BIOLOGICAL ACTIVITY OF IN VITRO SYNTHESISED PROTEIN: BINDING OF SEMLIKI FOREST VIRUS CAPSID PROTEIN TO THE LARGE RIBOSOMAL SUBUNIT

Niall Glanville and Ismo Ulmanen

Departments of Virology and Zoology, University of Helsinki

Received May 20,1976

SUMMARY

Cell-free translation of the Semliki Forest virus-specific 26S RNA yielded primarily capsid protein. After treatment of the protein synthesising reaction with 25 mM EDTA, the capsid protein cosedimented with the large ribosomal subunit in sucrose gradients, and banded with the subunit at a density of 1.54 gm/cm^3 in CsCl. Exposure to 0.5 M KCl released the protein from the subunit. Similar binding of the virus capsid protein to the large ribosomal subunit has been observed in infected HeLa cells, although its function is not clear. The nonstructural proteins, which are the major products translated from the virion 42S RNA, did not associate with sedimenting structures.

INTRODUCTION

Semliki Forest virus[x] is an alphavirus: its nucleocapsid (consisting of 42S RNA and capsid protein (1)) is surrounded by an envelope which contains three species of glycoprotein (2). The 42S RNA has molecular weight 4 x 10^6 (3). In the infected cell a second major virus-specific RNA is found. This 26S RNA, m.w. 1.6 x 10^6 (3), is a replica of about one third of the 42S RNA (4), and has been shown to encode the viral structural (i.e. C- and envelope) proteins (5,6). These proteins are synthesised as a 130,000 dalton polyprotein (7,8), but in vitro a 33,000 dalton protein which comigrates with, and contains the tryptic peptides of the C-protein is the major translational product (6,9). The cell-free translational products of the 42S RNA are predominantly nonstructural proteins (6), but in vivo synthesis of these proteins is shut off as infection progresses, indicating that some form of translational control operates in the infected cell (10).

Here we show that SFV C-protein translated in vitro associates with the large ribosomal subunit in the protein synthesising reaction.

[x]Abbreviations: SFV, Semliki Forest virus; C-protein, capsid protein.

METHODS

The preparation of virion 42S RNA and virus-specific 26S RNA from the cytoplasm of infected cells have been described (6). Conditions for cell-free protein synthesis were similar to those originally described for the preincubated wheat germ S30 extract (11). Spermine (50 µM) and spermidine (250 uM) were included and creatine phosphokinase was omitted from the reactions, which were incubated for 3h at 22°C, with ^{35}S methionine as the radioactive label. Addition of mRNA (40 ug/ml) resulted in a 10-20 fold increase in the incorporation of radioactivity into hot TCA insoluble form.

Reaction mixtures were treated with 25 mM EDTA and analysed on 15-30 % (w/w) sucrose gradients made in 50 mM Tris, pH 7.4, 100 mM NaCl, and 10 mM EDTA. For salt treatment, aliquots of the mixtures were mixed with 9 volumes of 0.2 M, 0.3 M or 0.5 M KCl and layered onto gradients topped with a 0.5 ml layer of 0.5 % sucrose containing the appropriate salt concentration. Centrifugation was in a SW41 rotor at 40,000 rpm for 5h at 4°C. Fractions were collected dropwise from below and the TCA insoluble radioactivity determined.

To determine the buoyant densities of sedimenting structures the material was fixed with 2.5 % glutaraldehyde and analysed on preformed CsCl gradients as described (12).

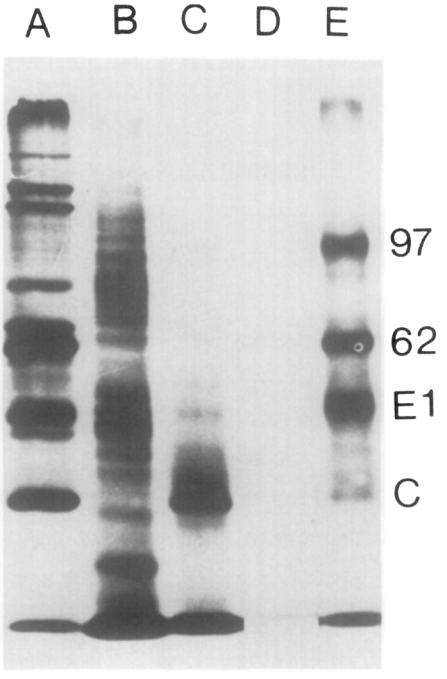

Fig. 1. SDS polyacrylamide gel electrophoresis of ^{35}S methionine containing protein synthesising reactions programmed with 42S RNA (B), 26S RNA (C), and control, without added RNA (D). Cytoplasmic extracts from cells infected with two SFV-mutants were included as markers (A,E): C = C-protein; E1 = envelope protein 1; 62 = precursor of envelope proteins m.w. 62,000 daltons; 97 = precursor of envelope proteins m.w. 97,000 daltons. Fluorography 2 days.

