Inhibition of Protein Synthesis in Reticulocyte Lysates by Poliovirus

(Accepted 11 December 1975)

SUMMARY

Addition of $1 \times 10^{10}$ p.f.u. purified poliovirus to 100 $\mu$l of a rabbit reticulocyte lysate protein synthesizing system causes a complete inhibition of initiation of protein synthesis. This inhibition is not due to the viral RNA nor to any contaminants of the preparation, but is most likely caused by the viral coat protein.

A well-established early phenomenon of picornavirus infection is a marked inhibition of host-cell protein synthesis (Levintow, 1974). The rapidity of onset and magnitude of this inhibition vary with different viruses, the multiplicity of infection, as well as the host cell type; both are especially pronounced in poliovirus infection of HeLa cells. Numerous mechanisms have been proposed to account for this virus directed inhibition; notable among them, the model in which poliovirus double-stranded RNA is the actual effector of host cell protein synthesis suppression (Hunt & Ehrenfeld, 1971). However, further studies on the role of double-stranded RNA in this inhibition (Celma & Ehrenfeld, 1974) did not bear out the mechanism proposed earlier (Hunt & Ehrenfeld, 1971).

Earlier work in this laboratory (Breindl & Koch, 1972) demonstrated that exposure of HeLa cells to hydroxylamine inactivated poliovirus or to isolated viral protein VP4, increased the competence of cells for infection by viral RNA. These observations suggested that viral coat proteins play an important role in the events leading to the initiation of the virus growth cycle, which include not only adsorption and penetration, but inhibition of host protein synthesis as well. Additional evidence implicating viral coat proteins in host cell suppression comes from studies with temperature-sensitive poliovirus mutants defective in repression of host protein synthesis (Steiner-Pryor & Cooper, 1973) in which the repression defective mutants were shown to carry temperature sensitive defects solely in structural protein. We present here some findings from a study on the effects of poliovirus coat proteins on a mammalian protein synthesizing system. It was found that purified whole poliovirus, when added to an in vitro protein synthesizing system of rabbit reticulocyte lysate, caused a complete shut-down of protein synthesis after a lag period of about 10 to 15 min at 26 °C.

Mahoney strain type 1 poliovirus was prepared from infected HeLa cells and purified on CsCl gradients as previously described (Koch, 1971). Reticulocytes were obtained from rabbits treated with 2-acetylphenylhydrazine (Sigma) as described by Villa-Komaroff et al. (1974). The lysate preparation and in vitro protein synthesis conditions were essentially the same as described by Villa-Komaroff et al. (1974), with a 100 $\mu$l final incubation volume of which 60 $\mu$l is reticulocyte lysate. Incubations were carried out at 26 °C and [$^{35}$S]-methionine (sp. act. 324 Ci/mmol) was used to follow incorporation of amino acids into protein. Protein synthesis was measured by the method of Mans & Novelli (1961). CsCl purified virus and empty capsids were dialysed in 25 mM-HEPES (Calbiochem), 50 mM-KCl, pH 7.0, before addition of the assay system.

As seen in Fig. 1, 10 $\mu$l of a purified, dialysed poliovirus preparation containing $1 \times 10^{10}$ p.f.u. (about 10 $\mu$g virus) was observed to cause a complete inhibition of protein synthesis when included in 100 $\mu$l of the reticulocyte lysate protein synthesizing system (about 12 mg
total protein). Heating the virus at 55 °C for 3 min, to disrupt the protein coat (Koch, 1960), prior to adding the lysate system results in a similar inhibition, indicating that intact virus is not necessary for the inhibition. Addition of an amount of phenol extracted purified virus RNA equivalent to that contained in $1 \times 10^{10}$ p.f.u. whole poliovirus only results in a slight decrease in the overall rate of protein synthesis, indicating that it is not the RNA in the virion that is responsible for the inhibition. Addition of an exogenous mRNA to a non-pre-incubated reticulocyte lysate extract usually does result in a reduced rate of overall protein synthesis (Lodish, Weinberg & Ozer, 1974). Analysis of the products by polyacrylamide gel electrophoresis did not show any virus RNA translation products being made under these conditions. Furthermore, there was no loss in infectivity after incubating poliovirus in the lysate for 30 min at 26 °C, as determined by plaque assay (Bishop & Koch, 1969), indicating that the virus is not disrupted and that the amount of free poliovirus RNA in this system is negligible. Pre-incubating the virus with the lysate for 15 min at 26 °C in the absence of other components necessary for protein synthesis, results in a more efficient inhibition. A similar pre-incubation with a control lysate, or with purified poliovirus RNA, has no appreciable effect.

