VESICULAR STOMATITIS VIRUS DEFICIENT IN PROTEIN KINASE IS INFECTIOUS

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1. Introduction

Several enzyme activities have been detected in purified vesicular stomatitis virus (VSV) and these include an RNA-dependent RNA polymerase, protein kinase, nucleoside triphosphate phosphotransferase, nucleoside triphosphatases and a proteinase (see review, ref. [1]). The RNA extracted from such virions is not infectious [2]. To exhibit infectivity, the virion RNA (negative-strand RNA) must be transcribed by the virion-associated transcriptase into a complementary RNA (positive-strand RNA) that serves as a messenger RNA for the synthesis of virus-specific proteins as well as a template for the production of virion RNA [1,3]. The transcriptase which has been purified was found to consist of two of the five viral structural polypeptides, the L and NS polypeptides [4]. Little is known about the other virion-associated enzymes. However, protein kinase has been the subject of numerous studies recently [5–9]. This enzyme is located within the virion and can be activated by mild disruption of the virion with non-ionic detergents, to phosphorylate the L, NS and M proteins in a cell-free system [6,7]. The protein kinase in VSV is probably of host cell origin [6,7]. The biological function of phosphorylated virion proteins is not known. One question which could be asked is whether the virion-associated protein kinase is required to initiate infectivity. We have attempted to answer this question by analyzing the protein kinase activity and transcriptase activity in relation to infectivity of a VSV preparation that is deficient in protein kinase.

2. Materials and methods

2.1. *Cells*

Baby hamster kidney (BHK-21) cells were propagated at 37°C and turtle heart (TH-1) [10] cells at 23°C in Eagle's minimum essential medium containing 10% calf serum.

2.2. *Virus*

Confluent monolayer cultures of BHK-21 or TH-1 cells were infected with 0.1–0.2 plaque forming units (p.f.u.) of VSV (Indiana serotype) per cell and incubated at 33°C for 24 h in medium containing 0.2% bovine serum albumin instead of calf serum [5]. Virus released into the culture medium was purified by precipitation with zinc acetate and banding in 10–60% (w/v) sucrose gradients as described by Sokol and Clark [15]. In the present study only B particles were used.

2.3. *Protein-kinase assay*

The reaction mixture contained 10 μmol Tris–HCl (pH 8.0), 2 μmol $MgCl_2$, 2 μmol dithiothreitol, 10 nmol [γ-^{32}P]ATP (New England Nuclear, Boston, Mass.) having a spec. act. 1125 cpm/pmol, 0.05% Nonidet P-40, 100 μg phosvitin (Sigma Chemical, St. Louis, Mo.) and 20 μg of virus or 30 μg of cell extract in a final volume of 200 μl. After incubation at 33°C for 30 min, acid-insoluble radioactivity was determined as described previously [9].

3. Results and discussion

VSV can replicate in a large variety of cells, and

protein kinase was readily detectable in virions obtained from VSV-infected mouse, chick embryo, hamster kidney, human and mosquito cells [6]. Similarly, we found that VSV grown in both African green monkey kidney and baby hamster kidney (BHK-21) cells had comparable protein kinase activities (K. B. Tan, unpublished). However, we found that virions purified from turtle heart (TH-1) cells had a 30-fold lower protein kinase activity compared with virions purified from BHK-21 cells (table 1). Previously we had shown that phosvitin added as a phosphate acceptor greatly increased the sensitivity of the protein-kinase assay [9]. The incorporation of [^{32}P]-phosphate into proteins was increased in the presence of phosvitin, and this increase was relatively greater for virions from BHK-21 cells compared to virions from TH-1 cells. Uninfected BHK-21 and TH-1 cells show a similar level of protein kinase activity (table 1).

Evidence has been presented to suggest that protein kinase may be required to activate the transcription of VSV, vaccinia, Rous sarcoma, and viper viruses in cell-free systems [8,11,12]. If this is true, then VSV grown in TH-1 cells (VSV-TH), being deficient in protein kinase, should exhibit lower transcriptase activity compared to VSV grown in BHK-21 cells (VSV-BHK). This was found to be the case (fig.1). The incorporation of [^{3}H]UMP by VSV-TH was detected only after a 1 h lag period and subsequently the rate of [^{3}H]UMP incorporation was about 9-fold

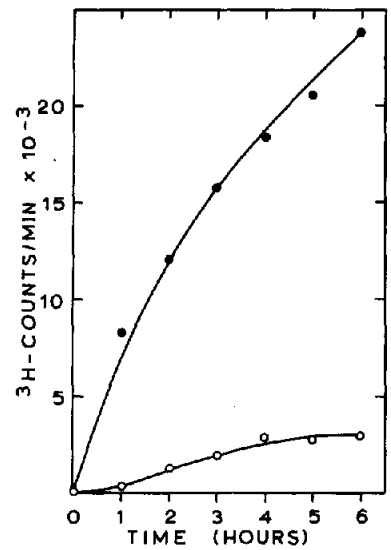

Fig.1. RNA polymerase activity in purified VSV. The RNA polymerase activity associated with purified VSV grown in BHK-21 (•—•) or TH-1 (○—○) cells was determined as described previously [13]. A scaled-up reaction mixture (750 μl) containing 48 μmol Tris–HCl (pH 8.2), 6 μmol $MgCl_2$, 12 μmol dithiothreitol, 0.6 μmol each of ATP, CTP and GTP, 0.06 μmol UTP, 100 μCi/ml of [^{3}H]UTP, 0.05% of Nonidet-P40 and 115 μg virus was incubated at 28°C. At the indicated times, 100 μl samples were withdrawn for determination of acid-insoluble radioactivity. The acid-insoluble radioactivity (1990 cpm) of a sample taken just before incubation at 28°C was subtracted from the rest of the samples.

