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REPLICATION OF MYCOPLASMAVIRUS MVL51: VI. ACRIFLAVINE STIMULATES GROWTH OF THIS SINGLE-STRANDED DNA VIRUS

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SUMMARY

Acriflavine inhibits the growth of a double stranded DNA mycoplasmavirus, but stimulates the growth of a single stranded DNA mycoplasmavirus. Maximal stimulation occurs when acriflavine is added late during infection and reflects an increased synthesis of viral relative to cellular DNA.

INTRODUCTION

Amino acridine dyes have been reported to inhibit cellular and viral growth (1-4). Studies on the effect of acriflavine on mycoplasmas have shown that cell growth is not significantly affected by incubation in up to 1 μg acriflavine/ml (5). However, in these cells 0.5 μg acriflavine/ml inhibits dark repair of UV induced DNA damage (6).

The present report describes the effect of acriflavine on the growth of single and double stranded DNA mycoplasmaviruses. The single stranded DNA virus, MVL51, is a bullet shaped particle containing circular DNA of molecular weight 1.5×10^6 (7) and the double stranded DNA virus, MVL2, is an enveloped virus containing supercoiled DNA of molecular weight 7.8×10^6 (8). Infection of both viruses is non-cytocidal: infected cells continue to grow and divide while producing viruses (9). The studies reported here found that, unlike other bacteriophages, the growth of single stranded DNA virus MVL51 is stimulated by acriflavine. In contrast to MVL51, acriflavine inhibited growth of the double stranded DNA virus MVL2.

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MATERIALS AND METHODS

Cells, viruses, media and buffer: Acholeplasma laidlawii JA1 was used for virus propagation and as an indicator host in these studies. The viruses used were MVL51, a single stranded DNA mycoplasmavirus, and MVL2, a double stranded DNA virus. Cells and viruses were cultivated using tryptose broth and agar plates as previously described (10). Viruses were assayed as plaque-forming-units (PFU) on A. laidlawii JA1 lawns (10): the turbid plaques are formed because infected cells grow slower than uninfected cells. β-Buffer used in these studies was 0.15 M NaCl, 0.05 M Tris and 0.01 M β-mercaptoethanol, pH 7.5.

Acriflavine treatment: Acriflavine hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO). A dye stock solution (100 µg/ml) was made in sterile distilled water and stored in the dark at 4°C.

To examine the effect of acriflavine on viral growth 0.5 or 1 µg dye/ml was added either immediately after infection or at a specified time during viral growth.

Macromolecule synthesis: In macromolecule synthesis experiments, cells were incubated with [^{3}H]-deoxythymidine (15 µCi/ml) and/or [^{14}C]-leucine (5 µCi/ml). Duplicate samples (50 µl) were removed at different times during growth and mixed with equal volumes of cold 10% trichloroacetic acid. The precipitates were filtered, washed, dried and assayed for radioactivity as described previously (11).

Analysis of viral and cellular DNA: Cell DNA was labeled by overnight growth in medium containing 5 µCi [^{14}C]-deoxythymidine/ml. MVL51 was added (moi 10) and after 2 hr infection acriflavine was added to a final concentration of 0.5 µg/ml. Thirty min after acriflavine addition [^{3}H]-deoxythymidine was added to a final activity of 40 µCi/ml and 1 hr later the infected cells were harvested (11). Cells were lysed and DNA analyzed by velocity sedimentation in high salt sucrose gradients as described previously (11). However, in these experiments the bottom of each tube contained a 3 ml shelf of 60% sucrose. By this protocol, viral DNA intermediates are found as peaks in each gradient and cellular DNA as a band on the shelf.

RESULTS

Effect of acriflavine on mycoplasmavirus growth:

A. laidlawii cells infected with the single-stranded DNA mycoplasmavirus MVL51 were incubated in medium containing acriflavine and virus growth was measured (Fig. 1a). No virus growth was observed at an acriflavine concentration (10 µg/ml) high enough to be mycoplasmacidal (5). However, lower dye concentrations (0.5 and 1.0 µg/ml), which have been shown not to significantly affect cell growth (5), cause a 2- to 3-fold stimulation in MVL51 virus yield relative to untreated infected control cells. At these lower acriflavine concentrations no change in the viral latent period was observed and the stimulation in viral growth was apparent by 2 hr after infection.

