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Preparation and Properties of an Inhibitory Extract from Frog Virus 3 Particles

A.-M. AUBERTIN, C. HIRTH, C. TRAVO, H. NONNENMACHER, AND A. KIRN

Université Louis Pasteur, Groupe de Recherches de l'I.N.S.E.R.M. sur la Pathogénie des Infections Virales, 67000 Strasbourg, France

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The structural constituents of the frog virus 3 particle were solubilized by treatment with a nonionic detergent followed by the addition of a high salt concentration. This soluble viral extract (SVE) inhibits host nucleic acid synthesis. Its activity on RNA synthesis was studied in KB cells and found to be dependent on the presence of DEAE dextran. Inactivation of the inhibitory properties of SVE were obtained by trypsin digestion, treatment with urea, or heat denaturation. Neutralization of the activity of SVE was obtained by anti-frog virus 3 serum but not by anti-BHK serum. In vitro a complex may be formed between polynucleotides and the inhibitor indicating a possible mechanism for vivo inhibition.

Infection of cell cultures with frog virus 3 (FV3) is rapidly followed by an important decrease in the synthesis of host nucleic acids. This inhibition occurs at permissive and nonpermissive temperatures for viral multiplication (1, 3, 7, 14, 15). In vivo inoculation of live or inactivated FV3 into mice lead to an acute toxic degenerative hepatitis which killed the animals within 18 to 36 h (2, 10). RNA synthesis in the liver of the mice was rapidly blocked, and a 50% decrease in the activities of RNA polymerases A and B were noticed as early as 3 h after inoculation (M. Elharrar and C. Hirth, *submitted for publication*). Reduction of DNA and RNA synthesis in the presence of cycloheximide and actinomycin D (7) indicated that the virus-directed inhibition was not dependent on de novo synthesis of macromolecules. Therefore, a viral component should be responsible for this inhibition. In an attempt to isolate the active molecules, we tried to solubilize the constituents of FV3 particles. In the present paper the properties of the soluble viral extract (SVE) and the conditions of its activity on cellular RNA synthesis are described.

MATERIALS AND METHODS

Cells. Monolayers of BHK-21 cells were grown in Stocker medium (Eurobio, Paris) supplemented with 5% calf serum. KB cells were cultivated in lactalbumin yeast extract Earle salt plus 5% calf serum.

Virus production and purification. FV3 was kindly provided by Dr. A. Granoff. The virus was propagated in BHK-21 cells. BHK monolayers grown in 2-liter roller bottles were infected with a multiplicity of 0.5 PFU/cell. After adsorption for 1 h, medium supplemented with 5% calf serum was added, and the cells were incubated at 26 C for 3 days. The cells were then collected, subjected to ultrasonic treatment (3 × 1 min, MSE disintegrator) to release cell-associated virus, and centrifuged at 1,500 × *g* for 15 min to remove cellular debris. The virus was pelleted by centrifugation at 45,000 × *g* for 45 min. The pellet was suspended in 0.001 M Tris-hydrochloride buffer (pH 9), subjected to a brief ultrasonic treatment, layered on a 10-ml sucrose cushion (43%, w/w), and centrifuged in a SW27 rotor at 120,000 × *g* for 4 h. The virus pellet was resuspended in Tris-hydrochloride, and layered onto preformed gradients of 10 to 40% (w/w) sucrose in Tris buffer. After centrifugation at 35,000 × *g* for 45 min, the virus band was collected, diluted in Tris-hydrochloride, and sedimented at 30,000 × *g* for 45 min. The resulting pellet was homogenized in 0.001 M triethanolamine (TEA), pH 9, and sonically treated for 1 min. In some cases, CsCl gradients (20 to 40% [w/w], 4 h, 200,000 × *g*) were made to obtain purified virus preparations exempt of cellular exonucleases (17) and ATPase (21). After dilution of virus particles to a concentration of 10^9 PFU/ml, the preparation was checked by electron microscopy after negative staining.