Table 1
Protein kinase activity in purified VSV and mock-infected cells[a]

Addition	[^{32}P]Phosphate incorporated[b] (cpm)			
	VSV grown in		Mock-infected cells	
	BHK-21	TH-1	BHK-21	TH-1
None	35 298	1141	12 498	12 040
Phosvitin	187 376	3542	82 759	38 328

[a] Protein kinase activity was assayed as described in Materials and methods

[b] Virus grown in BHK-21 or TH-1 cells was purified as described previously [9]. Confluent monolayer cultures were mock-infected and incubated at 33°C for 18 h in medium containing 0.2% bovine serum albumin instead of serum [5]. The cells were washed and suspended in a solution containing 0.5% Nonidet P-40, 5 mM $MgCl_2$, 0.13 M NaCl and 0.05 M Tris–HCl, pH 8.0. After incubation in an ice bath for 10 min, the cells were disrupted in a dounce homogenizer to separate nuclei from the cytoplasm. The cytoplasmic fraction was subjected to centrifugation (100 000 × *g*, 90 min, 4°C) and the supernatant fraction was assayed for protein kinase activity.

lower than that by VSV-BHK. Purified VSV-BHK and VSV-TH were analyzed on polyacrylamide gels and were found to have the same polypeptide profile. This suggests that the lower transcriptase activity exhibited by VSV-TH cannot be attributed to a decreased amount of the transcriptase proteins (L and NS).

The marked decrease in both protein kinase and transcriptase activities in VSV-TH was not accompanied, however, by a comparable decrease in the plaque-forming ability of the virions (table 2). The difference in specific infectivity (p.f.u./mg virus) between virus grown in the two cell types was only 2–2.5-fold. The kinetics of virus production in both BHK-21 and TH-1 cells was determined by harvesting cultures at different times after infection and quantitating the virus released into the culture medium by plaque assay on BHK-21 cells. The results presented in fig.2 show that although the final yield of both VSV-BHK and VSV-TH at 24 h was higher from BHK-21 than from TH-1 cells, the kinetics of virus production during the log phase were similar for both sets of cells infected with VSV-BHK or VSV-TH.

The ability of VSV-TH to replicate as efficiently as VSV-BHK in both BHK-21 and TH-1 cells is very intriguing since VSV-TH exhibits both lower protein kinase and transcriptase activities compared with VSV-BHK. The L and NS polypeptides of VSV, putative components of the viral transcriptase complex [4,14], can be phosphorylated in a cell-free system by the virion-associated protein kinase [6,7]. It is plausible to speculate that the transcriptase may thereby be activated as was suggested for other viral systems [8,11,12]. The above findings suggest that in cells infected with VSV-TH, the cellular protein kinase(s) induces the activation of the virion-associated transcriptase by phosphorylation to initiate virus

Table 2
Specific infectivity of purified VSV

	Plaque-forming units/mg virus assayed on	
Virus	BHK-21 cells	TH-1 cells
VSV-BHK	1.9×10^{10}	4.8×10^{9}
VSV-TH	1.0×10^{10}	1.9×10^{9}

The plaque-forming ability of purified virus derived from either BHK-21 or TH-1 cells was determined on both BHK-21 and TH-1 cells.

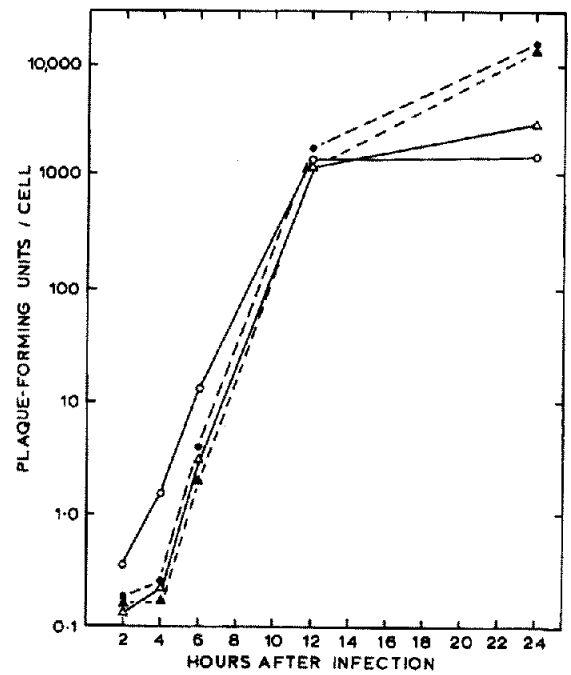

Fig.2. Growth curves of VSV in BHK-21 and TH-1 cells. Confluent BHK-21 and TH-1 cells were infected with 10–25 p.f.u. virus (stock suspension)/cell and incubated at 33°C. Virus released into the culture medium was quantitated by plaque assay on BHK-21 cells. BHK-21 cells infected with VSV-BHK (●—●) or VSV-TH (▲---▲); TH-1 cells infected with VSV-BHK (○—○) or VSV-TH (△—△).

replication. Thus it may be concluded that (a) VSV-TH is deficient in protein kinase but not transcriptase and (b) the virus-associated protein kinase may not be essential for initiating infectivity, this function can be provided by preformed host cell enzyme(s). The participation of host cell factors in the expression of VSV early functions has so far not been recognized. However, the possibility exists that the level of transcriptase associated with VSV-TH is adequate for initiating a normal yield of virus but this seems unlikely.

Acknowledgements

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