In order to ascertain that the inhibition was not caused non-specifically by CsCl still adsorbed to virions after dialysis, the purified virus preparation was checked for CsCl content by atomic absorption spectrophotometry. The concentration of CsCl detected in dialysed poliovirus preparations was $3.8 \mu g$ CsCl/10 µl, which is about two orders of magnitude below that necessary to cause inhibition of protein synthesis in this system. Similarly in order to rule out the possibility that sodium dodecyl sulphate (SDS) bound to viral proteins might play a role in this inhibition, poliovirus was prepared and purified.

**Fig. 1.** Effect of various poliovirus components on amino acid incorporation into TCA-precipitable material by rabbit reticulocyte lysates. [³⁵S]-methionine (5 µCi/100 µl incubation mixture) incorporation into TCA-precipitable material at 26 °C. All samples were pre-incubated 15 min at 26 °C with 60 µl reticulocyte lysate prior to addition of other components necessary for in vitro protein synthesis. ○—○, control sample containing 10 µl dialysis buffer; ●—●, 15 µg poliovirus RNA in 10 µl; △—△, 10 µl dialysed empty capsids (same E₁₀₀ as $1 \times 10^{10}$ p.f.u. virus); ★—★, $1 \times 10^{10}$ p.f.u. dialysed poliovirus in 10 µl; ■—■, 10 µl infected HeLa cell cytoplasm.
Short communications

without the use of SDS. This virus was found to be equally effective in shutting off protein synthesis in the lysate system.

Hunt & Ehrenfeld (1971) reported that cytoplasm from poliovirus-infected HeLa cells completely inhibits protein synthesis in a reticulocyte lysate system after 4 min. In the same report they also stated that purified virions added to the lysate have no detectable inhibitory activity. Cytoplasm was prepared from poliovirus-infected HeLa cells according to the method of Hunt & Ehrenfeld, and tested at the concentration described (Hunt & Ehrenfeld, 1971). Ten microlitres of the cytoplasmic extract included in the 100 μl system was indeed found to be inhibitory in our assay system and produced an inhibition identical, both in kinetics and magnitude, to that caused by the addition of 10^6 p.f.u. purified virus. The amount of virus in the infected cell is probably less than that used in this study; however, as a result of uncoating of infecting virions and de novo synthesis, the infected cell must contain free viral proteins, which might be more efficient in producing an inhibition. Empty viral capsids, isolated from the CsCl gradient, also display some inhibitory activity, though not as much as whole virions. This observation is not unexpected since the two species differ in their structural proteins; empty capsids containing three polypeptides, one of which, VP0, gives rise to VP2 and VP4 in the mature virions (Levintow, 1974). The inhibition observed in the presence of added poliovirus is concentration dependent and is not significant if less than 5 × 10^8 p.f.u. (0.5 μg) virus per 100 μl incubation volume is used.

The relatively high quantities of virus needed to cause an inhibition of protein synthesis in the reticulocyte lysate system does not necessarily reflect the in vivo situation where compartmentalization and membrane effects might dramatically alter the mechanism of action and magnitude of the effect. Studies on components of infected cells (Wright & Cooper, 1974) have indicated that there is an association of viral proteins and some ribosomal structures of the host cell. Based on some approximations on the ribosome content of rabbit reticulocyte lysates (Gross & Rabinowitz, 1973), one can calculate that the maximum inhibition in this system is achieved at a poliovirus particle to reticulocyte ribosome ratio of about 1 to 20. The shape of inhibition curves suggests an inhibition of initiation of protein synthesis. Furthermore, the lag period seen in this inhibition indicates that the mechanism of action is probably quite complex and that more refined studies will be needed to obtain a better understanding of the phenomenon.

We thank Dr H. Oppermann for providing the purified poliovirus, and Dr K. D. Gibson for assistance with the Cs determination by atomic absorption spectrophotometry.

Roche Institute of Molecular Biology
Nutley, New Jersey 07110, U.S.A.

J. Racevskis
S. S. Kerwar
G. Koch

REFERENCES


Short communications


(Received 16 September 1975)