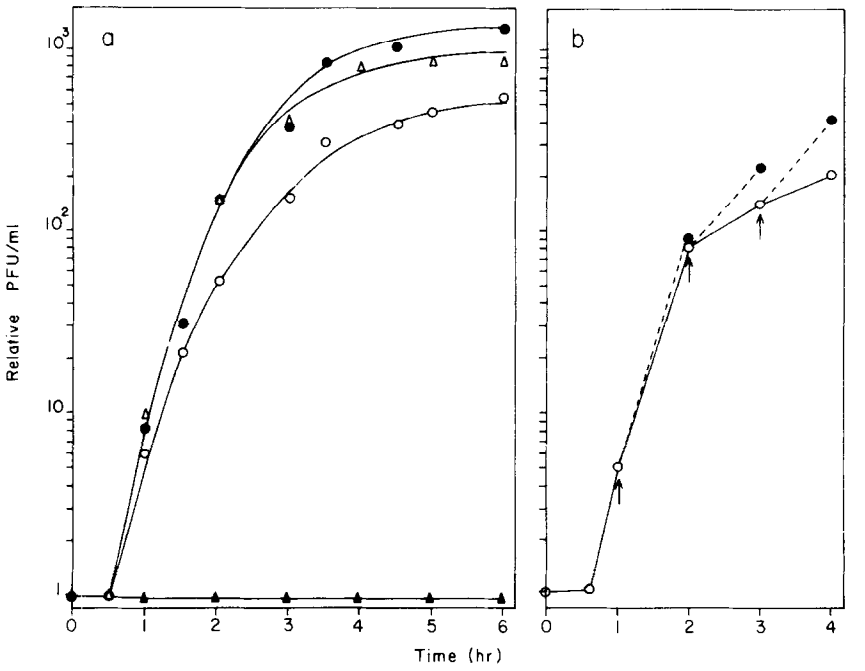

Fig. 1: Effect of acriflavine on virus growth. (a) Log phase cultures of JA1 in tryptose broth were infected with MVL51 at a multiplicity of infection (moi) of 10. After 10 min at 37°C, cells were centrifuged to remove unadsorbed virus. The infected cells were then resuspended in prewarmed (37°C) tryptose broth and diluted 10^4-fold in prewarmed tryptose broth to reduce reinfection. The diluted cells were divided into four parts. One part was kept as a control (○). To the other parts acriflavine was added to a final concentration of 0.5 µg/ml (●), 1.0 µg/ml (▲) and 10 µg/ml (▲). At different times after incubation at 37°C, samples were removed and assayed for PFU. Each point is the average of data from five independent experiments. (b) Cells were infected as in (a). At different times after infection (arrows), a sample of infected cells was removed, acriflavine added to 0.5 µg/ml, and incubated for 1 hr at 37°C. Virus titer was measured in untreated cells (○) and after 1 hr dye treatment (●). Each point is the average of data from five independent experiments.

The effect of acriflavine on the MVL51 growth cycle was examined by adding acriflavine (0.5 µg/ml final concentration) to infected cells 1, 2, or 3 hr after infection and measuring virus yield after a 1 hr incubation (Fig. 1b). No significant stimulation was seen in the 1 hr infected cells. However, acriflavine addition after 2 or 3 hr of infection produced about a 2-fold increase in virus production relative to untreated infected cells.

Treatment of MVL51 virus with 0.5 μg acriflavine/ml for 1 hr before infection produced no measurable effects: treated and untreated MVL51 had indistinguishable one-step growth curves (data not shown). In addition, no difference in virus adsorption or in the penetration of [^{32}P]-labeled viral DNA was observed between acriflavine treated and untreated cells (data not shown). This is consistent with the observation that acriflavine does not affect A. laidlawii membrane integrity (5).