Determination of cellular RNA synthesis. After infection with FV3 or treatment with SVE or the control preparation, KB cell monolayers were incubated for different times in minimal essential medium (MEM) plus 5% dialyzed calf serum at 37 C. At 0.5 h before the end of each period, the cells were pulse-labeled with ^{3}H-uridine at a final concentration of 0.5 μCi/ml (specific activity 20 Ci/mM). After trypsinization, labeled cells were collected by centrifugation at 1,000 × *g* for 5 min and washed twice with

cold phosphate-buffered saline. Cells were suspended in 5% trichloroacetic acid; nucleic acids were precipitated for 30 min at 0 C, and the acid-precipitable material was collected by centrifugation. The pellets were washed three times with cold 5% trichloroacetic acid. Finally, the precipitates were dissolved in 0.5 ml of digestin and diluted with 2 ml of ethanol and 10 ml of toluene scintillator fluid (PPO, 4 g, and POPOP, 0.4 g, per liter of toluene). The radioactivity of the solutions was determined in a Beckman scintillation counter LS 250.

Nucleotide phosphohydrolase assay. The reaction mixture contained the following, in a final volume of 250 μliters: 80 mM Tris-hydrochloride (pH 8.0), 6 mM $MgCl_2$, 0.2 mM ATP, 5 μCi of ^{3}H-ATP (specific activity 500 mCi/mM), 100 μliters of virus or soluble viral extract, both containing 50 μg of proteins. At the end of 30 min of incubation time at 37 C, 50-μliter samples were applied to DEAE cellulose paper (Whatman DE81). The chromatograms were developed by the procedure of Morrison (16), with 0.6 M ammonium formate (pH 3.1) for 5 to 6 h. The paper was dried overnight, and the chromatogram was cut into rectangles (4 by 2 cm), immersed in 12 ml of scintillator, and counted.

Endodeoxyribonuclease assay. ^{3}H-DNA of KB cells (specific activity 25×10^3 counts per min per μg of DNA) was used to measure the hydrolysis into shorter fragments. The reaction mixture contained 18 μg of DNA, 1 mM $MgCl_2$, 100 mM Tris-hydrochloride (pH 7.6), 25 μg of virus or SVE protein in 200 μliters. In the assay with virus, NP_{40} was added at a concentration of 0.2%. After incubation (1 h at 30 C), the reaction was stopped by adding 0.2% sodium dodecyl sulfate (SDS), and the products were analyzed by centrifugation in alkaline sucrose gradient (5 to 20% [w/v] sucrose in 0.9 M NaCl, 0.01 M EDTA, 0.1 M NaOH) for 3.5 h at 20 C and 40,000 rpm in an SW41 rotor (20 C). Radioactivity of the fractions was counted in a toluene Triton $\times$100 scintillator.

Protein kinase assay. The reaction mixture consisted of 50 mM Tris-hydrochloride (pH 8.5), 6.6 mM $MgCl_2$, 6 mM dithiothreitol, 50 μg of virus protein, or soluble viral extract protein, 0.1 μM ATP, 5 μCi [γ^{32}P] of ATP (specific activity 1,320 mCi/mmol) or 5 μCi [α^{32}P] of ATP (specific activity 1,380 mCi/mmol) in a total volume of 300 μliters.

NP_{40} was added to the virus assay at a final concentration of 0.5%. After incubation for the indicated intervals, the mixture was diluted with an equal volume of 10% trichloroacetic acid. After 20 min of storage in an ice bath, the precipitate was collected by filtration on a Whatman fiberglass filter (GF/C), washed, and dried, and the radioactivity was measured by liquid scintillation spectroscopy.

Assay of RNase. *Escherichia coli* 16*s* ^{32}P-RNA was used as substrate to measure the RNase activity. The reaction mixture contained RNA (0.5 μg) and virus or SVE added to Tris-magnesium chloride buffer (10 mM Tris, pH 7.5, 1 mM $MgCl_2$) in a final volume of 0.1 ml. Disruption of virus particles was obtained by adding NP_{40} to the assay at a concentration of 0.2%. After an incubation at 37 C for 1 h, the reaction was stopped by the addition of 0.5% SDS after the addition of cold RNA (20 μg). The samples were layered onto 15 to 30% sucrose gradients (in 10^{-2} M Tris [pH 7.5], 0.5% SDS, 0.1 M LiCl, 10^{-3} M EDTA) and centrifuged in a SW50 rotor at 30,000 rpm for 18 h. The radioactivity of the fractions was determined.

