RNA POLYMERASE ACTIVITY IN DOUBLE-STRANDED RIBONUCLEIC ACID VIRUS PARTICLES FROM ASPERGILLUS FOETIDUS

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Received July 31,1975

SUMMARY

RNA polymerase activities have been detected in purified particles of Aspergillus foetidus viruses S and F. Incorporation of [^{3}H]-UTP into acid insoluble RNA was dependent on ATP, GTP, CTP and magnesium ions. No pretreatment of the particles was required and the rate of reaction was proportional to the amount of virus added. In the conditions used RNA synthesis by A. foetidus virus S was complete in 4 h. The reaction could be stimulated by Triton X-100, but was unaffected by heat shock, dithiothreitol, potassium chloride or ammonium chloride; it was inhibited by ethidium bromide but not by actinomycin D. The major reaction product was single-stranded RNA, as indicated by its sensitivity to degradation by ribonuclease A. This is the first report of synthesis of single-stranded RNA by a double-stranded RNA mycovirus.

INTRODUCTION

Double-stranded RNA viruses have been found in many genera of fungi (1). RNA polymerase activity has been reported in purified virus particles in only three cases, Penicillium stoloniferum viruses S and F (2,3) and Penicillium chrysogenum virus (4). In the case of P. chrysogenum virus the products of polymerase action were not characterized, but the products of RNA synthesis by P. stoloniferum viruses S and F were shown to be double-stranded RNA molecules, which remained within the virus particles (3). Two serologically unrelated double-stranded RNA viruses have been isolated from Aspergillus foetidus, namely A. foetidus virus S and A. foetidus virus F (5,6). In the present communication we report the detection of RNA polymerase activity in these two viruses and show that the major product in the case of the virus S polymerase is single-stranded RNA.

MATERIALS AND METHODS

Chemicals. [^{3}H]-UTP was from the Radiochemical Centre, Amersham, Bucks, U.K.; unlabelled nucleoside triphosphates from P-L Biochemicals, Milwaukee, Wisconsin, U.S.A.; dithiothreitol and ethidium bromide from B.D.H. Biochemicals, Poole, Dorset, U.K.; actinomycin D (dactinomycin, actinomycin C_1) from Merck, Sharp and Dohme, West Point, Pennsylvania, U.S.A.; ribonuclease A (deoxyribonuclease free) from Worthington Biochemical Corporation, New Jersey, U.S.A.

Preparation of virus. Virus preparations were obtained from Aspergillus foetidus, Strain IMI 41871, grown in 5 l fermenters as described by Ratti and Buck (5), except that all buffers contained additionally 10 mM EDTA and the virus-yeast RNA precipitation stage was omitted. Instead homogenates were made 6% w/w with respect to polyethylene glycol 6000 and after 18 h at 4°C the resultant precipitate was recovered by low speed centrifugation and resuspended in 0.03 M-sodium phosphate buffer, containing 0.15 M-KCl + 10 mM-EDTA, pH 7.6. Virus preparations were purified by sucrose density gradient centrifugation and A. foetidus viruses S and F were separated by DEAE cellulose chromatography (6). Preparations were dialysed against 0.05 M-tris buffer, pH 7.9, containing 0.15 M-KCl + 0.1 mM-EDTA (for virus S), or 0.05 M-tris buffer, pH 7.9, containing 0.1 mM-EDTA (for virus F), and stored at 4°C.

RNA polymerase assay. RNA polymerase activity in A. foetidus virus S was assayed in the following complete reaction mixture (final concentrations): 0.05 M-tris-HCl buffer, pH 7.9; 0.15 M-KCl; 0.1 mM-EDTA; 3 mM-$MgCl_2$; 0.15 mM-GTP; 0.15 mM-CTP; 0.15 mM-ATP; 0.15 mM-[^{3}H]-UTP (51 mCi/mmole); virus (2 to 30 µg) in a total volume of 0.2 ml. Incubations were carried out for 30 min at 30°C, unless otherwise stated, and incorporation of [^{3}H]-UTP into acid-insoluble RNA was measured as described by Chater and Morgan (3). RNA polymerase activity in A. foetidus virus F was assayed as above except that KCl was omitted from the reaction mixture and the $MgCl_2$ concentration was 5 mM.

