RIBONUCLEIC ACID POLYMERASE ACTIVITY IN PURIFIED INFECTIOUS PANCREATIC NECROSIS VIRUS OF TROUT.

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Received December 2,1974

SUMMARY

Purified infectious pancreatic necrosis virus of trout was found to have associated with it a polymerase activity, capable of catalysing the synthesis of single stranded ribonucleic acid (RNA) from the double stranded RNA genome.

Recent results (1) have shown that Infectious Pancreatic Necrosis (IPN)virus contains a segmented double stranded RNA genome. Initial steps of viral replication of double stranded RNA viruses have been extensively studied with the reovirus (2). Within this system it has been shown that messenger RNA synthesis is directed by a structural enzyme of the virion, which catalyses, in vivo as well as in vitro, the transcription of one strand of the viral RNA. Similar results have been published with several reo-like viruses : Wound Tumor virus (3), Blue Tongue virus (4, 5) and Cytoplasmic Polyhedrosis virus (6).

Very little is known of the biochemical steps involved in IPN virus replication. In this study we establish that the pathogenic agent of the trout, also contains an RNA polymerase which is active in vitro. The characteristics of this in vitro synthesis system are described.

METHODS

All techniques concerning the production of IPN virus in tissue culture, the purification of the virions, and infectivity assay have been described in a previous publication (1). In this work, fractions of sucrose gradients containing the virus were pelleted and the pellet resuspended in 0.1 M Tris-HCl pH 8 buffer. The RNA polymerase activity was assayed as follow : the standard reaction mixture was adjusted to a final volume of 0.250 ml and contained :

Table 1

Characteristics of the in vitro synthesis of RNA by purified IPN virus

Reaction condition (a)	[^{3}H]UTP incorporated CPM *	Reaction condition (a)	[^{3}H]UTP incorporated CPM *
Complete	7463	Complete	15084
+ Actinomycin D (10 μg/ml)	7340	+ KCl 150mM ; NaCl 12mM	2525
+ Rifampicin 10 μg/ml	6527	+ RNase 10 μg/ml (c)	3614
+ Uridine 1 mg/ml	7045	+ RNase 10 μg/ml (d)	3178
+ Chymotrypsin (b)	1013	+ Triton X100 0.1%	10804
- CTP	505	+ NaCl 100mM	2023
- CTP, - GTP	483	+ NaCl 200mM	395

(a) Standard assay conditions were used as described in Methods, except where noted..

(b) Virions were pretreated for 30 min./37°C with 100 μg/ml of chymotrypsin.

(c) RNase was added after incubation in the standard condition and the reaction mixture was further incubated at 37°C for 15 min.

(d) Same as (c) except that the reaction mixture was adjusted to 0.2 M NaCl. before addition of RNase.

* The values indicated represent the mean of duplicate determination. A background incorporation of radioactivity of 120 CPM was obtained for 0°C incubated controls, and was substracted to obtain the values listed above.

Tris-chloride (pH 8) 25 μmole ; adenosine triphosphate 2.5 μ mole ; cytidine triphosphate 0.6 μ mole ; guanosine triphosphate 0.6 μ mole ; [^{3}H]uridine triphosphate 10 μCi (1 Ci/mmole) ; $MgCl_2$, 2.5 μ mole. Incubation was performed at 24°C and the reaction was terminated by adding to 1 vol. of reaction mixture, 1 vol. of an aqueous solution containing bovine serum albumin (2 mg/ml) and yeast RNA (8 mg/ml), and 3 ml of 5% trichloroacetic acid containing 3 mg/ml of sodium pyrophosphate. The precipitate was collected on glass fiber filters

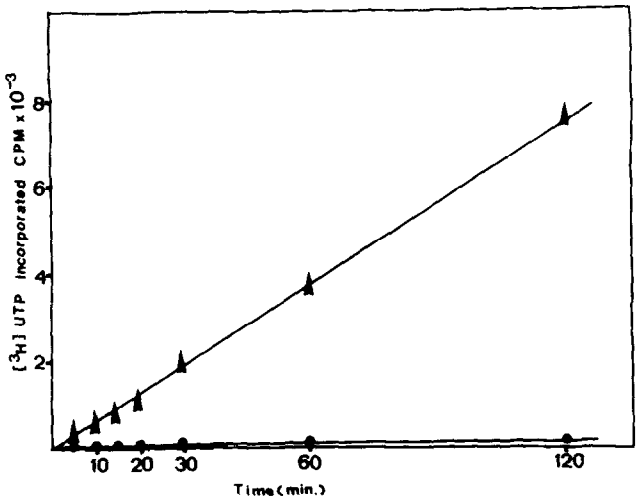

Figure 1. Dependence of polymerase activity on length of incubation. Enzyme consists of a pool of three peak fractions of a gradient, similar to that in figure 2 (▲——▲). The control consists of three optical inactive fractions obtained at the right of the peak (●——●).

and washed with cold 5% trichloroacetic acid containing 10 g/l sodium pyrophosphate. Dried filters were suspended in a toluene based scintillation fluid (0.33% PPO and 0.0083% POPOP) and counted in an "Intertechnique" SL 30 liquid scintillation spectrometer.