Antigen-antibody precipitation. Rabbit anti-FV3 serum was prepared as described by Kirn et al. (11). To obtain anti-BHK serum, rabbits were immunized by BHK cells (1.2×10^7) supplemented with complete Freund adjuvant once a week for four consecutive weeks. They were then killed 10 days after a booster inoculation.

The virus-soluble extract and the control preparation were diluted with the same volume of different dilutions of either anti-FV3 serum or anti-BHK serum (dilutions made in 0.10 M NaCl). These solutions were incubated for 30 min at 37 C, and the precipitates formed were sedimented at $1,500 \times g$ for 30 min. These supernatant fluids were assayed for their inhibitory activity on KB RNA synthesis.

Enzymes and chemicals. DNase I was obtained from Worthington Biochemical Corp. and trypsin from Sigma Chemical Co. Iniprol was a gift from Laboratoire Choay (Paris); nonionic detergent P_{40} (NP_{40}) was purchased from B.D.H. (England); and digestin ($C_{16}H_{37}NO$) was a product from Merck.

Polydeoxyadenylate-thymidylate (poly dAT), polydeoxyguanylate-polydeoxycytidylate (poly dG, dC), and poly-L-ornithine were purchased from Miles Laboratories. DEAE dextran was a product from Pharmacia-Uppsala. ^{3}H-ATP (specific activity 500 mCi/mmole), α^{32}P-ATP (specific activity 1,380 mCi/mmol) and γ^{32}P (specific activity 1,320 mCi/mmol) were obtained from the Radiochemical Center Amersham, and ^{3}H-uridine (specific activity 20 Ci/mmol) was from the Commissariat à l'Energie Atomique (Saclay, France).

RESULTS

Preparation of the virus-soluble extract. A purified virus suspension (approximately 1 mg of protein/ml) was treated with NP_{40} (final concentration 0.5%) for 30 min at room temperature. The mixture was then cooled at 0 C, and solid LiCl was added to attain 20% (w/w). To avoid an increase in temperature, the adjunction of salt was made progressively. After 5 to 6 h, the incompletely disrupted particles were eliminated by centrifugation at $200,000 \times g$ for 1 h. The supernatant fluid was dialyzed for 36 h against 10^{-3} M TEA, pH 9, and then centrifuged at $1,000 \times g$ for 15 min to remove the precipitated proteins. The supernatant fluid, called SVE, had a protein content of approximately 0.5 mg/ml. Since the results reported here showed that a viral protein was responsible for the inhibition, a control preparation was prepared by incubation of bovine serum albumin (1 mg/ml) with NP_{40} and LiCl in the same conditions as these described for the virus. The concentration of protein recovered after the

treatment was about 0.4 to 0.6 mg/ml. This solution was used instead of SVE in the control test.

Penetration of SVE into the cells by means of basic polymers. To facilitate the penetration of SVE into the cells, basic polymers were added to the extracts. With poly-L-ornithine, no inhibition of incorporation of ^{3}H-uridine could be demonstrated, whereas with DEAE dextran the incorporation of the radioactivity into RNA was greatly reduced (Table 1). This indicates that DEAE dextran is necessary for inhibition to occur. Inhibition is observed if SVE and DEAE dextran are incubated together with the cells. Addition of SVE followed by its removal and the simultaneous adjunction of DEAE dextran does not result in an inhibition of RNA synthesis. It should be noted that the adjunction of DEAE dextran to the adsorption medium also enhanced the inhibitory effect of the virus particles on cellular RNA synthesis. The optimal concentrations of DEAE dextran have been studied. With 50 μg of DEAE dextran per ml, no effect on the metabolism of uninfected cells was observed, whereas the inhibitory capacity of SVE was greatly enhanced (Fig. 1). Therefore, the concentration of 50 μg of DEAE dextran per ml was routinely used in the following experiments. In these conditions, when SVE (50 μg of proteins) prepared with protein-labeled virus was incubated with 5.10^6 cells, 3% of the radioactivity became cell associated within 1 h.