Proteins were analysed using the discontinuous SDS polyacrylamide system of Neville (13), with 7.5 % acrylamide in the separating gel. Gels were stained, destained and fluorographed (14). To quantitate the radioactivity, dried gels were cut into 2 mm slices, treated with NCS and counted in toluene Permablend.

RESULTS

The proteins synthesised in the wheat germ cell-free system programmed with SFV 42S and 26S RNAs are shown in Fig. 1. In the 26S RNA directed product the major band seen comigrates with the viral C-protein (Fig. 1C). Minor bands, including one which comigrates with the 97,000 dalton precursor of the three envelope proteins, are also detected. The C-protein accounted for about 40 % of the radioactivity in the products with m.w. > 25,000, and the 97,000 dalton protein for about 4 %. The 42S RNA products are more heterogeneous (Fig. 1B) and have been shown to contain minimal amounts of peptides from structural proteins (unpublished data). Almost no protein synthesis is seen in the control incubation which lacks exogenous RNA (Fig. 1D).

After treatment of these reaction mixtures with EDTA to dissociate the polysomes and 80S monosomes, they were analysed on sucrose gradients. In the reaction programmed with 26S RNA there are clear peaks of radioactivity which cosediment with the 40S and 60S ribosomal subunits (Fig. 2A). Only small amounts of radioactivity were associated with sedimenting structures in the 42S RNA directed reaction, most of the radioactivity being found on the top of the gradient (Fig. 2B).

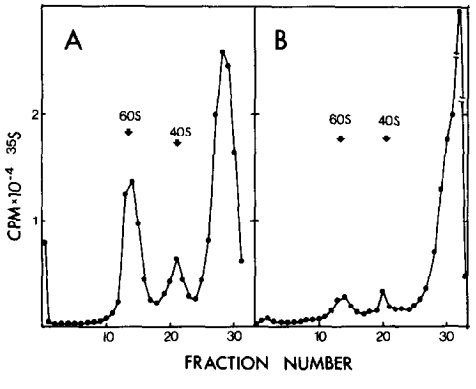

Fig. 2. Sucrose gradient analysis of [^{35}S] methionine containing reactions programmed with 26S RNA (A) and 42S RNA (B), and treated with EDTA. Arrows indicate the positions, determined from the absorbance $A_{260:280}$ profile, of 40S and 60S ribosomal subunits from non-incubated, EDTA treated wheat germ extract centrifuged in a companion gradient.

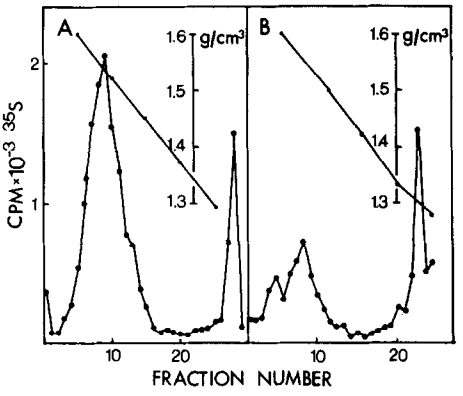

Fig. 3. CsCl gradient analysis of peak fractions from the 26S RNA programmed reaction analysed in Fig. 2A. 60S material (A); 40S material (B).

Samples from the peak fractions sedimenting at 40S and 60S in 26S RNA programmed reactions were fixed with glutaraldehyde and their buoyant densities determined on CsCl gradients. Most of the material from the 60S region banded at a density of 1.54 gm/cm^3 (Fig. 3A), the density determined for wheat germ 60S subunits in control experiments. The material from the 40S region was more heterogenous with density ranging from 1.52 to 1.58 gm/cm^3 (Fig. 3B). This was also true of wheat germ 40S subunits which had not been incubated. A significant part of the radioactivity was evidently not fixed to the 40S subunits and remained on the top of the gradient.

