INTERACTION BETWEEN SINDBIS VIRUS RNA AND RIBOSOMES FROM CHICK FIBROBLASTS

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Summary: After a short time incubation with ribosomes from chick embryo fibroblasts, the purified RNA from Sindbis virus is bound in a complex which sediments as a 70 - 75 S structure.
Aurintricarboxylic acid, but not NaF, inhibits this interaction.
The addition of GTP, tRNA and energy leads to the formation of a heavier complex (about 100 S) but only when the ribosomes have been extracted from the cells with a nonionic detergent.
It is suggested that the first complex is constituted by viral RNA bound to the light ribosomal subparticle, whereas the heavier complex is formed by the additional binding of a heavy subunit.

There are some indications that the synthesis of polypeptide chains in eukaryote cells is initiated, like in bacterial cells, by the binding of one light ribosomal subparticle to the mRNA molecule. Among these, studies with vaccinia virus infected Hela cells (1) or poliovirus infected cells (2) have shown that the viral RNA becomes associated with the small subunit prior to entering polysomes. Also, in vitro, incubation of ribosomes with mRNA's of myosin (3) or of globin (4) results in the formation of a complex which sediments with the 40 S ribosomal subunit. In the case of the myosin - mRNA, a heavier structure, sedimenting at 80 S with the monosomes, is formed when tRNA and GTP are added to the system (3). Under similar conditions, a complex of about 85 S was also obtained with TMV - RNA and ribosomes from wheat germ (5).
Aurintricarboxylic acid (ATA) and NaF are known to inhibit specifically the binding of a phage mRNA to E. Coli ribosomes without affecting the elongation of polypeptide chains (6). These inhibitors permitted to demonstrate the sequential attachment of one light and one heavy ribosomal subparticle to the mRNA in a reticulocyte lysate: ATA blocks the first step while NaF inhibits only the binding of the 60 S subunit (7).
We report here that the infectious, single stranded RNA (8) extracted from the Sindbis virus particle, can interact in the same way with ribosomes from chick embryo fibroblasts. These findings give further support to the hypothesis that, in the infected cell, the RNA from the parental virion of arboviruses enters into polysomes and takes part in protein synthesis, as does poliovirus RNA (9).

MATERIALS AND METHODS.

Radioactive viral RNA: Labelling of Sindbis virus with ^{3}H-uridine and virus purification were done according to Marcus and Salb (10) with the following modification: the virus was concentrated from the clarified culture medium by sedimentation through a 15% sucrose layer for 2 hours at 30.000 rpm and 4°C. The resuspended virus was immediately purified by centrifugation to equilibrium in a sucrose-D_2O gradient. The ^{3}H-labelled RNA was extracted from the concentrated virions according to Kerr et al. (11). The aqueous layer of the phenol extract was immediately centrifuged in a linear sucrose gradient (15 - 30% sucrose in 0.01 M Tris-HCl pH 7.6, 01 M LiCl, 0.001 M EDTA, 0.1% SDS). After 16 hours centrifugation at 35.000 g in the 9740 rotor of a Martin Christ ultracentrifuge, fractions containing the peak of ^{3}H-RNA sedimenting at 42-44S were pooled and precipitated twice with two volumes of ethanol in the presence of 0.2 mgr/ml bentonite and 0.2 M potassium acetate and redissolved in TKE buffer (0.01 M Tris HCl pH 7.6, 0.1 M KCl, 0.001 M EDTA).

Ribosomes: Primary cultures of chick embryo cells were scraped from the glass after 40 hours. After thorough washing in saline solution, they were broken in a Dounce homogenizer in buffer B (0.02 M Tris, HCl pH 7.6, 0.15 M KCl - 0.005 M Mg acetate), and the ribosomes were isolated by the method of Heywood and Nwagwu (12). Alternatively the cells were broken open with Nonidet P 40 (13). This gave a four or five time higher yield of ribosomes. In both cases, the ribosomal pellet (4 hours at 150.000 g) was suspended in buffer B + 10% glycerol, dialysed overnight against this medium and stored at -70°C.

Binding assay: Ribosomes (5 to 7 O.D. 260 units) were incubated for 10 min. at 35°C with ^{3}H-labelled RNA in a total volume of 500 µl buffer B, containing 0.5 µmole dithiothreitol and 10 µl of a 20% éthanol solution of diethylpyrocarbonate (DEP) in a plastic vial. When this strong ribonuclease inhibitor (14) was omitted, the RNA was immediately degraded when mixed with the ribosomes. ATA or NaF were added to a final concentration of 0.4 mM and 10mM respectively, 5 minutes before addition of the labelled RNA. 0.25 µmole GTP, 100 µg unstripped tRNA (prepared from muscle of 12 days chick embryos), 0.5 µmole ATP and an ATP-genrating system (4 µmole creatine phosphate, 8 µg creatine phosphokinase) were included when indicated in the legends.

