The Effects of Actinomycin D on RNA Synthesis in Measles Virus-Infected Cells

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SUMMARY

The effects of actinomycin D on the production of genomic RNA, messenger RNA and protein in measles virus-infected cells were investigated. It was found that whereas high concentrations of the drug inhibited genome synthesis, the production of messenger RNA was unaffected and in some cases enhanced. These effects were shown, at least in part, to be due to a secondary effect of the drug which caused an inhibition of virus-specific protein synthesis.

Over the last decade, there have been conflicting reports on the effects of actinomycin D on measles virus replication. Early investigations showed that the addition of a low concentration (less than 0.1 μg/ml) to measles virus-infected cells resulted in an increase in virus production (Anderson & Atherton, 1964; Matumoto, 1966). In direct contrast to these findings, other reports have demonstrated that concentrations greater than 1.0 μg/ml decreased the virus yield (Mirchamsy & Rapp, 1969; De Jong & Winkler, 1970). However, an exact interpretation of the latter results was difficult due to varying toxicity effects on the infected cells.

More recently, Schluederberg, Williams & Black (1972) demonstrated that concentrations of actinomycin D which inhibited RNA synthesis in Vero cells suppressed the yield of progeny virus by more than 99%. It was also found that the incorporation of 3H-uridine into the genomes of released virus particles was decreased by approx. 50%.

The present communication describes the results of experiments which were designed to consider the effects of different concentrations of actinomycin D on the production of genomic RNA, messenger (m) RNA and protein in measles virus-infected cells.

In all experiments, Vero cells (a green monkey cell line) were grown in plastic Petri dishes (100 mm diam.) in MEM supplemented with 5% (v/v) foetal calf serum and antibiotics (penicillin 100 units, streptomycin and refobacin 100 μg/ml). The Edmonston strain of measles virus was inoculated at a multiplicity of 10 p.f.u./cell and allowed to absorb at 37°C for 1 h. Infected cells were maintained in MEM containing 2% foetal calf serum and antibiotics at 37°C. In this particular cell line, 10 μg/ml actinomycin D were required to completely suppress host cell RNA synthesis; maximum virus specific RNA synthesis occurred between 17 and 20 h post-infection (p.i.). When actinomycin D was used it was added to infected cells in MEM without serum at 16 h.p.i. after incubation for 1 h at 37°C, 3H-uridine (sp. act. 20 to 30 mCi/mmol) at 50 μCi/ml and/or 14C-protein hydrolysate (sp. act. 54 mCi/mAtom) at 4 μCi/ml were added. Cultures were maintained at 37°C for a further 3 h before harvesting. RNA was isolated as follows. Medium was poured off and the cells were washed twice with ice-cold NTE buffer (0.01 M-tris-HCl at pH 7.5, 0.1 M-NaCl and 0.001 M-EDTA). Cell monolayers were then scraped from the glass with a rubber policeman into fresh, cold NTE buffer and collected by centrifuging at 2000 g for 10 min at 4°C. Pellets were resuspended in sterilized distilled water (0.4 or 0.8 ml) and allowed to swell on ice for 15 min. Cells were disrupted with ten strokes of a Dounce homogenizer.
Fig. 1. Sucrose gradient sedimentation patterns of RNA synthesized in measles virus-infected and
and uninfected Vero cells. (a) ●—●, ^3H^-uridine in virus-infected cells; ○—○, in uninfected cells.
(b) ●—●, ^3H^-uridine in infected cells treated with 10 µg/ml actinomycin D; ○—○, uninfected
cells treated with actinomycin D.

and nuclei were removed by centrifuging at 1000 g for 15 min. Supernatant fluids were
removed, made 2 % in SDS and heated at 56 °C for 2 min. Released RNA was either
examined directly by sedimentation analyses or frozen at −20 °C. Sedimentation was
carried out on linear 15 to 30 % (w/w) sucrose gradients (16 or 36 ml) in NTE buffer
containing 0.5 % (w/v) SDS. Gradients were centrifuged for 17 h at 20000 rev/min at 20 °C.
in either a Beckman SW 27·1 or SW 27 rotor. Fractions (0·55 or 1·1 ml) were collected from the bottom of the tube using an LKB pump and collected using an ISCO fractionater which measured extinction continuously at 254 nm.

The effect of actinomycin D on protein synthesis was measured by the uptake of ¹⁴C-protein hydrolysate into uninfected or infected cells. At the times shown in the Figures, cultures were washed with ice-cold NTE and removed from Petri dishes by washing with NTE containing 2 % SDS. Samples were boiled for 10 min, cooled on ice and the level of radioactivity precipitated by trichloroacetic acid was measured.

Fig. 1(a) shows typical sedimentation patterns of RNA synthesized in uninfected and infected cells in the absence of actinomycin D. The typical 28S and 18S ribosomal RNA species predominate. In addition, a minor peak of RNA sediments at 50S in the infected cells. Fig. 1(b) shows typical profiles of RNA synthesized in the presence of 10 μg/ml actinomycin D in both uninfected and infected cells. In the former, only slowly sedimenting RNA is present and neither of the ribosomal species are labelled. In the infected cells four species are labelled. The largest RNA again sedimented at 50S and is thought to be progeny genome RNA. Annealing experiments have shown that over 90 % of this RNA is negative stranded and only a small amount represents 'positive strand' intermediates (Hall & ter Meulen, unpublished data). The RNAs sedimenting at 12 to 28S and 28 to 36S are considered to be the virus messengers since we have shown that these are associated with polyribosomes, they contain poly(A) segments on their 3' end and are complementary in base composition to genome RNA (Hall & ter Meulen, unpublished data). The 4S RNA is probably a mixture of both virus and cellular material since it is also present in drug-treated uninfected cells.