To eliminate the possibility that the stimulation of MVL51 viral growth might be due to induction of some virus that may be carried by these host cells, acriflavine treated uninfected cells were assayed for PFU. No PFU was observed in acriflavine treated uninfected cells (data not shown).

The growth of double stranded DNA mycoplasmavirus MVL2 in 0.5 μg acriflavine/ml was also examined (Fig. 2). Both the rate and total yield of MVL2 virus are reduced in the acriflavine treated cells.

Effect of acriflavine on macromolecule synthesis in infected cells:

An infected cell suspension was divided into two parts. Acriflavine was added to one part at a final concentration of 0.5 μg/ml and the other part was kept as an untreated control. [^{3}H]-deoxythymidine and [^{14}C]-leucine were added to both parts to measure DNA and protein synthesis respectively. At different times after infection samples were removed and acid precipitable radioactivity was assayed. DNA synthesis in infected cells was slightly reduced by 0.5 μg acriflavine/ml (Fig. 3a), while protein synthesis was not affected by the drug (Fig. 3b). This result was not unexpected since (i) MVL51 infection is non-lytic and infected cells continue to grow and divide while producing virus (9), and (ii) at 0.5 μg acriflavine/ml cellular DNA and protein synthesis were reported not to be significantly affected (5). To investigate this further cell lysates were analyzed.

Analysis of DNA replication in infected cells:

Cells infected with MVL51 for 2 hr were treated for 30 min with acriflavine (0.5 μg/ml) and nascent DNA was then labeled for 1 hr by addition

