FURTHER EVIDENCE ON THE ROLE OF THE A PROTEIN IN BACTERIOPHAGE MS2 PARTICLES

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1. Introduction

Bacteriophage MS2 is one of the group of closely related small icosahedral RNA phages. The coat protein shell is composed of 180 subunits [1] and one A-protein molecule [2,3]. The A-protein is in some way required for proper assembly since defective particles are formed during the growth of A-cistron amber mutants in non-permissive cells [4,5]. These defective particles contain originally an intact RNA-molecule, but which protrudes through the capsid and is accessible to nucleases present in the solvent [6,7]. Furthermore infective phage is only obtained in reconstitution experiments when A-protein is present [8].

It is not clear, however, whether the A-protein is still required once a complete and perfect virion has been formed. We found that in high salt the A-protein is preferentially lost from the viral particles. Some hints for this effect have previously been published in the literature [9]. The A-protein-less particles remain physically intact, unlike "defective particles", but are not infective. We conclude that A-protein is not only essential for proper assembly, but also for subsequent infection.

2. Materials and methods

Growth and purification of the virus was essentially as previously described [10,11]. ^{14}C-labeled phage was grown in TPG medium [12] containing 0.01% (w/v) Casamino acids. Immediately after infection (m.o.i. = 5), a [^{14}C] protein hydrolysate (Schwarz Bio Research) was added to 4 μCi/ml. The last step in the purification consisted of a centrifugation on a linear 10–30% glycerol gradient in 5×10^{-3} M Tris buffer, 10^{-3} $MgCl_2$ and 10^{-4} M EDTA, adjusted to pH 7.4 with HCl, instead of banding in a CsCl-gradient. The gradients were centrifuged for 2.5 hr at 40,000 rpm in the SW41 rotor at 2°.

For density gradient centrifugation, the virus suspension was added to 1.65 g CsCl and the volume was adjusted to 3 ml with buffer (10^{-3} M Tris, 10^{-3} M $MgCl_2$ and 10^{-4} M EDTA, pH 7.4). Centrifugation was carried out for 30 hr at 2° in the SW65 rotor at 37,500 rpm. Fractions were collected by piercing the bottom of the tube.

Total phage protein was analyzed on 10% polyacrylamide gels containing sodium dodecyl sulfate [11,13]. The gels were prerun with 0.1 M phosphate buffer, pH 7.2 containing thioglycolic acid to remove the persulfate. Prior to electrophoresis the proteins were heated in 0.01 M phosphate buffer, pH 7.2 in the presence of 2% SDS and 0.14 M β-mercaptoethanol.

3. Results and discussion

In order to calculate changes in the number of A-protein molecules per virus particle we used phage uniformly labeled with a [^{14}C] protein hydrolysate. When the purified phage preparation was subjected to a CsCl-gradient centrifugation, a decrease in specific infectivity (being the ratio of infectivity to radioactivity) was observed (table 1). The top fractions of the gradient (fig. 1A) were collected and a second centrifugation was performed (fig. 1B). The two peak fractions (indicated by the arrows) had a 2-fold difference in spe-

Table 1
Correlation between infectivity of MS2 phage and A-protein content.

	(Specific infectivity)[a]	(%)[b]	moles A-protein[c] / virus particle	(%)[b]
Purified phage	5.8×10^5	100	0.96	100
After first CsCl gradient centrifugation[d]	2.8×10^5	48.9	0.44	45.8
After second CsCl gradient dentrifugation (fig. 1,B) :				
Fraction no. 10	7.4×10^4	12.7	0.13	13.5
Fraction no. 9	3.87×10^4	6.7	0.08	8.3

[a]Ratio of pfu over cpm. [b]The value for the original preparation of purified phage is taken as 100. [c]Uniform labeling of both proteins is assumed. [d]Fractions indicated in fig. 1A.