These results on the sedimentation patterns provided the basic information for further studies. The total ct/min. in 50S in RNA in Fig. 1(a) represents 100 % synthesis, whereas 100 % mRNA synthesis represents the total ct/min in 12 to 36S RNA in untreated infected cells minus the total ct/min sedimenting in the same region in untreated uninfected cells. In the present experiments we examined the effect of different concentrations of actinomycin D on the production of the 50S and 12 to 36S virus-specific RNAs. Fig. 2(a) shows a typical set of results. At the highest concentrations (50 and 25 μg/ml), the incorporation of ³H-uridine into 50S RNA was inhibited by over 90 %. At 10 μg/ml, 50S RNA synthesis was inhibited by approx. 55 %, and at the lower concentrations a dose-dependent effect on genome synthesis was observed. In contrast, the incorporation of ³H-uridine into virus mRNA in the presence of 50, 25 or 10μg/ml was increased up to 14 % over the controls. Below 10 μg/ml the radioactivity incorporated into the 12 to 36S mRNAs remained at approx. 100 % with little or no enhancement being observed.

We recently found that cycloheximide completely inhibits measles virus 50S RNA synthesis whereas the levels of mRNA are increased threefold (Hall, Genius & ter Meulen, unpublished data). It seemed possible that the inhibition of 50S RNA relative to the 12 to 36S mRNA could be due to a secondary effect of actinomycin D on protein synthesis. We therefore compared the effect of different concentrations of the drug on the incorporation of ¹⁴C-protein hydrolysate into uninfected and infected cells. Fig. 2(b) shows that the addition of 50, 25 or 10 μg/ml of the drug reduced the radio-isotope incorporation by 50 to 60 % after a 3 h labelling period in infected cells. Below these concentrations, a dose-dependent relationship between concentration and inhibition was observed. These values are much higher than in the uninfected cells where the maximum inhibition of protein synthesis reached a range of 11 to 18 %. Therefore, it does seem that high concentrations of actinomycin D can produce a secondary effect(s) resulting in the inhibition of protein synthesis in virus-infected cells. Since measles virus RNA replication, but not transcription,
Fig. 2. (a) Effect of actinomycin D on the incorporation of $^3$H-uridine into 50S RNA (●—●) and 12 to 36S mRNA (○—○). (b) Effect of actinomycin D on the incorporation of $^{14}$C-protein hydrolysate into infected (●—●) and uninfected (○—○) cells. Each value represents the mean ± S.E.M. of four experiments.

requires active protein synthesis such a secondary effect would be expected to cause an inhibition of 50S RNA synthesis. Previous reports on RNA synthesis in measles-infected cells (Carter, Schluederberg & Black, 1972; Winston, Rustigian & Bratt, 1973; Kiley & Payne, 1974) have emphasized the inability or difficulties to incorporate $^3$H-uridine into 50S genome RNA. An examination of the experimental methods in these investigations shows that actinomycin D had been used at a concentration of 25 $\mu$g/ml or higher. In the light of our findings it seems possible that the drug was inhibiting protein synthesis in these systems.

The inhibition of paramyxovirus replication by actinomycin D does not seem to be a
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general phenomenon. However, Granoff & Kingsbury (1964) demonstrated that the replication of one strain of Newcastle disease virus was affected. More recently it has been shown that mumps virus (East & Kingsbury, 1971) and parainfluenza 3 virus (Dubois, 1975) replication are also inhibited.

At present, it is still not known how actinomycin D could elicit such a secondary effect. It is possible that the drug could bind to double-stranded RNA intermediates as suggested by Barry (1964) for influenza virus. This is supported by the fact that actinomycin D can bind to RNA \textit{in vitro} but with much less efficiency than to DNA (Dubois, 1975). Such a binding in the case of transcriptive intermediates could result in an inhibition or a slowing down of the ensuing translation process.

In general, the replication of RNA viruses (with the exception of oncornaviruses) are unaffected by actinomycin D. One exception, however, is the myxovirus group. The sensitivity of the myxoviruses and its relation to the involvement of the host cell nucleus is not completely understood (Kelly, Avery & Dimmock, 1974). A similar sensitivity of measles virus and its proven nuclear involvement (Norrby, Chiarini & Marusyk, 1970; Schluederberg & Chavanich, 1974) may indicate that some similarities may exist between the replication of the two virus systems.

Finally, recent reports (Zhdanov, 1975) have indicated that under certain conditions measles virus genome can be incorporated into the DNA of the host cell. The data described in this report might provide some indirect evidence of the involvement of host cell DNA. At present however, we have no evidence to confirm these reports (Hall & ter Meulen, unpublished) and it is more likely that if host cell DNA is involved at all in measles virus replication then it is not in the same or in such a direct manner as has been described for the oncornaviruses.

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