RIFAMPICIN INHIBITION OF VACCINIA REPLICATION

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Received August 18, 1969

The mechanism of rifampicin inhibition of poxvirus replication has been investigated. At doses that selectively block viral replication there is complete inhibition of increase of a particulate RNA polymerase activity. Early mRNA synthesis by cores is resistant as is synthesis of the early induced enzyme thymidine kinase and of viral DNA. Late mRNA synthesis also was not significantly inhibited by the drug. The effect of rifampicin on the activity of the structural virion polymerase and of a soluble vaccinia induced RNA polymerase is contrasted with its inhibition of E. coli polymerase.

Rifampicin inhibits the replication of the poxvirus vaccinia (1,2). Since its action on vaccinia replication appears to be selective (2), an interesting question is its target in the replication cycle. Poxvirus, a DNA virus, possesses a DNA dependent RNA polymerase as an integral part of the virion (3). This polymerase transcribes early poxvirus mRNA *in vitro* if virions are converted to the sub-structures referred to as cores (4)--i.e., a state in which viral DNA is still encased in its protein coat. In addition, poxvirus infection elicits two polymerase activities in cells. One, an early function, is soluble and primed most efficiently by the polymer dAT (5). The other, a late function, is particulate but non-primable (3). The latter probably represents the polymerase activity of progeny particles although special conditions are required to unmask its activity in cell extracts (5). This report describes the effect of rifampicin on the activity of these species of polymerase and on transcription of early and late poxvirus mRNA.

MATERIALS AND METHODS

Growth and infection of HeLa-S_3 cells in suspension have been described

in detail (6). The virus used was vaccinia WR. Assays for thymidine kinase, dAT primed polymerase, core polymerase, E. coli polymerase, and the late particulate polymerase were essentially as reported (5,7,12). The activity of the latter activity was markedly enhanced by reducing the detergent used in the original assay to 0.05%. Highly purified E. coli polymerase was a gift from Dr. J. Kates. Viral cores were prepared from purified virus as follows: virions were dispersed in TRIS-Detergent (0.05M Tris-HCl, pH 8.4, containing 0.075M mercaptoethanol and 0.5% Nonidet P40 Shell) and incubated (30 minutes at 37°C). The mixture was chilled, centrifuged (35,000 x g, 30 minutes) and the pellet redispersed at 10^{11} particles per ml in TRIS (0.05M Tris-HCl, pH 8.4, containing 0.015M mercaptoethanol). About 10^{10} particles were used per assay.

To label viral RNA and DNA, samples of 5×10^6 infected cells in 10 ml of growth medium were pulsed either with H^3-uridine (1 µc/ml for 10 minutes) or with H^3-thymidine (2 µc/ml, 60 minutes). Pulsed cells were washed and disrupted (4), and radioactivity of the acid precipitable cytoplasmic fraction determined by scintillation spectrometry. Only viral mRNA and viral DNA are detected by these procedures.

Generous gifts of rifampicin were received from Dr. Justus Gelzer, CIBA Pharmaceutical Co., Summit, N.J. and Dr. Paul Hoeprich, University of California, Davis. The antibiotic was dissolved in a few drops of dimethylsulfoxide and diluted with buffer or water as required.

RESULTS

The incorporation of H^3-uridine into cytoplasmic mRNA of WR-infected cells shows two clearly defined peaks (8). The first represents early mRNA made from viral cores and from viral DNA that has uncoated. The second represents predominantly late mRNA and is dependent on DNA synthesis (9). The actual pattern of incorporation into HeLa-S_3 cells infected with 1000 WR particles per cell is shown (Fig. 1a). Rifampicin (100 µg/ml) added at the time of infection caused no significant reduction of uridine uptake into late mRNA

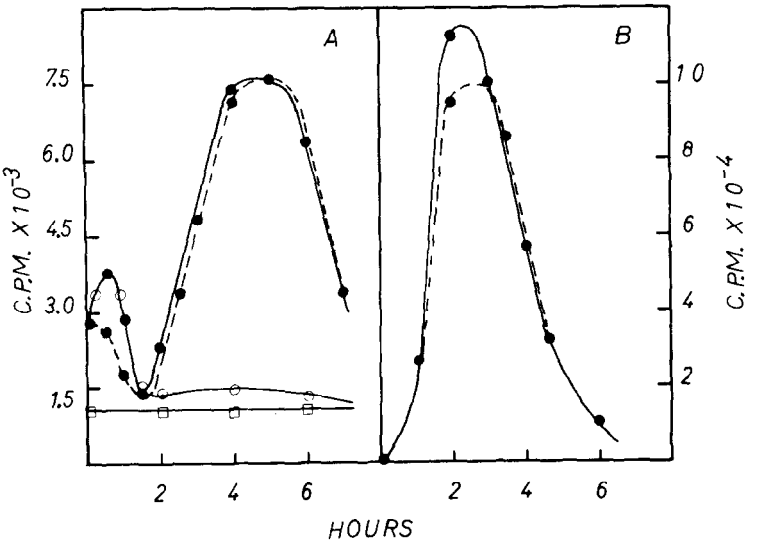

Fig. 1a. Incorporation of H^3-uridine into cytoplasm of WR-infected cells. (—●—) without rifampicin; (— — —) rifampicin (100 μg/ml) at time 0; (□) uninfected cells with or without rifampicin; (O) in the presence of FUdR to block DNA and late mRNA synthesis (cf., Fig. 2b).