RESULTS

1) Uptake of tritiated UTP by purified IPN virions :

The results in table 1 show that purified virions are capable of polymerising ribonucleoside triphosphates. This incorporation requires the presence of the four ribonucleosides. The omission of CTP or CTP-GTP, considerably diminishes the RNA synthesis. In contrast with reovirus, the enzymatic activity of chymotrypsin treated virions is considerably decreased. It is also worthy to note that triton X 100 does not activate the polymerase, as it does with Influenza virus.

The polymerase activity is insensitive to actinomycin D and rifampicin . In absence of an ATP regenerating system, the reaction continues up to two hours ; at which time all experiments were terminated . In order to ascertain the correlation between the presence of the virions, and the RNA polymerase activity, we determined the amounts of $[^3H]$UTP incorporated in various fractions of the purification gradients. These fractions were also assayed for infec-

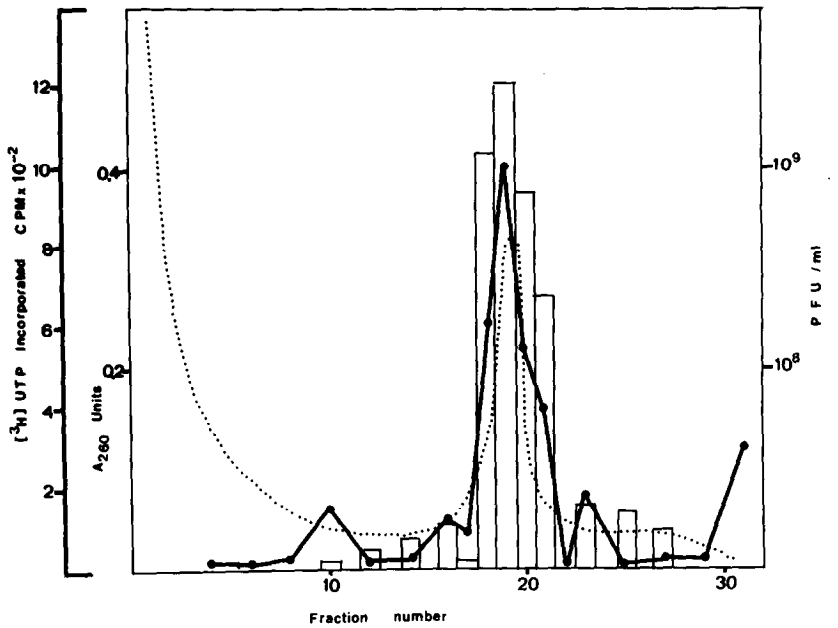

Figure 2. Cosedimentation of IPN virions and polymerase activity
1 ml of concentrated virus was layered on a 15% to 45% W/V linear sucrose gradient in 10 mM Tris-HCl buffer pH 7.6 (1mM EDTA ; 100mM NaCl) and was sedimented (24000 rev/min. ; 4°C ; 150 minutes), using a SW 25.1 rotor in a Spinco L2 centrifuge. Optical density (dotted line) was monitored with an Isco UA4 spectrometer. Infectivity (histogramme) and polymerase activity after pelleting (●——●) were assayed in various fractions. Sedimentation was from left to right.

tivity. The peaks of polymerase activity, optical density, and infectivity are shown in figure 2. Electron microscopic examination of peak samples, by means of negative staining, confirmed the presence of large quantities of virus and only very little cell debris. 80% of the RNA synthesized during a 1 hour incubation period with IPN virus, in a standard reaction mixture, was degraded by ribonuclease A in 0.1 M Tris-HCl pH 7.8, 10 mM Mg^{2+} ; or in the same buffer, plus 0.2 M NaCl, indicating that most of the newly synthesized RNA is accessible to RNase A and that it is single-stranded.

2) Temperature dependence of the polymerase activity :

The kinetics of RNA synthesis at 6 different temperatures are illustrated in figure 3. Several features of the IPN virus polymerase are immediately apparent. Ribonucleoside incorporation was greatly enhanced when the temperature increased from 10°C to 40°C. The activity recorded at 40°C was twice that observed at 24°C, which in turn was three times that observed

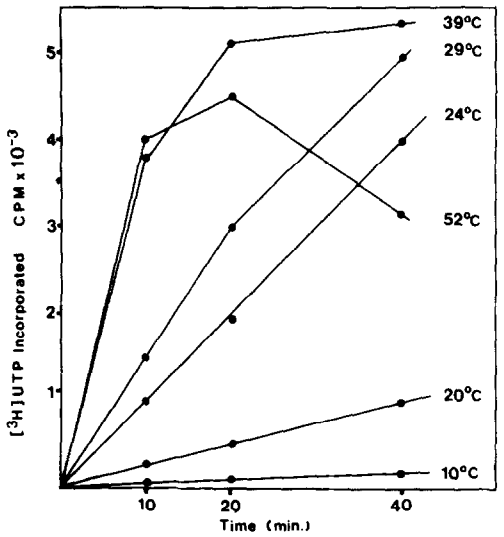

Figure 3. Kinetics of RNA synthesis with IPN polymerase at different temperatures.

at 20°C. At 10°C the reaction rate was negligible. The rate of polymerisation at high temperature (39°C and 52°C) was constant for only 20 minutes. After 20 minutes there was a distinct block on RNA synthesis at 39°C. At 52°C we observed a block as well and a degradation of the newly formed RNA.