TABLE 1. *Requirement of basic polymers for the expression of the inhibitory capacity of the soluble viral extract (SVE) on RNA synthesis*[a]

Polymers	Uridine incorporation in cellular RNA (counts per min per 5×10^6 cells)		
	SVE	Control	Uninfected cells
No	182,238	186,379	187,809
Poly-L-ornithine[b]	185,796	182,214	186,122
DEAE dextran[c]	59,691	107,048	116,789

[a] Monolayers of KB cells (5×10^6 cells per flask) were incubated either with SVE or control (100 μliters/flask) in a final volume of 0.5 ml of medium containing basic polymers. After 1 h of adsorption at 37 C, the solutions were replaced by MEM plus 5% dialyzed calf serum; 4 h later, cultures were labeled for 45 min with ^{3}H-uridine (0.5 μCi/ml), and the radioactivity incorporated into RNA was measured. The results reported correspond to the mean of two determinations.

[b] Poly-L-ornithine concentration, 10 μg/ml.

[c] DEAE dextran concentration, 200 μg/ml.

The relationship between the inhibition and the amount of protein was studied. Figure 2 shows that, with the concentration of DEAE dextran determined above, the extent of inhibition increases with the amount of viral protein.

Time course of RNA inhibition by SVE and virus particles. To determine whether the effect of SVE was similar to that of virus particles, we compared their kinetics of inhibition of RNA synthesis. At 4 to 5 h after adsorption, SVE produced the same inhibition as virus particles when used at the same protein concentration (Fig. 3). However, the action of SVE was faster during the first hours of postinfection. This could be explained by the fact that release of viral components from the particles does not occur immediately after infection.

Effect of enzymes and denaturating agents on the activity of SVE. Further experiments were conducted to determine the nature of the viral component producing the inhibition. The effect of DNA could be ruled out because (i) only 2% of ^{3}H-DNA of the original virus was recovered in SVE, and (ii) DNase treatment did not reduce the inhibitory activity of SVE (Table 2).

We then studied the effect of trypsin digestion on the inhibitory capacity of SVE. It is clear that the treatment with trypsin greatly reduced the inhibitory capacity of SVE (Table 2). Furthermore, the activity of SVE was partially destroyed by heating at 56 C for 30 min and completely lost when the temperature was raised to 70 C (Table 3).

Denaturation by dialysis against 7 M urea was not reversible in spite of a subsequent dialysis against 10^{-3} M TEA (Table 3). These results imply that one or several structural proteins could be responsible for the inhibitory effect of SVE.

Recovery of virus-associated enzyme activities in SVE. Several virus-associated enzymes have been demonstrated in FV3 particles (6, 17, 18, 21). It was of interest to know if they were still present in SVE and if they could contribute or be responsible for the inhibition. Therefore, we checked for the presence of ATPase, DNase, RNase, and protein kinase in SVE.

With regard to ATP phosphohydrolase activity, for the same amount of protein, 63% of the ATP was hydrolyzed by the virus whereas only 29% was hydrolyzed by SVE. The ratio of SVE activity to virus activity is about 0.45. This ratio is the highest value found; with some SVE preparations, it was as low as 0.15. However, SVE as well as virus produced a reduction in the molecular size of single-stranded RNA (Fig. 4), but acid-soluble products were never formed. To detect the presence of protein kinase, we