RESULTS AND DISCUSSION

Incorporation of [^{3}H]-UTP into acid insoluble material by A. foetidus virus S was proportional to the amount of virus added up to 30 µg of virus. The reaction was dependent on magnesium ions with an optimum magnesium ion concentration of 3 mM using nucleoside triphosphate concentrations of 0.15 mM (Fig. 1). [^{3}H]-UTP incorporation was not linear and terminated after 4 h (Fig. 2).

The effects on RNA polymerase activity of omissions and additions to the complete reaction mixture are shown in Table 1. The dependence of [^{3}H]-UTP incorporation on ATP, GTP and CTP and the sensitivity of the reaction to ribonuclease A indicates that the reaction product is RNA. The lack of effect of actinomycin D shows the absence of any contaminating

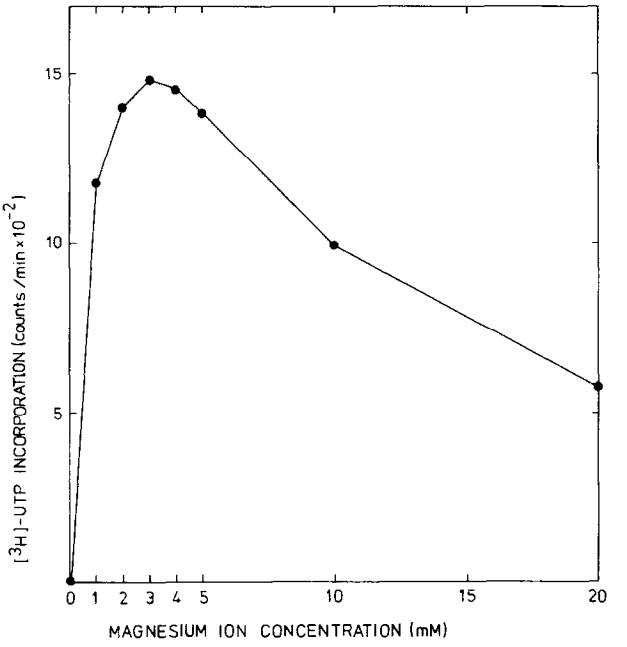

Fig. 1. The effect of magnesium chloride concentration on [^{3}H]-UTP incorporation by A. foetidus virus S particles. Each assay contained 4 µg of virus and was carried out at 30°C for 15 min as described in the text.

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cellular DNA dependent RNA polymerase (7). Ethidium bromide is known to bind strongly to double-stranded DNA and RNA (7,8) by intercalating between adjacent base pairs in the double helix and inhibits DNA dependent DNA and RNA polymerases (7) and the synthesis of double-stranded RNA by P. stoloniferum viruses (3). The insensitivity to actinomycin D and the inhibitory effect of ethidium bromide on the RNA polymerase activity of A. foetidus virus S suggests that the reaction is dependent on double-stranded RNA. Potassium chloride and dithiothreitol caused only small changes in [^{3}H]-UTP incorporation at the concentrations tested. In this respect this RNA polymerase resembles that of P. stoloniferum virus particles (3), but differs from the potassium chloride, dithiothreitol dependent RNA polymerase detected in P. chrysogenum virus particles (4).

No pretreatment of the virus particles with a proteinase was necessary

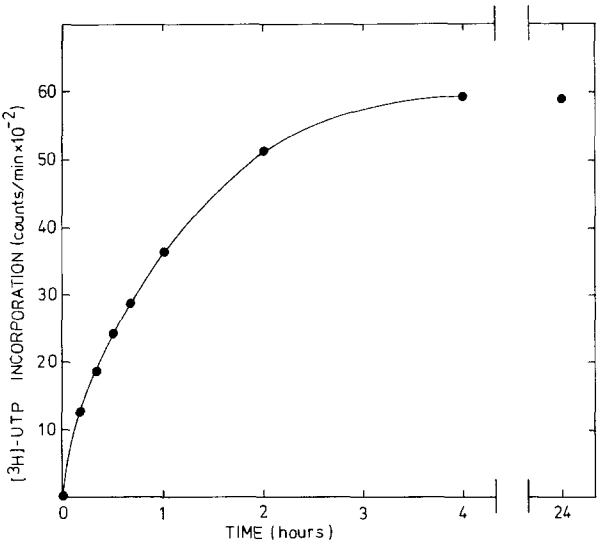

Fig. 2. [^{3}H]-UTP incorporation by A. foetidus virus S particles as a function of time. Each assay contained 4 µg of virus and was carried out for the times indicated as described in the text.