3) Effect of metal ions.

The polymerase activity is completely dependent upon a concentration of 5-20 mM of Mg^{2+}. In absence of magnesium the incorporation is virtually nil, showing no difference with a control incubated at 0°C. When Mg^{2+} was replaced by Mn^{2+}, enzyme activity was considerably lower. Additionally, the Mn^{2+} had an inhibitory effect at concentrations higher than 2.5 mM (fig. 4). In reactions primed by IPN polymerase, the activity diminished when monovalent cations (Na^{+} of K^{+}) were present (table 1).

DISCUSSION

As has been shown with various viruses having a double-stranded RNA genome (for a general review see (7)), it is clear from our results that IPN virions carry an RNA dependent RNA polymerase. On the basis of the requirement of four ribonucleosides for the RNA synthesis, the non inhibiting effect of Rifampicine or Actinomycine D, and the cosedimentation of RNA-polymerase-activity with infective virions, it seems clear that the ability of incorporating [^{3}H]UTP is due to virions, and not to contaminating micro-organisms.

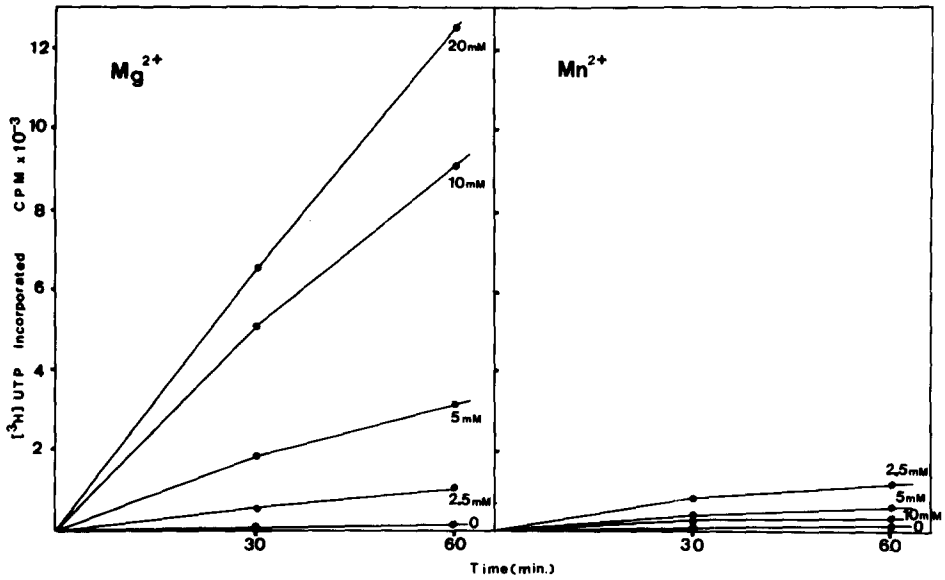

Figure 4. Effect of divalent cations on RNA polymerase activity of IPN virus. Except for Mn^{2+} and Mg^{2+}, the standard reaction mixture was used.

Although the presence of the IPN virus polymerase is now firmly established, its location in the virion is not known yet. In contrast to purified reovirus, whose structure must first be alterred to allow expression of polymerase activity (8), purified IPN virions are already active without any treatment. Moreover, pretreatment with chymotrypsine or with Triton X 100 reduces the $[^3H]$UTP incorporation. Other reolike viruses having a single shelled capsid, such as Cytoplasmic Polyhedrosis Virus (6) or Penicillium Stoloniferum virus (9), exhibit like IPN virus, a polymerase activity without any pretreatment.

As with Influenza virus (10), or reovirus (11) there is no correlation between the optimum temperature for in vitro RNA synthesis and the optimum temperature for viral replication. With IPN virus we observe that there is no detectable viral RNA synthesis in infected cells maintained at 28°C (12), although the virion associated polymerase is highly active at this temperature in an in vitro system. Additionnal studies (in preparation) are aimed to characterise the $[^3H]$ labelled product synthesized in vitro, and to compare it with viral 24 S messenger RNA which appears (unpublished data) in infected cells.

ACKNOWLEDGEMENTS

I am grateful to Mrs C. Feynerol for her technical assistance and Mr L. MALLORY for his assistance in translation of the manuscript.

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