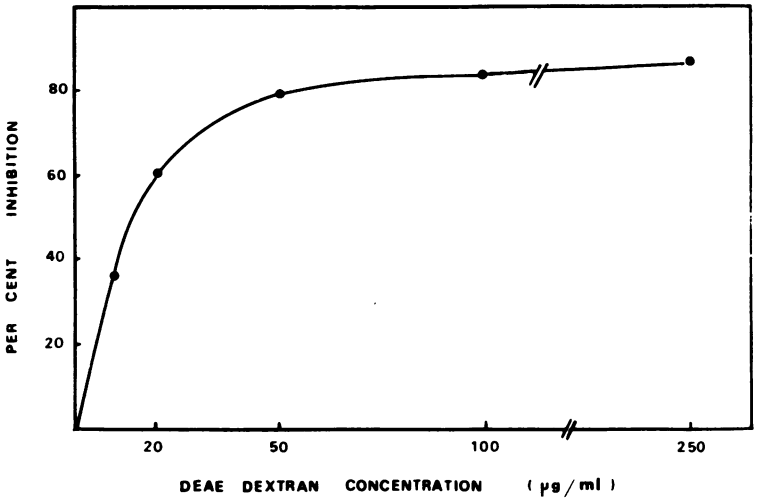

FIG. 1. *Effect of different concentrations of DEAE dextran on the inhibition of cellular RNA synthesis by SVE. RNA synthesis in KB cells treated with SVE or control in the presence of DEAE dextran was followed by the incorporation of ^{3}H-uridine. For each DEAE dextran concentration, RNA synthesis in SVE-treated cells was expressed as a percentage of inhibition against synthesis in control cells.*

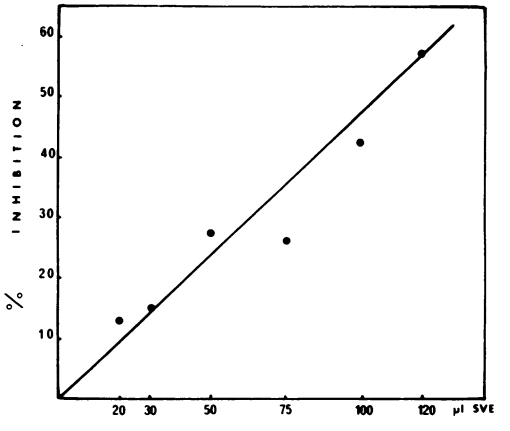

FIG. 2. *Relationship between the amount of SVE and the extent of inhibition. RNA synthesis in KB cells was measured after a 3-h, 30-min treatment.*

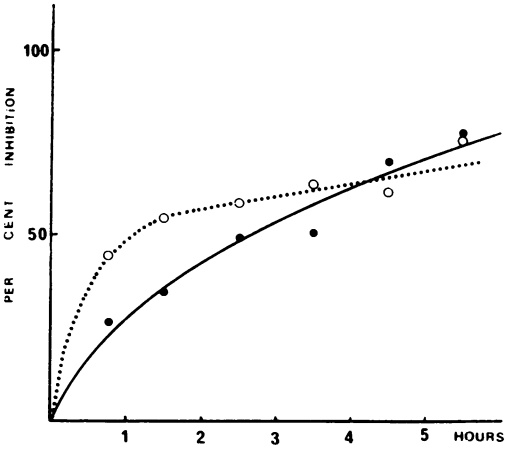

FIG. 3. *Kinetics of RNA synthesis inhibition. KB cells were incubated with an equivalent amount of protein (45 μg/5 × 10^6 cells) of FV3 or SVE in the presence of 50 μg of DEAE dextran per ml. After 1 h of adsorption, FV3 or SVE was replaced by culture medium, and the cultures were incubated at 37 C. At different times, the cultures were pulse-labeled for 0.5 h with ^{3}H-uridine, and the incorporation was measured as described. Results are expressed as a percentage of inhibition produced by FV3 (●) or SVE (○).*

measured the ability of γ^{32}P-ATP to give an acid-precipitable product labeled with ^{32}P. The formation of an unspecific complex containing ATP rather than a covalent link between the γ phosphate and a protein was excluded by the absence of labeled precipitate when γ ^{32}P-ATP was replaced by ^{32}P-ATP α. There was no conversion of γ^{32}P-ATP into an acid-insoluble form catalyzed by SVE, whereas, with a corresponding protein concentration of virus particle, an activity was obtained (600 pmol of phosphate incorporated during 10 min/50 μg of protein). Thus no protein kinase activity is present in SVE. It was also found that the endodeoxyribonuclease activity was completely absent in SVE.