After incubation, the reaction mixtures were cooled to 0°C and layered on 30 ml linear sucrose gradients (10 - 30% in buffer B) and centrifuged for 15 hours at 16.000 rpm at 4°C. The absorbance was determined at 260 nm.

Each sample was filtered through a nitrocellulose membrane filter (Sartorius SM 0.45 μ). The filters were washed with 3 ml buffer B, dried and counted in toluene based scintillation fluid in a Packard spectrometer.

Results and discussion

The purity of the viral ^{3}H-RNA preparation was tested by sedimentation in a 15 - 30% linear sucrose gradient in TKE buffer, with ribosomal RNA as a marker. More than 90% of the radioactivity was found in a narrow peak corresponding to 43 S as calculated according to Martin and Ames (15). A similar sedimentation constant was previously described for Sindbis virus RNA (10).

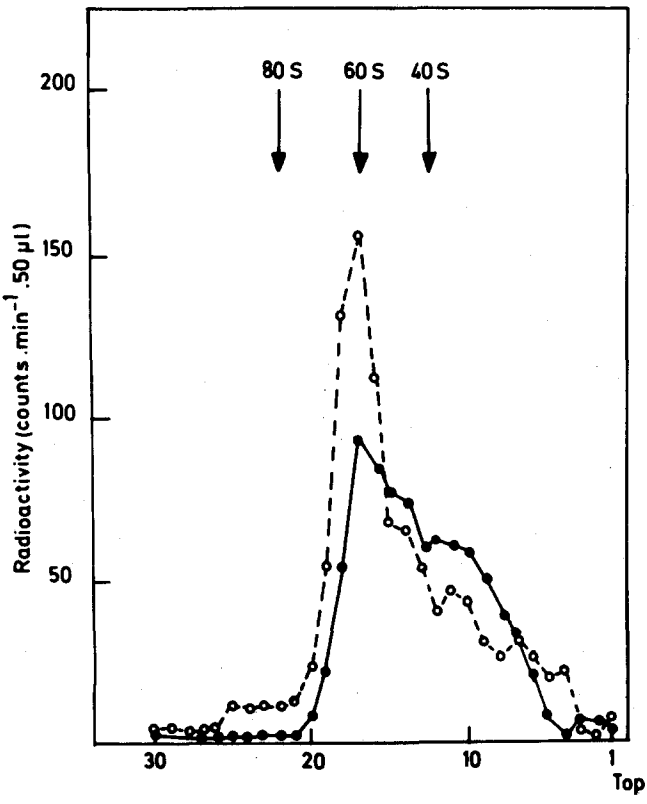

Fig. 1. Sedimentation profile in a sucrose gradient of ^{3}H - labelled RNA (15.000 cpm) in buffer B

o ——— o ^{3}H-labelled RNA

o - - - - o ^{3}H-labelled RNA + GTP + tRNA + ATP + ATP - generating system.

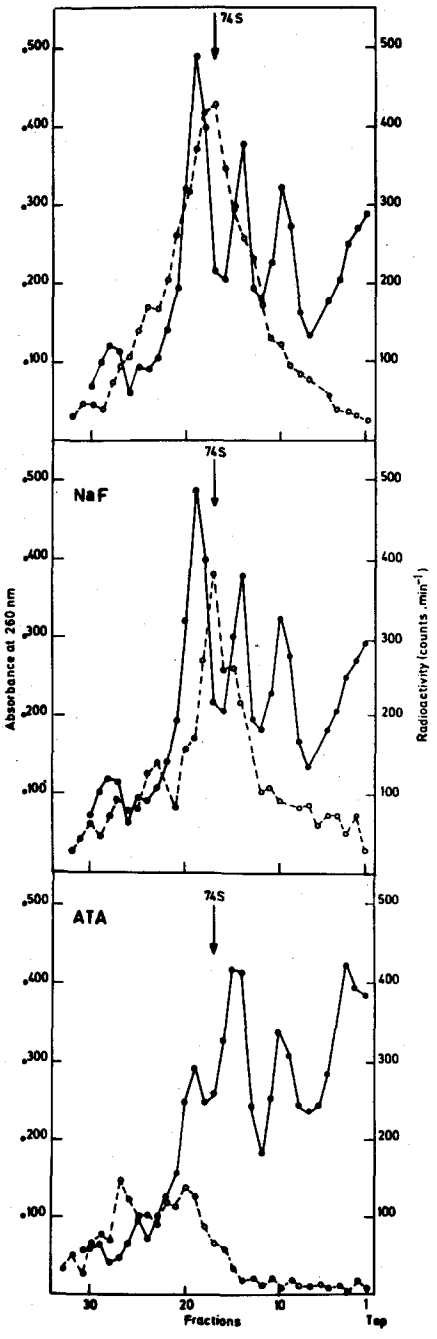

Fig. 2. Sedimentation profiles in sucrose gradients of ribosomes incubated with ^{3}H-labelled RNA (upper curve), or in the presence of 10 mM NaF or 0.4 mM ATA

o ——— o Absorbance at 260 nm

o - - - - o ^{3}H activity retained on nitrocellulose filters

The addition of 0.4%* diethyl pyrocarbonate to the RNA preparation did not modify this peak (not shown).