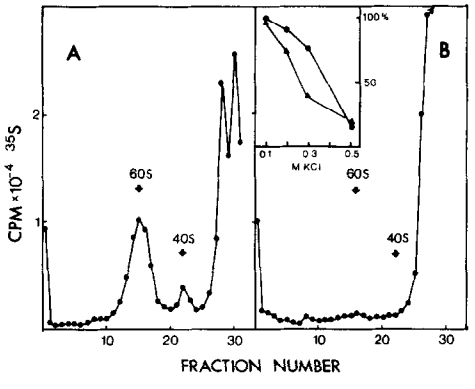

Fig. 4. Sucrose gradient analysis of aliquots from the 26S RNA programmed reaction analysed in Fig. 2A, after exposure to 0.3 M KCl (A) and 0.5 M KCl (B). Inset: Percentage reduction in radioactivity sedimenting at 40S (▲—▲) and 60S (●—●) following treatment with KCl. Arrows indicate the positions of the 40S and 60S ribosomal subunits (see legend of Fig. 2).

The sedimentation pattern obtained after exposure of the 26S RNA directed reaction to 0.2 M (not shown) and 0.3 M KCl (Fig. 4A) was fairly similar to that obtained without salt treatment, although the 40S bound material was noticeably less salt-resistant than the 60S bound material (Fig. 4B, inset). However, exposure to 0.5 M KCl released virtually all the radioactivity from sedimenting structures (Fig. 4B, and inset).

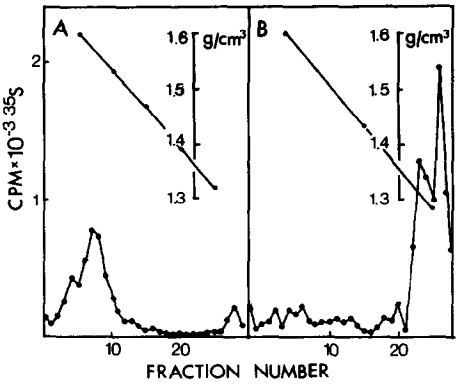

Fig. 5. CsCl gradient analysis of peak fractions from the 26S RNA programmed reaction exposed to 0.3 M KCl and analysed in Fig. 4A. 60S material (A); 40S material (B).

Density analysis of the sedimenting material after salt treatment revealed a peak from the 60S material with a slightly increased density (1.57 gm/cm^3, Fig 5A). The radioactivity from the 40S region was not fixed to RNA containing structures and was recovered from the top of the gradient (Fig. 5B).

In polyacrylamide gel electrophoresis a discrete C-protein band was detected in the 60S material from sucrose gradients (Fig 6B). This band was absent from both the 40S and top materials (C and D). However, after exposure to KCl a clear C-protein band was present in the top material (E).

DISCUSSION

In HeLa cells infected with SFV, labelled C-protein is found bound to the 60S ribosomal subunit after short pulses with ^{35}S methionine. Treatment with KCl releases a part of the bound C-protein (about 40 % after exposure to 0.4 M KCl and about 70 % with 0.8 M KCl) (Ulmanen et al., submitted for publication).

Here we present results showing that a similar but less salt-resistant

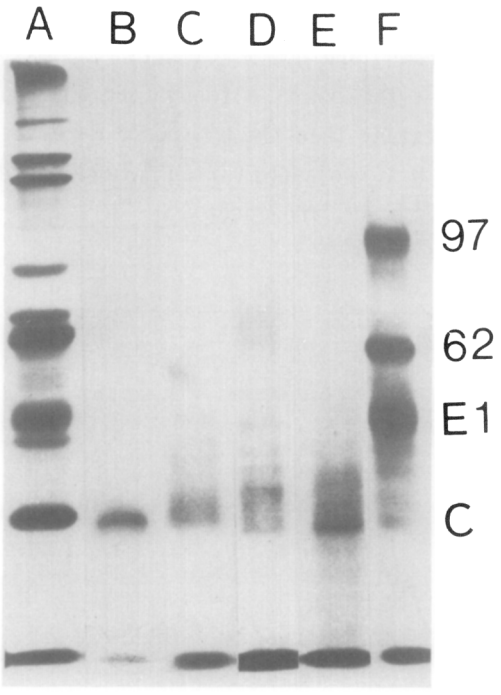

Figure 6. SDS polyacrylamide gel electrophoresis of peak fractions from 26S RNA programmed reactions fractionated on sucrose gradients. 60S material (B), 40S material (C) and top fraction (D) from gradient shown in Fig. 2A. Top fraction after exposure to 0.3 M KCl (E) from gradient shown in Fig. 4A. Markers (A,F) are those used in Fig. 1. Fluorography 14 days.

