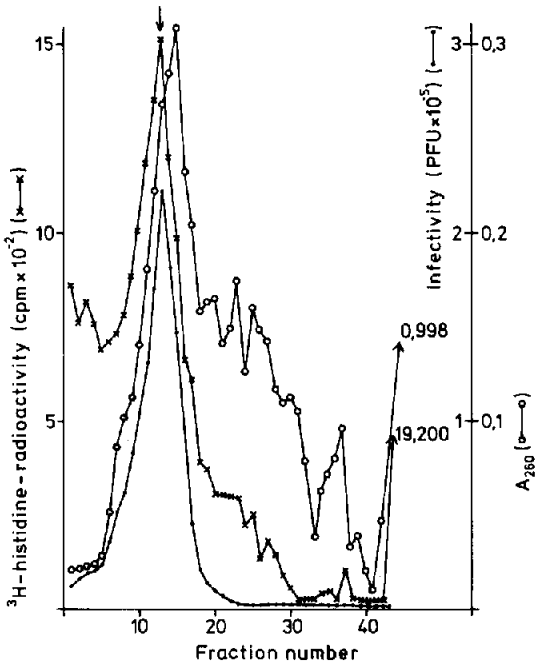

Fig.1. Sucrose gradient centrifugation of solubilized acetic acid pellet prepared from [^{3}H]histidine-labelled phage (1.2×10^{13} pfu). The solution (0.25 ml) was layered on the gradient and centrifuged as described in Materials and methods. The RNA, the A-protein and the infectivity of the gradient fractions were determined as described in Materials and methods. The arrow indicates the position of intact phage ^{32}P-RNA in a parallel gradient. Centrifugation was from right to left.

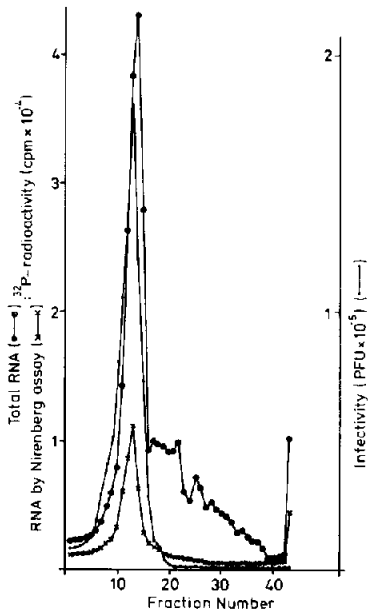

Fig.2. Sucrose gradient centrifugation of solubilized acetic acid pellet prepared from ^{32}P-labelled phage (1.2×10^{13} pfu). The solution was layered on the gradient and centrifuged as described in Materials and methods. The total RNA, the RNA bound to protein and the infectivity of the gradient fractions were determined as described in Materials and methods. Centrifugation was from right to left.

observed approx. 10^{-7} pfu per original infectious unit. However, this infectivity is not due to intact phage particles, because it was destroyed on incubation with only 5–10 μg/ml pancreatic RNAse, at 37°C for 30 min. Under these conditions, intact phage particles retain their total infectivity. Evidence is presented that the infectious agent is an RNA–A-protein complex. First, a coincident peak of [^{3}H]histidine-labelled A-protein, RNA and infectivity was observed, when the redissolved acetic acid pellet was analysed on sucrose gradient (fig.1). Second the binding of A-protein to RNA was demonstrated by the Nirenberg filter binding assay. The protein bound ^{32}P-RNA co-sedimented with infectivity on sucrose gradients (fig.2). By contrast, when RNA was extracted from the acetic acid precipitate with phenol [12], it was neither infectious nor was significant binding of ^{32}P-RNA to filters observed. It can be seen from figs.1 and 2 that degraded RNA was also present (fraction 15–43), which however was not complexed to A-protein. Since approx. 50% of the A-protein sedimented to the bottom of the gradient and it was also found in fractions containing no protein-bound RNA, it appears, that A-protein occurs in aggregates of various sizes.

The molar ratio of RNA to protein in the complex was found to be 1:1. The number of RNA molecules in the complex was calculated from the known specific radioactivity of the RNA bound to protein in the infectivity peak (fig.2). Since the number of A-protein molecules in the original phage was known, the number in the complex could be estimated from the proportion of A-protein in the infectivity peak (fig.1), after correction for aggregated material.

The infectivity of the complex was found to be 10^{-7} pfu per complex molecule. This could be increased 20-fold by brief incubation in urea [13].

While this paper was in preparation, it was reported, that infectivity for intact *E. coli* cells co-sediments with phenolized MS2-RNA, provided the RNA is mixed with A-protein [14]. Since infectivity disappeared on treatment with RNAse or proteolytic enzymes, it was suggested that it is due to an RNA–A-protein complex. We demonstrate here the existence of such an infectious complex. It was shown [13], that RNA and A-protein are held together by ionic bonding; also, that infection takes place only via F-pili and that the infectivity of the initial phage preparation and the amount of complex derived from it are closely correlated.

Acknowledgements

We thank Miss S. Döhring for her excellent technical assistance and Dr B. Martin for reading this manuscript.

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