Inhibition of the elongation step of protein synthesis by vaccinia virus

P.W.L. Tas and O.H.W. Martini

Institute for Virology and Immunology, University of Würzburg, Versbacher Straße 7, D-8700 Würzburg, FRG

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Vaccinia cores inhibit translation in cell-free protein synthesis systems at two stages: initiation; and, as shown here, elongation. The former effect tends to obscure the latter. Elongation control could, however, be revealed as follows: when, in a reticulocyte of L-cell lysate, initiation was blocked by a drug (edein), the residual [^{35}S]methionine incorporation was severely reduced by the subsequent addition of vaccinia cores. The elongation block could also be demonstrated by analysis of ribosome profiles: treatment with edein alone permitted ribosomal run-off; treatment with either the elongation inhibition anisomycin or with cores preserved the polyribosomes.

Vaccinia core *Control, of protein synthesis* *Cell-free translation system* *Elongation inhibition*

1. INTRODUCTION

The mechanism by which vaccinia virus inhibits protein synthesis in vivo is largely unknown. Several authors have observed the shut-off of host protein synthesis in the absence of viral RNA and protein synthesis [1–3] which suggests that a component of the virion is involved. Further work has shown that the addition of vaccinia cores to cell-free systems results in the inhibition of protein synthesis [4,5].

Evidence has been obtained that inhibition by vaccinia cores occurs at the initiation step of protein synthesis [4–7]. Here, we present evidence that vaccinia cores can also affect the elongation step of protein synthesis.

2. MATERIALS AND METHODS

2.2. *Preparation of vaccinia cores*

The WR strain of vaccinia virus was propagated in L_{929} cells and purified as in [8]. Cores were prepared by dissolving purified virus in 25 mM Tris buffer (pH 7.5) containing 0.25% Nonidet P40 (NP-40) and 50 mM 2-mercaptoethanol (2-ME) followed by centrifugation at 20000 rev./min for 45 min in an SW 50 rotor. The cores were resuspended in 10 mM Tris buffer (pH 7.5) and recentrifuged to remove traces of NP-40. The final pellet was dissolved in 10 mM Tris buffer (pH 7.5) to give 10^{12} core particles/ml.

2.2. *Cell-free systems*

The rabbit reticulocyte lysate was prepared and treated with micrococcal nuclease as in [9,10]. The reaction mixture for studying endogenous protein synthesis in reticulocyte lysates was as in [9] with the following modifications: unlabeled amino acids were added at 200 μM each, [^{35}S]methionine at 5 μCi/incubation mixture, glucose and 2-aminopurine at 2 and 6 mM, respectively. Protein synthesis was assayed by determining the incorporation of [^{35}S]methionine into protein as in [4].

An S 30 from L-cells was prepared as follows: cells were washed 3 times and resuspended in 2 vol. buffer (10 mM Tris–HCl (pH 7.5), 1.5 mM $MgCl_2$, 15 mM KCl and 5 mM 2-ME), left on ice for 10 min and then broken by 20 strokes in a tight fitting Dounce homogenizer. The lysate was made isotonic by addition of buffer A containing

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200 mM Hepes (pH 7.5), 800 mM KCl and 30 mM $MgCl_2$. The S 30 was obtained by centrifugation for 10 min at 10000 rev./min and chromatographed over Sephadex G-25 coarse equilibrated with buffer A (1:10 diluted and containing 5 mM 2-ME). The peak of the void volume fraction was collected and frozen in liquid nitrogen. The conditions for protein synthesis in the L-cell lysate were as in [10].

2.3. *Materials*

Micrococcal nuclease was obtained from PL Biochemicals, NP-40 from Fluka, edeine from Calbiochem and anisomycin from Sigma.

3. RESULTS

3.1. *Effect of vaccinia cores on in vitro protein synthesis in the presence of edeine*

The study of the effect of vaccinia cores on elongation requires a cell-free system that incorporated [^{35}S]methionine into protein in the presence of an inhibitor of initiation.

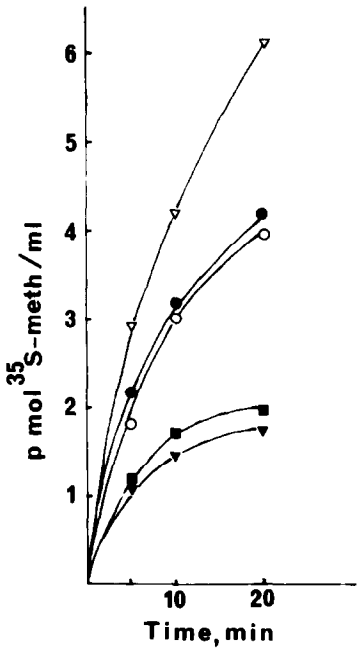

Fig. 1. Effect of vaccinia cores on endogenous protein synthesis in a cell-free system from L_{929} cells: (▽—▽) no addition; (●—●) 1 μM edeine; (○—○) 2 or 5 μM edeine; (■—■) 1.6×10^{11} vaccinia cores/ml; (▼—▼) 1.6×10^{11} vaccinia cores/ml + 2 μM edeine.