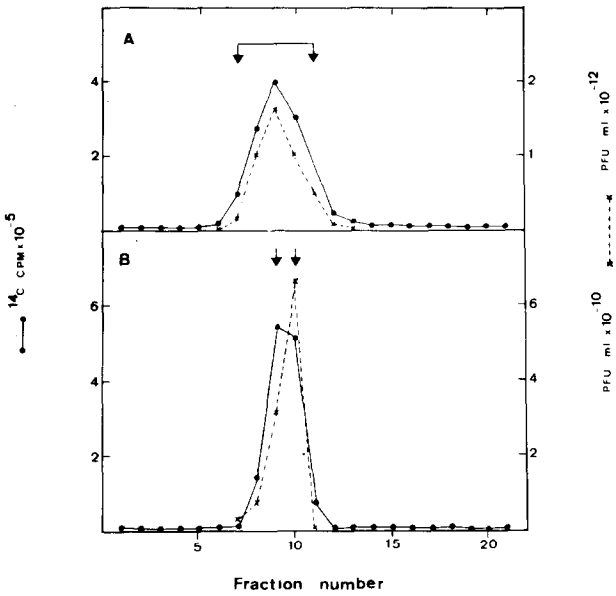

Fig. 1. CsCl density gradient centrifugation of purified MS2 virions uniformally labeled with a [^{14}C]-protein hydrolysate. A) 1 ml of the phage preparation was mixed with a CsCl solution and centrifuged as described in Materials and methods. B) The top fractions indicated in fig. 1A were collected and 1 ml was subjected to a second CsCl-gradient centrifutation. Radioactivity (●—●—●); infectivity (X--X--X).

cific infectivity.

All samples with different specific infectivity were concentrated by centrifugation for 2.5 hr at 50,000 rpm and 2° for further analysis. The pellet was resuspended, heated in detergent-buffer, and the proteins were separated by electrophoresis on polyacrylamide gels (fig. 2.). More than 96% of the radioactivity was found in the two peak fractions, the main component being the coat protein while the minor band with a mobility of 0.4 relative to that of the coat protein corresponds to the A-protein. It must be noted that 1.6 to 2.5% of the total radioactivity remained at the interphase of the gels. This was due to some insoluble material which could be removed by prior centrifugation of the sample before loading. When this pellet was solubilized with 8 M urea and analyzed again by electrophoresis, the same ratio of coat protein to A-protein was found as in the original sample.

The A-protein being approx. 3 times as large as the coat protein, the number of A-protein molecules per virion could be estimated from the radioactivity found in the peak fractions. The results of these experiments are summarised in table 1.

Virions which have not been centrifuged through a CsCl gradient contain – within the limitations of the method used – one A-protein molecule per particle. By centrifugation in CsCl, this A-protein is gradually lost, and there is a very satisfactory correlation between loss of A-protein and drop in specific infectivity. It should be noted that the A-protein-less particles, formed in this manner, are different from the defective particles formed by A-amber mutants in non-permissive cells. The former remain structurally nearly intact and still sediment at approx. 80 S, while the latter sediment at 69 S [7] because of a higher frictional coefficient due to protruding RNA-