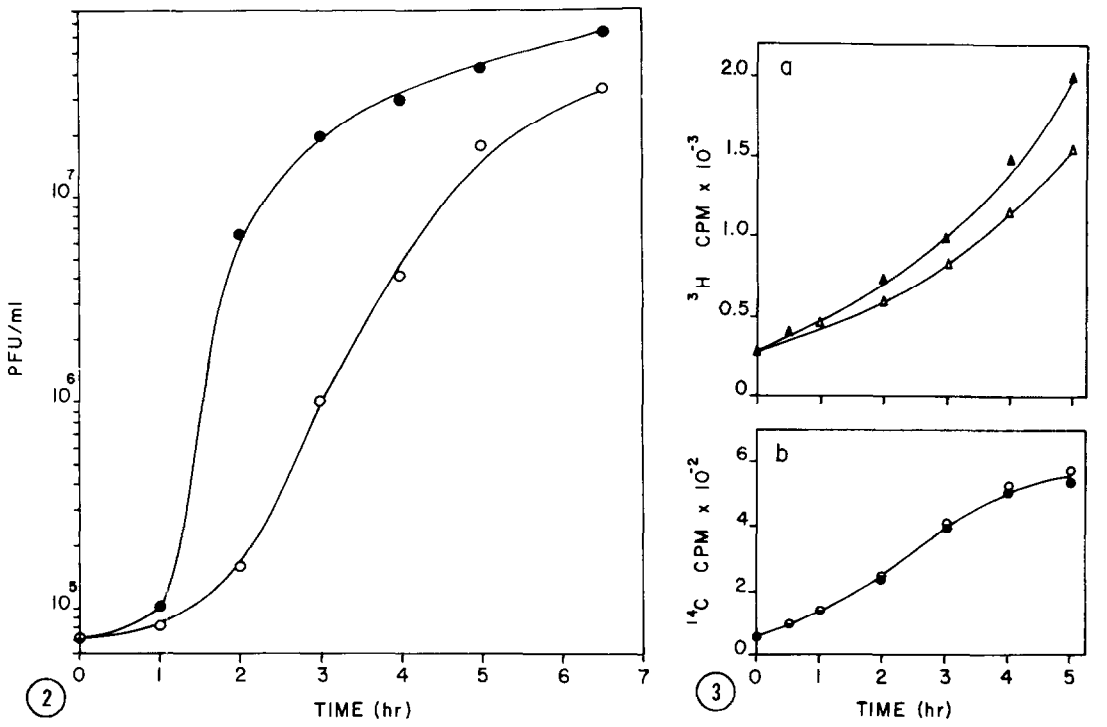

Fig. 2: Effect of acriflavine on the growth of mycoplasmavirus MVL2. JA1 cells were infected with MVL2 at an moi of 10. After 15 min of adsorption at 37°C, cells were centrifuged and diluted 10^4-fold, resuspended in prewarmed tryptose broth (37°C) and then divided into two parts. One part was untreated (●). To the other, acriflavine was added to a final concentration of 0.5 µg/ml (○). At different times after incubation at 37°C samples were taken and assayed for PFU.

Fig. 3: Effect of acriflavine on DNA and protein synthesis in infected and uninfected cells. DNA and protein synthesis were measured as the incorporation of [^{3}H]-deoxythymidine and [^{14}C]-leucine, respectively, into acid-insoluble material. Cells were infected with MVL51 at an moi of 10. (a) DNA synthesis in untreated infected cells (▲) and in infected cells in 0.5 µg acriflavine/ml (△). (b) Protein synthesis in untreated infected cells (●) and in infected acriflavine treated cells (○).

of [^{3}H]-deoxythymidine. Control cells were treated identically, but no acriflavine was added. DNA was then analyzed in sucrose gradients, which allowed the measurement of the [^{3}H]-deoxythymidine incorporated into cellular DNA and into the three viral DNA replicative intermediates: RFI, supercoiled covalently closed double-stranded DNA molecules; RFII, relaxed forms of RFI; and SS, circular single-stranded progeny viral chromosomes.

Although acriflavine treatment caused a reduction in total DNA synthesis in infected cells (Table 1; cf. also Fig. 3a), it increased the synthesis of viral relative to cellular DNA. Hence, the net effect is that the total amount of [^{3}H]-deoxythymidine incorporated into viral DNA intermediates is similar in acriflavine treated and untreated cells. Since the rate of viral production is greater in these acriflavine treated cells than in untreated cells (Fig. 1), this indicates that the turnover of viral DNA intermediates is greater in acriflavine treated cells than in untreated cells.

DISCUSSION

Amino acridine dyes have been reported to inhibit a variety of bacteriophages (12-15). This is also true for the double stranded DNA mycoplasmavirus MVL2 (Fig. 2). In contrast, growth of the single stranded DNA mycoplasmavirus MVL51 is stimulated by acriflavine treatment of infected cells (Fig. 1). This effect involves intracellular viral replicative events because (i) no stimulation was observed if acriflavine treated viruses were used for

Table 1: Viral and cellular DNA synthesis in infected acriflavine treated and untreated cells.

Nascent DNA	No acriflavine		Acriflavine	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Total [^{3}H] cpm	1.6×10^5	1.3×10^5	8.9×10^4	9.4×10^4
% [^{3}H] in cell DNA	40.4	43.2	34.4	24.7
% [^{3}H] in total viral DNA	40.2	41.8	52.6	61.5
% [^{3}H] in: RFI	5.8	6.3	6.7	7.9
RFII	16.5	18.1	23.4	28.1
SS	17.9	17.4	22.5	25.5

An overnight incubation in medium containing [^{14}C]-deoxythymidine was used to prelabel cellular DNA, which allowed the [^{14}C]-labelled cell DNA to be used as an internal standard for the normalization of the [^{3}H] incorporation data. The high salt sucrose gradient sedimentation conditions used in these studies (11) allowed the recovery of 80-86% of the acid precipitable [^{3}H]-cpm in cell and viral RFI, RFII and SS DNA peaks. The remaining [^{3}H]-cpm was found as material trailing from the cell DNA peak at the bottom of the gradient.

infection, (ii) significant stimulation is only observed when the acriflavine treatment occurs late in infection (Fig. 1b), and (iii) acriflavine treatment causes an increase in viral DNA synthesis relative to cell DNA synthesis (Table 1). The data suggest an increased turnover in MVL51 DNA replicative intermediates in acriflavine treated cells.

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