Fig. 1b. Incorporation of H^3-thymidine into cytoplasm of WR-infected cells. (●) Without rifampicin; (— — —) rifampicin (100 μg/ml) at time 0.

(Fig. 1a). Synthesis of viral DNA is also not significantly depressed by rifampicin (Fig. 1b). In agreement with others (2), we find that rifampicin (100 μg/ml) inhibited the one-step growth of WR in HeLa-S_3 a hundred fold.

In cells pretreated with actidione (100 μg/ml) to inhibit protein synthesis, viral replication is arrested at the core stage--i.e., viral DNA is not released from its inner protein coat and early mRNA is transcribed continuously from these cores at a much higher rate than in the uninhibited system (4). Rifampicin added at the start of infection of actidione-treated cells did not inhibit mRNA transcription from cores (Fig. 2a).

Parallel experiments were conducted using fluorodeoxyuridine (FUdR, 10^{-4}M) to inhibit DNA synthesis but permit uncoating of the virus. A burst of early mRNA synthesis was detected followed by a drop in transcription rate (cf., Woodson (9)) (Fig. 2b). Certain intermediate functions not expressed by viral cores are expressed by uncoated DNA prior to its replication (3). In cells

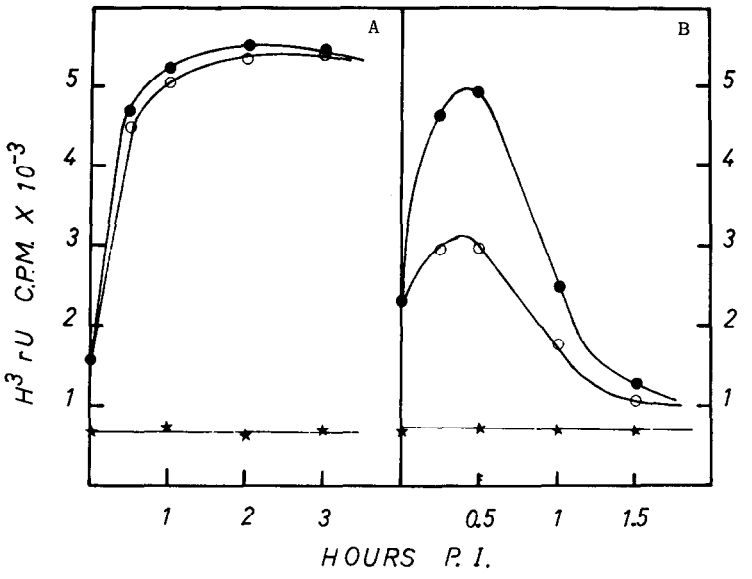

Fig. 2a. Transcription by viral cores in vivo. Cells were pretreated with actidione (100 µg/ml) for 2 hours prior to infection with 2000 virus particles/cell and pulse labeled with H^3-uridine. The experiment was conducted in the presence (O) or absence (●) of rifampicin (100 µg/ml) added at time zero. (✱) Uninfected cells with or without actidione plus rifampicin.

Fig. 2b. Uridine incorporation in the presence of FUdR (●) or FUdR plus rifampicin (O). (✱) Uninfected cells with or without FUdR plus rifampicin.

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infected in the presence of FUdR, rifampicin inhibition of mRNA was apparent (Fig. 2b).

Induction of an early enzyme. Thymidine kinase is an early enzyme induced by poxvirus infection (10) and the messenger for its synthesis is made while virus is at the core stage (4). Rifampicin (100 µg/ml) added at the time of infection did not inhibit synthesis of this enzyme (Fig. 3a).

Induction of a late enzyme. The particulate RNA polymerase induced in poxvirus-infected cells is a late function whose synthesis depends upon prior synthesis of viral DNA (5). Despite the fact that viral DNA and late mRNA is made, increase in activity of the late polymerase is abolished when rifampicin was added at the start of infection (Fig. 3b).