After the addition of edeine to our L_{929} cell-lysate at a concentration that completely blocks initiation, [^{35}S]methionine incorporation still continues at 0.3 $pmol.min^{-1}.ml^{-1}$. Vaccinia cores were added to this lysate in the presence and absence of 2 μM edeine (fig. 1). If inhibition were only at the level of initiation the curve of cores plus edeine should coincide with the 2 μM edeine curve. However, since the combined action of cores plus edeine is clearly more inhibitory than edeine alone it would appear that vaccinia cores affect the elongation step of protein synthesis.

In addition we analyzed the effect of vaccinia cores on elongation in a micrococcal nuclease-treated reticulocyte lysate supplemented with encephalomyocarditis (EMC) RNA. Addition of 5 μM edeine together with EMC RNA at the start of incubation blocks the incorporation of [^{35}S]-methionine into protein almost completely (fig. 2A). To study the effect of vaccinia cores on elongation it was necessary to add the edeine 15 min after the start of incubation to enable initiation to take place and thus elongation to occur (fig. 2B). The combined action of cores and edeine was again more inhibitory than edeine alone, confirming our findings with the L_{929} cell lysate.

Control experiments showed that NP-40 in the concentrations used to disrupt cores did not affect the initiation or elongation step of protein synthesis in L-cell lysate. NP-40 up to 0.2% was not in-

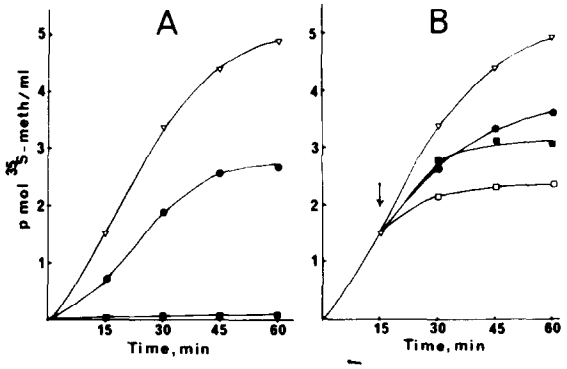

Fig. 2. Inhibition of encephalomyocarditis (EMC) RNA translation in nuclease-treated reticulocyte lysate by vaccinia cores and edeine: (▽—▽) no addition; (●—●) 3 μl cores; (■—■) 5 μM edeine; (□—□) 3 μl cores + 5 μM edeine. In A additions were made at zero time, in B additions (↓) were made 15 min after the start of incubation.

hibitory for reticulocyte lysate. Tris and 2-ME did not inhibit protein synthesis in the concentrations used in our experiments.

3.2. *Effect of vaccinia cores on the polyribosome profile of reticulocyte lysates*

An inhibitor of initiation should cause the disappearance of polyribosomes when initiation is the rate-limiting step of protein synthesis. However, an inhibitor of elongation should result in an increase in polysomes but only if free ribosomal subunits and mRNA are still available.

Fig. 3 shows the effect of vaccinia cores and the initiation inhibitor edeine on the polyribosome profile of a reticulocyte lysate after 10 min incubation. The addition of edeine at 0.4 μM which inhibited protein synthesis less than cores (fig. 3A),