association occurs between the in vitro synthesised C-protein and wheat germ ribosomal subunits in the protein synthesising extract. Most of the C-protein translated from the 26S RNA appears to bind to the 60S subunit: about 30 % of the radioactivity recovered from the gradient sedimented at 60S (Fig. 2A). Polyacrylamide gel analysis showed that this material consisted almost only of a protein comigrating with the C-protein (Fig. 6), whereas little if any of this protein was present in the top gradient fraction prior to treatment with KCl. In the unfractionated product about 40 % of the radioactivity was found at this position of C-protein (see also Fig. 1).

A smaller amount of radioactivity cosedimented with the 40S subunit (Fig. 2A). Protein analysis showed this material to be heterogeneous and lack a discrete C-protein band (Fig. 6C). Its behaviour on CsCl gradients (Figs 3 and 5) and resistance to salt treatment (Fig. 4) was different from that of the 60S material. Together these findings indicate that this association is different from that between the C-protein and the 60S subunit.

Further indications of the specificity of the C-protein binding to the 60S subunit come from the translation of the 42S RNA. The products include only minimal amounts of C-protein and only very small amounts of radioactivity were associated with sedimenting structures (Fig. 2B).

The findings presented show that one biological activity of the C-protein (that of binding to the 60S subunit) is mimicked by the *in vitro* synthesised protein. The function of this binding is not yet clear. A role in the control of translation has been suggested for the binding of picornavirus (15,16) and influenza virus proteins (17) to ribosomes. On the other hand, the rapid incorporation of labelled C-protein into the SFV nucleocapsid (18) and experiments showing that label can be chased from the 60S subunit to the cytoplasmic nucleocapsid (19), suggest that the C-protein-60S ribosomal subunit complex plays a role in the formation of nucleocapsid.

ACKNOWLEDGEMENTS

The authors thank Drs. Leevi Kääriäinen and Hans Söderlund for helpful discussions and criticism of the manuscript, Ms Ritva Rajala and Ms Mirja Salonen for excellent technical assistance. This work was supported by grants from the Sigrid Juselius and Alfred Kordelin Foundations.

REFERENCES

1. Laine, R., Söderlund, H. and Renkonen, O. (1973) Intervirology 1,110-118.
2. Garoff, H.K., Simons, K. and Renkonen, O. (1974) Virol. 61,493-504.
3. Levin, J.G. and Friedman, R.M. (1971) J. Virol. 7,504-514.
4. Simmons, D.T. and Strauss, J.H. (1972) J.Mol.Biol. 71,599-613.
5. Clegg, J.C.S. and Kennedy, S.I.T. (1975) J.Mol.Biol. 97,401-411.
6. Glanville, N., Morser, J., Uomala, P. and Kääriäinen, L. (1976) Europ.J. Biochem. 64,167-175.
7. Keränen, S. and Kääriäinen, L. (1975) J. Virol. 16,388-396.
8. Lachmi, B., Glanville, N., Keränen, S. and Kääriäinen, L. (1975) J.Virol. 16,1615-1629.
9. Wengler, G., Beato, M. and Hackemack, B.A. (1974) Virol. 61,120-128.
10. Kääriäinen, L., Lachmi, B. and Glanville, N. (1976) Ann. Microbiol. (Inst. Pasteur) 127 A, 197-203.
11. Roberts, B.E. and Paterson, B.M. (1973) Proc.Natl.Acad.Sci. U.S.A. 70,2330-2334.
12. Baltimore, D. and Huang, A.S. (1968) Science 162,572-574.
13. Neville, J.M. (1971) J.Biol. Chem. 246,6328-6334.
14. Bonner, W.M. and Laskey, R.A. (1974) Europ.J. Biochem. 46,83-88.
15. Manak, M.M., Abreu, S.L. and Lucas-Lenard, J. (1975) INSERM 47,387-396.
16. Wright, P.J. and Cooper, P.D. (1974) Virol. 59,1-20.
17. Compans, R.W. (1973) Virol. 51,56-70.
18. Söderlund, H. (1973) Intervirology 1,354-361.
19. Kääriäinen, L., Keränen, S., Lachmi, B., Söderlund, H., Tuomi, K. and Ulmanen, I. (1975) Med.Biol. 53,342-352.