Rifampicin and activity of poxvirus polymerases. The effect of rifampicin

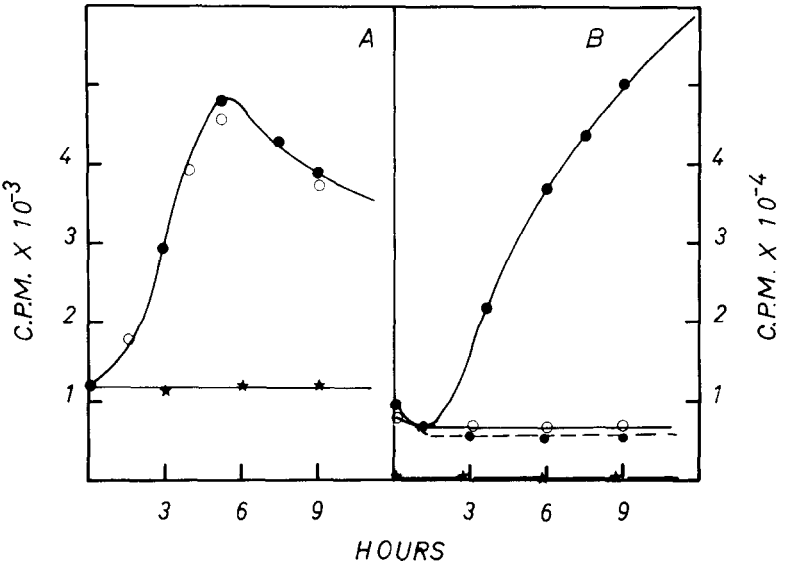

Fig. 3a. Induction of thymidine kinase in WR-infected cells. Activity is expressed as C.P.M. of C^{14}-thymidine phosphorylated. (●) Uninhibited system; (O) rifampicin treated; (✱) uninfected cells.

Fig. 3b. Induction of RNA polymerase in WR-infected cells. Activity is expressed as C.P.M. of H^3-UTP converted to acid precipitable material. Enzyme activity of uninfected cells (✱); of infected cells (●); of cells infected in rifampicin (— - —); of cells infected in FUdR (O), i.e., represents enzyme level due to residual activity of input particles.

on the in vitro activity of the following polymerases was tested: (a) purified dAT primed polymerase from infected cells (5,13), (b) active viral cores, detergent cores, prepared as described in Materials and Methods, (c) crude cyto-

Table I. Effect of rifampicin on poxvirus polymerases*

Rifampicin (μg/ml)	0	5	25	50	100	200
(a) dAT polymerase	6,000	-	5,900	5,000	4,100	3,900
(b) Detergent cores	17,800	-	17,500	17,800	16,750	14,500
(c) Natural cores	12,300	-	12,300	12,400	12,300	9,300
(d) E. coli polymerase	6,900	1,500	500	100	100	50

* Results are expressed as C.P.M. of H^3UTP converted to acid precipitable form.

plasmic extracts from cells infected in the presence of actidione to accumulate "natural" cores, (d) E. coli polymerase. Rifampicin was added to the assay mixtures several minutes prior to adding substrates or primers which were dAT in the case of (a) or heat-denatured calf thymus DNA in the case of (d)(Table I).

DISCUSSION

Despite the fact the rifampicin caused no inhibition of transcription of late poxvirus mRNA, induction of at least one late function, the particulate polymerase, appeared to be completely blocked by the drug. At least one early enzyme, thymidine kinase, was induced; the virus was uncoated, functions essential for DNA synthesis (11) were expressed and viral DNA was synthesized at a rate similar to that found in the uninhibited system. Early mRNA synthesis by cores in vitro was also unaffected by high concentration of rifampicin. Such cores thus act like DNA-polymerase complexes that have initiated RNA synthesis (12). Some rifampicin inhibition of mRNA transcription under conditions where viral DNA uncoats, was observed repeatedly (Fig. 2b). This might be because of increased accessibility of the structural RNA polymerase to the drug at that stage of the cycle. On the other hand it might represent inhibition of transcription of intermediate functions (cf. ref. 3) by another form of the RNA polymerase. The dAT primed polymerase is the only soluble vaccinia induced RNA polymerase known to date. In vitro it shows little capacity to use viral DNA as a template and is in any case not readily inhibited by rifampicin (Table I). Too little is known about the sub-viral structures involved, the regulation of core transcription during uncoating or even the number and state of RNA polymerases involved to speculate further on this point.

Assuming that the virion polymerase is essential to the viability of progeny virions, inhibition of this one enzyme activity or its synthesis would explain the rifampicin inhibition of vaccinia replication. However, there remains the problem of the mechanism by which rifampicin blocks increase in late enzyme activity even though there is no significant reduction of late

mRNA synthesis. Although induction appears to be blocked, one possibility under investigation is that the polymerase is synthesized but is inactive because it binds rifampicin prior to packaging into sub-viral particles.

ACKNOWLEDGMENTS

The expert technical assistance of Miss Nancy Quintrell is gratefully acknowledged. This work was supported by a grant from Hoffman LaRoche, Inc.

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