Neutralization of the activity of SVE by antiviral serum. SVE and the control preparation were incubated with antisera as described. RNA synthesis in cells treated with the SVE supernatant fluid was compared to the control treated with the same serum dilution and expressed as a percentage of inhibition (Fig. 5). As can be seen, neutralization of the inhibitory activity is specifically obtained with antiviral

TABLE 2. *Effect of hydrolytic enzymes on the activity of SVE*[a]

Enzymes	Uridine incorporation (counts per min per 5 × 10⁶ cells)		Percent inhibition[b]
	SVE	Control	
No	13,088	72,712	82
DNase[c]	11,150	74,336	85
Trypsin + iniprol[d]	45,670	63,591	28
Iniprol + trypsin[e]	16,266	71,201	77

[a] SVE or control were incubated with the enzymes and then added to the KB cells in the conditions described in Table 1, experiment a.

[b] 100 = SVE (counts/min)/control (counts/min) × 100.

[c] DNase used at a concentration of 25 μg/ml in the presence of 10^{-2} M $MgCl_2$ was incubated with SVE or control for 1 h at 37 C.

[d] SVE or control were preincubated at 37 C with trypsin (600 μg/ml). For trypsin inactivation, iniprol was added after 1 h (200 UIP of iniprol for 1 μg of trypsin).

[e] Trypsin and iniprol were first mixed in the proportion indicated in footnote *d* and then incubated with SVE or control for 1 h.

TABLE 3. *Effects of protein denaturating agents on the inhibitory activity of SVE*

Treatment	Uridine incorporation (counts per min per 5 × 10⁶ cells)		Percent inhibition
	SVE	Control	
None (expt 1)	132,070	431,956	69
56 C 30 min (expt 1)	324,364	456,089	29
70 C 30 min (expt 1)	417,366	364,325	0
Dialysis against TEA buffer only (expt 2)	27,826	195,120	90
Dialysis against 7 M urea and TEA buffer (expt 2)	209,908	207,278	0

serum. Anti-BHK serum did not affect SVE inhibitory properties.

Interaction of SVE with polynucleotides. Preincubation of SVE with DNA resulted in the formation of a complex when the reaction mixture was made in a saline solution. This complex was removed by centrifugation (1,000 × *g*, 30 min), and the supernatant fluid was tested for remaining activity. The results expressed in Table 4 (experiment a) indicate that, in the presence of salt, the inhibitory component of SVE interacted with calf thymus DNA and could be partially eliminated with it. The reduction of the inhibitory activity is dependent on the incubation medium; however, we observed that the amount of labeled DNA binding to the cells in the various conditions is unchanged (*unpublished data*). This indicates that the inhibition is not prevented by the interaction of DNA to DEAE dextran. Similar results were obtained with vaccinia or KB DNA. RNA, however, seemed to be less active (Table 4). In the same manner, interaction of synthetic polynucleotides with SVE lead to a decrease in the supernatant activity.

DISCUSSION

Infection of cells with viruses often results in the switch-off of cellular functions. The inhibition of host macromolecular metabolism has been studied with DNA viruses (8, 9, 13, 19) as well as with RNA viruses (4, 5, 22). In the case

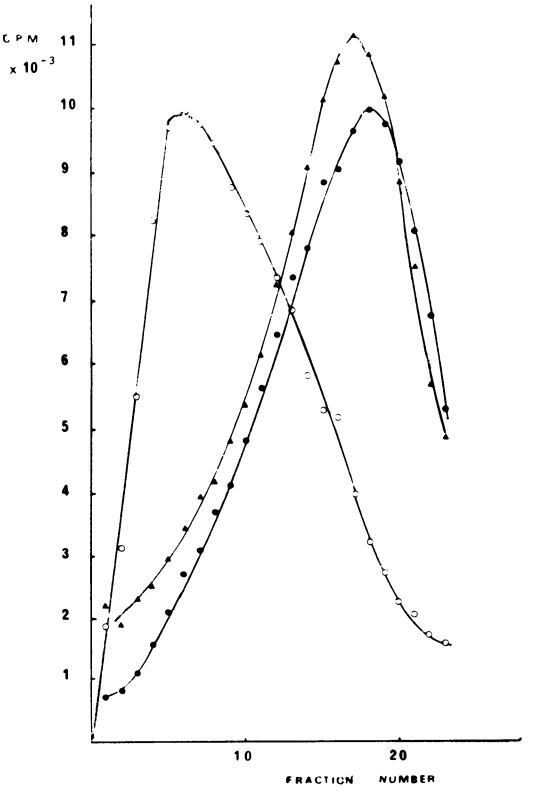

FIG. 4. *Endoribonuclease assay. Modification of the molecular weight of 16S RNA after incubation with FV3* (▲) *or SVE* (●). *Sedimentation of 16S RNA alone* (○).