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to activate the RNA polymerase of A. foetidus virus S as is the case with certain other double-stranded RNA viruses e.g. reovirus (9), and heat shock treatment (60°C for periods of 30 to 60 sec), which was found to activate the virion polymerase of reovirus (10) and of the double-stranded RNA bacteriophage ø 6 (11), had no effect on activity. Treatment of the virus particles with 0.1% Triton X-100 had a stimulatory effect on RNA polymerase activity, the amount varying with the virus preparation up to a maximum of 30% increase in activity. Stimulation by Triton X-100 was observed also for the RNA polymerase activity of P. chrysogenum virus particles (4), but not of that of P. stoloniferum virus F particles (3).

Addition of ribonuclease A to the reaction mixture after 30 min incubation resulted in a 65% reduction of acid insoluble radioactivity (Table 1). At the ionic strength of the assay mixture double-stranded RNA is resistant to the action of ribonuclease A (5). It may be deduced therefore that 65% of the reaction product is single-stranded RNA, which

Table 1

A. foetidus virus S associated RNA polymerase activity under different conditions

Reaction mixture	Relative [^{3}H]-UTP incorporation
Complete	100
- ATP	3
- GTP	1
- CTP	5
- KCl	90
+ actinomycin D (125 µg/ml)	100
+ ethidium bromide (125 µg/ml)	4
+ 0.25 mM dithiothreitol	110
+ 25 mM ammonium chloride	83
+ ribonuclease A*	30
+ ribonuclease A**	35

Reactions were carried out at 30°C for 30 min as described in the text with the omissions or additions indicated.

* Ribonuclease A (6 µg/ml) was added to the complete reaction mixture at zero time

** Ribonuclease A (6 µg/ml) was added to the complete reaction mixture after 30 min incubation, and incubation was carried out at 30°C for a further 30 min before assay for acid insoluble [^{3}H]-UTP incorporation.

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is probably released from the virus particles. The remaining 35% of ribonuclease resistant RNA synthesis (note that 30% of the RNA synthesis is resistant even when if ribonuclease is added at zero time) may represent synthesis of double-stranded RNA or possibly synthesis of single-stranded RNA within the virus particle and protected from ribonuclease by the capsid proteins. This is the first report of the synthesis of single-stranded RNA by a double-stranded RNA mycovirus and the reaction is clearly quite different from that of the polymerases of *P. stoloniferum* viruses (3) in which the reaction product after 30 min incubation, using conditions similar to those described here, was predominantly double-stranded RNA.

The total amount of RNA synthesis achieved by A. foetidus virus S after complete reaction (4 h) represents only a 35% net RNA increase. However it has been shown that preparations of this virus are heterogeneous, being comprised of a large number of particle types, some of which are believed to be intermediates of virus replication (6). It is possible, therefore, that some particles are totally inactive, while others achieve a much greater net RNA synthesis.

Recently we have detected RNA polymerase activity in A. foetidus virus F also. [^{3}H]-UTP incorporation into acid insoluble RNA was proportional to the amount of virus added up to 30 µg of virus and was dependent on ATP, GTP and CTP, incorporation being reduced to 5%, 2% and 5% of the control in the absence of each nucleoside triphosphate respectively. The reaction was dependent on magnesium ions with an optimum magnesium ion concentration of 5 mM using nucleoside triphosphate concentrations of 0.15 mM.

ACKNOWLEDGEMENTS

We thank Mr. G. T. Banks for Pilot Plant fermentations. This work was supported by a grant from the Science Research Council which is gratefully acknowledged. We thank Mr. G. McGregor for experimental assistance.

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