Fig. 1 shows the sedimentation profile of ^{3}H-labelled RNA in buffer B, alone or after addition of GTP, tRNA, ATP and an ATP-generating system. In the ionic environment of the "binding reaction", the viral RNA now sediments as a single peak at 60 S. It was verified that this radioactive RNA passed quantitatively through the nitrocellulose filters.

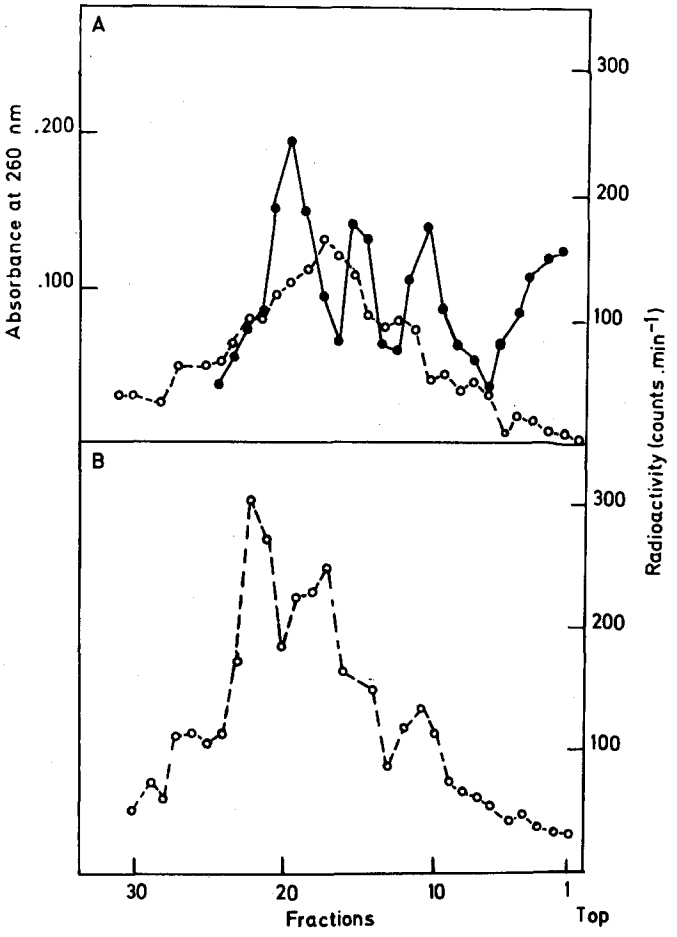

Fig. 3 Sedimentation profiles in sucrose gradients of ribosomes incubated with ^{3}H-labelled RNA (A) and tRNA, GTP and energy (B)

o ——— o Absorbance at 260 nm

o - - - - o ^{3}H activity retained on nitrocellulose filters

* Since diethyl pyrocarbonate reacts and is inactivated by plastic (16), it is likely that, in our experimental conditions, the effective concentration of this inhibitor was much lower than 0.4%.

After incubation with ribosomes, the ^{3}H-labelled RNA is associated with a component which displaces it to the 74 S region and which allows its retention on a nitrocellulose filter (Fig. 2, upper curve). This reaction was inhibited by ATA but not by NaF (Fig. 2), indicating that the associated component was the light ribosomal subunit. A heavier and heterogenous complex is formed in the presence of ATA, the nature of which remains unexplained. As already noticed by others, (4), ATA induced an important lowering of the sedimentation constant of 80 S ribosomes which sediment now at 60S.

A second radioactive complex of about 95 - 100 S is formed in addition to the 74 S structure when tRNA, GTP and energy are supplied (Fig. 3). ATP is necessary for this reaction, in agreement with observations in the TMV - RNA - wheat germ system (5) but not with those in the myosin RNA - muscle system(3)

Also, the 95-100 S complex was only observed with ribosomes obtained from Nonidet P40 - treated cells. This suggests that most of the "free" ribosomes are inactive or lack some of the initiation factors necessary for translation whereas the nonionic detergent might extract functionnally active ribosomes and subunits. It may be objected that a non specific association of the viral RNA with ribosomes had occured here and, in particular, the role of DEP must be controled. It is known that DEP reacts with aminoacids (14) and inactivates the infectivity of single-stranded TMV - RNA (16) and poliovirus RNA, while it does not alter the biological activity of double-stranded nucleic acids (17).

Nevertheless, the action of ATA and NaF on the formation of the 74 S peak, and the requirements for tRNA, GTP and ATP to obtain the 100 S compound indicate that a specific initiation complex is observed here.

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