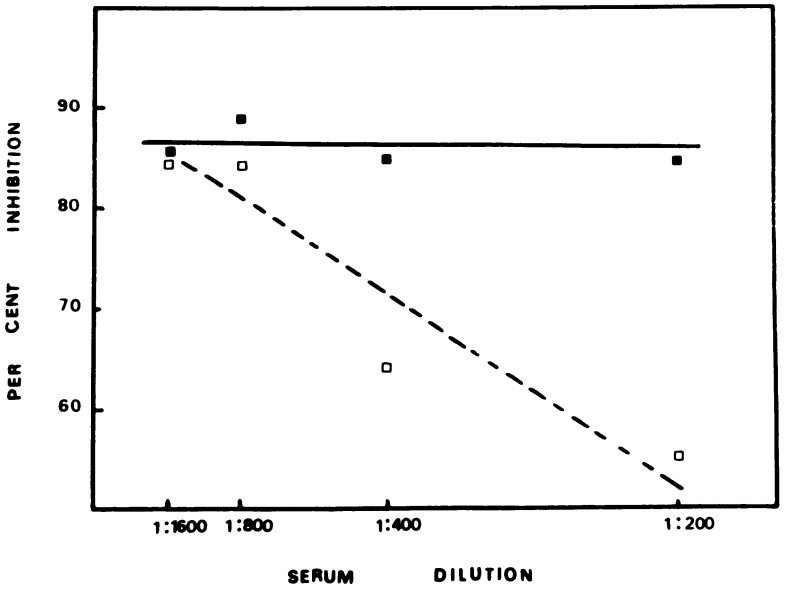

FIG. 5. *Neutralization of the inhibitory activity of SVE by preincubation with different dilutions of anti-FV3 serum* (□). *Controls were incubated with anti BHK serum* (■).

of adenovirus, a structural protein, the fiber antigen, has been shown to be involved in this inhibition (12, 13). The present study describes the solubilization of an inhibitory component associated with the FV3 particle by treatment of the virion with a detergent, followed by a high increase in the ionic strength. The resulting soluble extract produced an inhibition of cellular RNA synthesis similar to that obtained with the intact virus. SVE blocked host cell DNA synthesis as well as vaccinia DNA replication (*unpublished data*). Inhibitory activity was observed only when the assays were carried out in the presence of DEAE dextran, thus indicating that basic polymers must be necessary for SVE to penetrate into the cells. Poly-L-ornithine, which is frequently used at low concentrations to facilitate the penetration of proteins into cells (20), was not efficient. Since the activity of SVE was destroyed by trypsin digestion as well as by antibody treatment, one can assume that a protein of the viral particle could be responsible for the inhibitory effect. In contrast with the results obtained with complete virus (7), the inhibitory activity was lost when the soluble extract was submitted to incubation at 56 C for 30 min. This difference may be due to the fact that the virion structure stabilizes the inhibitory protein. In regard to their inhibitory properties, the precipitated proteins eliminated during the preparation of SVE were also tested but were found to be inactive. This is not surprising, since the results of heat or urea denaturation showed that a particular conformation of the protein is required for the inhibitory property to be conserved. Since the inhibition could be brought about by one of the enzymes associated with the virion, we checked for the presence of different enzymatic activities in SVE. Endodeoxyribonuclease activity is absent in SVE and thus, cuts into host DNA do not seem to be responsible for the inhibition of transcription and replication. On the other hand, since no protein kinase activity was recovered in SVE, the inactivation of the enzymes involved in RNA and DNA synthesis by phosphorylation of the active sites could not account for the observed effect. In a study published recently on polyhedral cytoplasmic deoxyribovirus associated protein kinase, Gravell and Cromeans (6) also came to the conclusion that this enzymatic activity is not responsible for the inhibition of cellular macromolecule synthesis. The absence of these two enzyme activities could be due either to an inactivation or to an elimination in the course of SVE preparation.