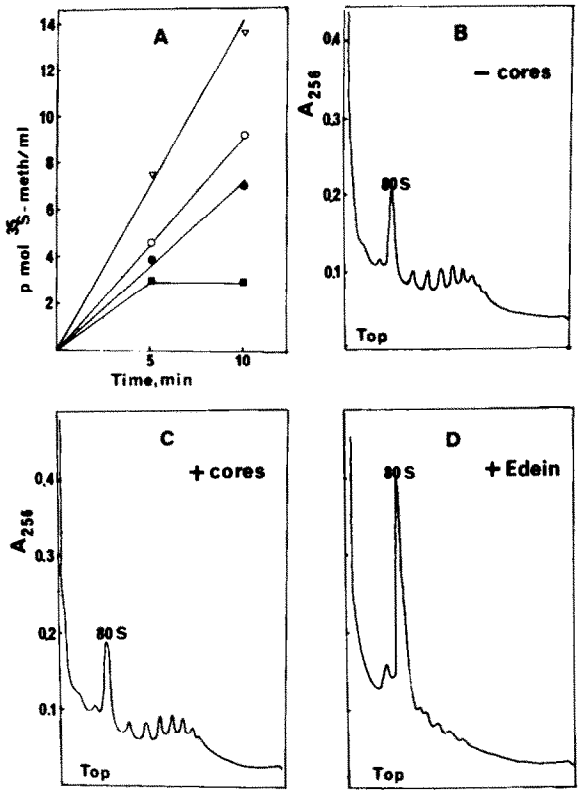

Fig. 3. Effect of vaccinia cores and edeine on endogenous protein synthesis and polyribosome profile of reticulocyte lysates. The inhibition of the protein synthesis by cores and edeine is shown in (A): (▽—▽) no addition; (○—○) 0.4 μM edeine; (●—●) 1.6×10^{11} cores/ml; (■—■) 2 μM edeine. Reticulocyte lysate (60 μl), in 100 μl final vol. was incubated in the absence (B) or in the presence (C) of 1.6×10^{11} cores/ml or with 0.4 μM edeine (D). After 10 min the reaction was stopped with 200 μl gradient buffer (50 mM Tris–HCl (pH 7.5), 100 mM KCl, 5 mM $Mg(AC)_2$ and 5 mM 2-ME) containing 400 μg cycloheximide/ml. Samples were loaded onto 10–50% isokinetic sucrose gradients in gradient buffer (containing 75 μg cycloheximide/ml) and centrifuged at 36000 rev./min for 110 min in a Beckman SW 41 rotor. The contents of the tubes were monitored at 256 nm using an Isco type 6 optical unit.

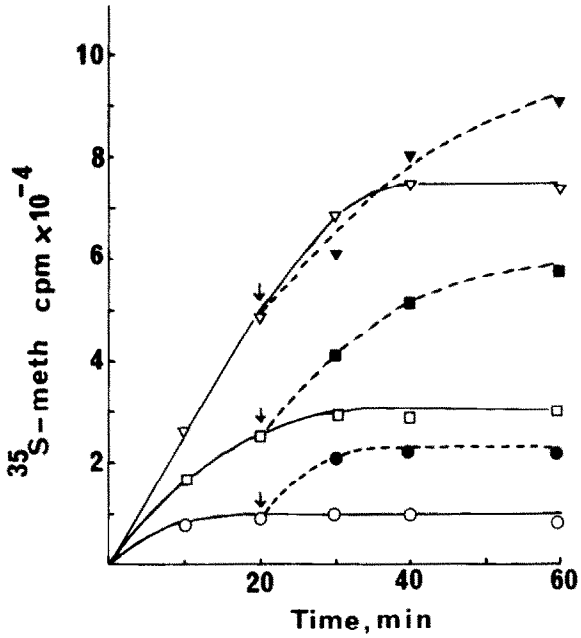

Fig. 4. Effect of addition of extra lysate on protein synthesis in reticulocyte lysates incubated in the presence and absence of mRNA and cores. Duplicate samples of a micrococcal nuclease-treated reticulocyte lysate were incubated respectively without mRNA, with mRNA and with mRNA plus cores (final vol. 30 μl). After 20 min incubation 20 μl nuclease-treated lysate (containing all ingredients for protein synthesis except mRNA) was added to one set of incubation mixtures (thereby doubling the volume). Samples of 5 μl were taken at intervals to measure the incorporation of [^{35}S]methionine into protein. Samples of 10 μl were taken when extra lysate had been added to the incubation mixture: (○—○) – mRNA; (●---●) – mRNA + 20 μl nuclease-treated lysate at 20 min; (▽—▽) + mRNA; (▼---▼) + mRNA + 20 μl nuclease-treated lysate at 20 min; (□—□) + mRNA + 1.5×10^{11} core particles/ml; (■---■) + mRNA + 1.5×10^{11} core particles/ml + 20 μl nuclease-treated lysate at 20 min.

caused an almost complete loss of polyribosomes (fig. 3D). The addition of cores, however, did not change the profile (fig. 3C), as was the case with anisomycin, an elongation inhibitor (not shown). From this we conclude that in this system it is mainly the elongation step of protein synthesis which is inhibited by cores. An effect on the termination step of protein synthesis seems less likely because an accumulation of large polysomes was not found.

The possibility that protein synthesis is inhibited by degradation of mRNA seems unlikely in view of fig. 3C. Additional evidence that mRNA is not degraded by cores is presented in fig. 4. Lysates were incubated with vaccinia cores for 20 min at which time protein synthesis proceeds at a low rate. Addition of nuclease-treated lysate (-mRNA) to this core-inhibited lysate at 20 min resulted in an increased rate of protein synthesis. At 60 min, an extra 28 000 cpm were incorporated due to the addition of nuclease-treated lysate. Part of this increase was caused by background incorporation (13 000 cpm). The difference of 15 000 cpm has to be ascribed to translation of the added mRNA into protein. This indicates that mRNA is still biologically active in the core-inhibited lysate.

4. DISCUSSION

Here, we show that the elongation step of protein synthesis is inhibited by vaccinia cores in two different cell-free systems where initiation was blocked by edeine. Analysis of polyribosome profiles of lysates incubated in the presence and absence of cores confirmed that inhibition occurred at the elongation step of protein synthesis (fig. 3). This result seems to contradict the observation [4] that vaccinia cores fail to inhibit poly(Phe) synthesis under the direction of poly(U). At present we cannot explain this discrepancy.

The fact that inhibition at the elongation step of protein synthesis was overlooked till now is probably caused by the fact that under normal condition (fig. 2A) we cannot discriminate between initiation and elongation inhibition.

Since vaccinia cores inhibit both the initiation and elongation step of protein synthesis, the possibility exists that more than one component of the translational machinery is affected. Furthermore, it would be interesting to determine whether a reduced rate of elongation also exists in cells shortly after infection with vaccinia virus.

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