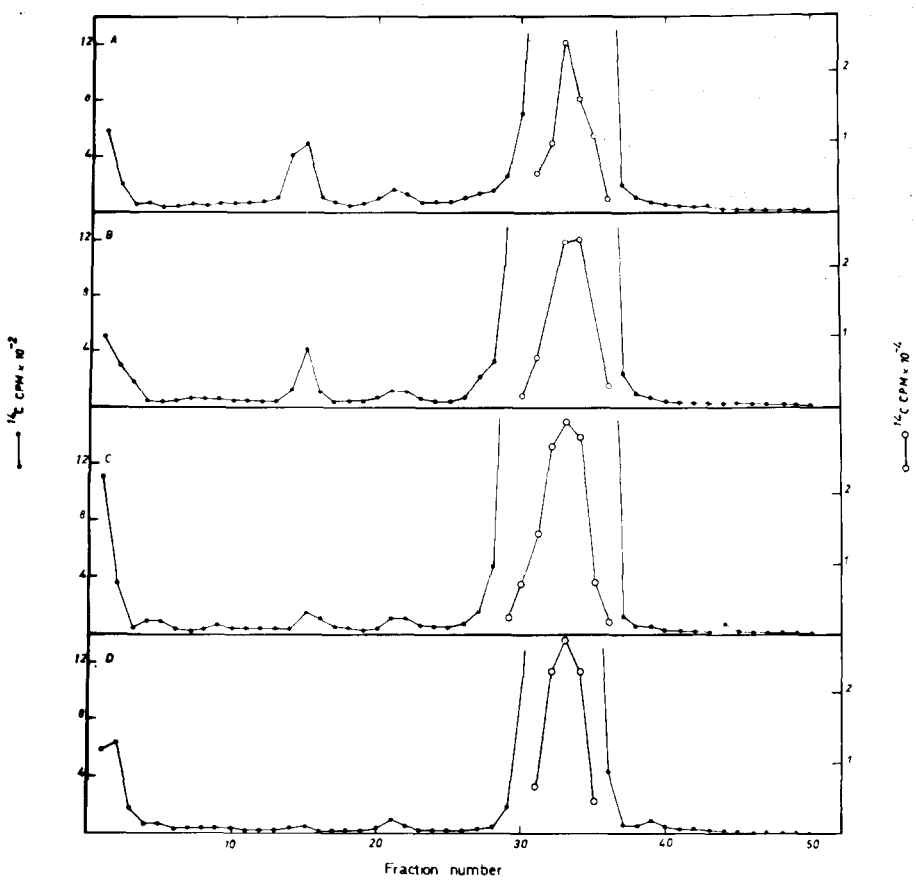

Fig. 2. Analysis of the phage proteins by electrophoresis on acrylamide gel. Proteins from virus samples with different specific infectivity were electrophoresed for 5 hr on 10% acrylamide gels at 7 mA/tube as previously described. The gels were then sliced and assayed for radioactivity. The anode is to the right. (A) Original preparation. (B) Fractions indicated in fig. 1A (purified virion after first CsCl-gradient centrifugation). C) Fraction number 10 and D) fraction number 0 of fig. 1B (purified particles after a second CsCl-gradient centrifugation). The specific infectivity of the virions is indicated in table 1.

segments. The fact that A-protein can be dissociated from the virion in high salt suggests that ionic bonds are involved in its interactions, either with the capsid proteins or with the RNA. Direct binding with the RNA is in agreement with the recent finding that upon infection the A-protein penetrates into the host cytoplasm together with the RNA-molecule [14, 15].

We conclude that A-protein is necessary, not only for proper assembly during maturation, but also for one or more subsequent processes, e.g. adsorption, penetration or early eclipse phase.

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References

[1] C. Vasquez, N. Granboulan and R.M. Franklin, J. Bacteriol. 92 (1966) 1779.
[2] J.A. Steitz, J. Mol. Biol. 33 (1968) 923.

[3] J.A. Steitz, J. Mol. Biol. 33 (1968) 937.
[4] H.F. Lodish, K. Horiuchi and N.D. Zinder, Virology 27 (1965) 139
[5] M. Heisenberg and J. Blessing, Z. Naturforch. 20B (1965) 859.
[6] J.E. Argetsinger and G.N. Gussin, J. Mol. Biol. 21 (1966) 421.
[7] M. Heisenberg, J. Mol. Biol. 17 (1966) 136.
[8] J.W. Roberts and J.A. Steitz, Proc. Natl. Acad. Sci. U.S. 58 (1967) 1416.
[9] T. Sugiyama and D. Nakada, J. Mol. Biol. 31 (1968) 431.
[10] W. Fiers, L. Lepoutre and L. Vandendriessche, J. Mol. Biol. 13 (1965) 432.
[11] E. Verbraeken and W. Fiers, Virology, in press.
[12] R.L. Sinsheimer, B. Starman, C. Nagler and S. Guthrie, J. Mol. Biol. 4 (1962) 142.
[13] K. Weber and M. Osborn, J. Biol. Chem. 244 (1967) 4406.
[14] J.M. Kozak and D. Nathans, Nature New Biol. 234 (1971) 209.
[15] J.W. Paranchych, S.K. Answorth, A.J. Dick and P.M. Krahn, Virology 45 (1971) 615.