ATP phosphohydrolase specific activity found in SVE was lower than that found in the intact virion. For the RNase activity, no difference was noticed between SVE and virus suspensions. Cellular RNA synthesis and its inhibition were measured by the level of acid-insoluble material, whereas FV3 endonuclease did not hydrolyze RNA into acid-soluble material. Thus it is unlikely that the endonucleolytic activity found in SVE produces the inhibitory effect on RNA synthesis.

Our findings indicate, without question, that

TABLE 4. *Reduction of the inhibitory activity of SVE by preincubation with polynucleotides.*

Polynucleotides	Uridine incorporation (counts per min per 5×10^4 cells)		Percent inhibition
	SVE	Control	
Expt 1[a]			
No DNA	17,343	189,753	91
DNA (H 20)	15,938	142,216	89
DNA (PBS)	102,466	149,703	32
DNA (MEM)	93,990	144,931	35
Expt 2			
No[b]	14,574	65,077	78
DNA[b]	39,724	59,954	34
RNA[b]	24,012	58,011	59
Expt 3			
No[c]	87,503	350,012	75
Poly dG, dC[c]	330,814	349,081	6
Poly d (AT)[c]	262,199	341,813	23

[a] SVE or control was mixed with an equal volume of calf thymus DNA (1 mg/ml) dissolved in water, PBS, or MEM. Interaction was allowed for 30 min at 37 C, and the complex was eliminated by centrifugation at $2,000 \times g$ for 30 min. The remaining activity of the supernatant fluid was measured.

[b] DNA and RNA were present at a final concentration of 500 μg/ml. Polynucleotides dissolved in 0.1 M Tris-hydrochloride (pH 8), 10^{-2} M $MgCl_2$ were mixed with SVE or control as described in *a*.

[c] Poly d (AT) and poly dG, dC were used at a final concentration of 4 OD 260/ml. Polynucleotides treated as in *b*.

the inhibitory component can interact with polynucleotides in vitro and can be removed by elimination of the complex formed after incubation with DNA. This occurred with native and with denatured DNA, whereas RNA was less effective. It is likely that either the presence of deoxyribonucleotide residues or specific sequences of nucleotides could be required for a strong binding. Unfortunately, the results obtained when polydeoxyribonucleotides were used did not allow us to demonstrate any preferential interaction with one type of base pair.

The observed difference should not be emphasized, since it is difficult to control rigorously the concentration of poly dG·poly dC present in the assay, because this product adheres tenaciously to glass surfaces. Furthermore, the secondary structure of poly dG·poly dC molecules is known to be very complex, and it cannot be excluded that the interaction of SVE with polynucleotides is dependent on their structure. It is interesting to compare the effect of adeno-5 fiber antigen and SVE on the cells. Fiber antigen binds very slowly to KB cells and produces an inhibition of DNA and RNA synthesis 20 to 30 h after it has been added to the culture (12). At this time, the antigen can still be removed, and the cells can recover their ability to form viable colonies. The effect of SVE is very different; after the adsorption period (1 h), enough protein is associated with the cells so that its removal does not reverse the inhibition. The amount of pure adeno fiber antigen bound per cell was about 2 to 3 pg (12), which represents about 10 times more than SVE. Based on the percentage of SVE protein bound to DNA in vitro, the inhibitory protein represents less than 10% of the mixture and, assuming that every protein of SVE may interact with KB cells in the presence of DEAE dextran, the inhibition of nucleic acids in one KB cell would be produced by less than 3.10^{-8} μg of inhibitor.

If interaction of the viral component occurs in vivo with DNA and RNA, as it does in vitro, it is not unlikely that transcription and DNA replication can be modified. However, up to now there is no definite proof that the inhibition takes place by this mechanism.

Purification of the active molecule from SVE should make possible its analysis and should lead us to a better understanding of this inhibition.

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