EFFECT OF RIFAMPICIN ON BACTERIOPHAGE PM2 BIOGENESIS

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1. Introduction

PM2 bacteriophage has been demonstrated to possess a membrane like structure as an outer coat [7] composed of lipids and proteins. The two major phospholipids species are the same as those present in the host *Pseudomonas* BAL-31 [4], although they display quite different proportions [1]. Furthermore, it has been demonstrated that purified PM2 is 60% neutralized by antibody prepared against purified BAL-31 membranes [8], suggesting a structural similarity between the phage outer coat and the cytoplasmic membrane of its host.

The DNA of PM2 approximately codes for 10 proteins. The mature virus contains 4 proteins which would leave about six non-structural proteins to participate in viral formation. This means that host components are likely to be responsible for some steps in the process.

A ribonucleic acid (RNA) polymerase has been detected in purified PM2 [3] but it is not known whether this protein is virus-specific. One approach to this problem is to study if the bacteriophage uses the cell polymerase or synthesizes a new one or a sub-unit of it after infection. It is known [9] that the antibiotic rifampicin is bound to the beta sub-unit of RNA polymerase in *Escherichia coli*, thus preventing the enzyme from initiating RNA biosynthesis. Consequently, this drug could be a useful tool to distinguish between host and a possible phage induced polymerase with a different sensitivity. Therefore, experiments with rifampicin added at different times, before and after PM2 infection were done.

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2. Materials and methods

Pseudomonas BAL-31 was grown in minimal medium at 28°C [6]. Rifampicin Lepetit was kindly supplied by the Instituto Bacteriologico de Chile. For the studies of phage production in the presence and absence of the antibiotic, cultures with a cell density of 10^8 cells/ml were used and aliquots distributed in different flasks. At time zero the cells were infected with PM2 at a multiplicity of infection (m.o.i.) of 10; phage was allowed to adsorb for 15 min and anti-PM2 serum was added, immediately diluted 10^3 times; a sample from each flask was taken, assayed for infective centres and plated in rich medium plates. The concentration of rifampicin was readjusted to the original concentration and the incubation continued for 150 min. Aliquots from each of the flasks were then assayed for phage production as usual.

The [^{3}H]uracil incorporation experiments were done with actively growing cells in minimal medium, having a cell density of 10^8 cells/ml. Aliquots of 1 ml were distributed in test tubes. When required 0.3 μg/ml of rifampicin was added 15 min prior to infection and PM2 added at time zero at a multiplicity of 10. Thirty min after infection 0.06 μCi of [^{3}H]uracil were added to all incubation mixtures; 10 min later the incorporation was stopped with 1 ml of 10% cold trichloroacetic acid (TCA). Samples were filtered on Whatman fiberglass paper, dried and counted in toluene-fluor in a liquid scintillation counter.

Aminoacids incorporation was performed with actively growing cells. When required the cells were

infected at time zero with PM2 at a m.o.i. of 10. At different times post infection 1 ml from each culture was taken and 0.06 μCi of [^{3}H]amino acids mixture was added. Incorporation was stopped after 5 min by the addition of 1 ml of 10% cold TCA containing 2 mg/ml of casamino acids. The precipitates were collected on 'millipore' filters, washed 10 times with 5% cold TCA containing 2 mg/ml of casamino acids, dried and the remaining radioactivity was determined in toluene-fluor in a liquid scintillation counter.

3. Results and discussion

In order to select the concentration of rifampicin to be used in the experiments, rich medium plates [4] were prepared with different antibiotic concentrations. An actively growing culture was spread on the agar plates and incubated at 28°C for two days. Poor growth was observed in the presence of 0.30 μg/ml of rifampicin. Concentrations between 0.5 and 30 μg/ml of the drug were tested and none of them allowed *Pseudomonas* to grow under these same conditions. Fig.1 shows the sensitivity of BAL-31 to 0.3 μg/ml of rifampicin as a function of time.

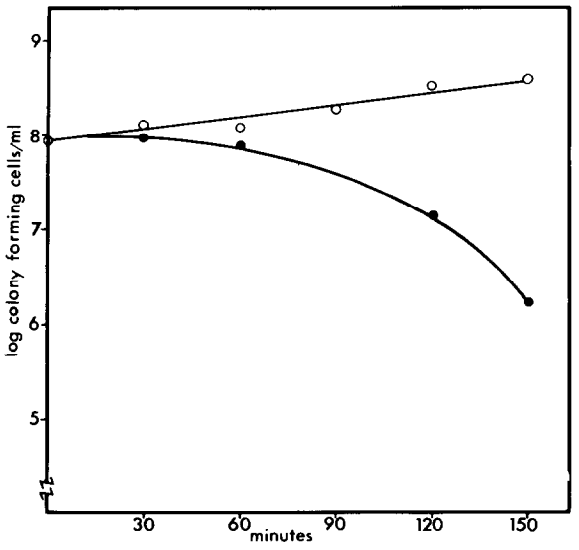

Fig.1. At time zero two aliquots of actively growing Ps. BAL-31 in minimal medium were placed in separate flasks, 0.3 μg/ml of rifampicin was added to one of them. Aliquots were taken for viable counts at the indicated times and plated in rich medium. ○ untreated cells, ● rifampicin treated cells.

Table 1
PM2 formation in the presence of rifampicin

Time of rifampicin addition* (min)	Infective centres pfu/ml × 10^{-7}	Free mature virus pfu/ml × 10^{-7}	Burst size
−30	0.25	0.53	2
0	2.5	3.0	1.2
+15	3.2	70	22
+30	2.5	160	64
+60	1.9	320	168
−	2.6	900	346

* 0.3 μg/ml of rifampicin was added before or after infection at the indicated times. The sign − stands for time before infection and + for time after infection. Details of the experiment are given in the text.

The effect of rifampicin on PM2 development was subsequently studied by the addition of 0.3 μg/ml of the drug at different times before or after infection. Table 1 shows that *Pseudomonas* are not capable of forming mature phage when the antibiotic is added before or at the time of infection. Late addition allows the bacteria to produce mature PM2, but consistently less phage is produced than in cultures without rifampicin.

It was interesting to investigate how RNA synthesis was affected by the presence of rifampicin. Table 2 shows that at 30 min post-infection, RNA synthesis in infected cells treated with the antibiotic was decreased to a fifth of that in untreated infected cells, although in uninfected cells the RNA synthesis only

Table 2
Effect of rifampicin on RNA synthesis

Incubation medium	[^{3}H]-uracil incorporation cpm/ml
Ps. BAL-31	2256
Ps. BAL-31 + rifampicin	1272
Ps. BAL-31 + PM2	2056
Ps. BAL-31 + PM2 + rifampicin	420

When necessary 0.3 μg/ml of rifampicin was added 15 min before infection. At time zero the bacteria were infected and 30 min post-infection the labelled tracer was added. The experiment is described in the text.

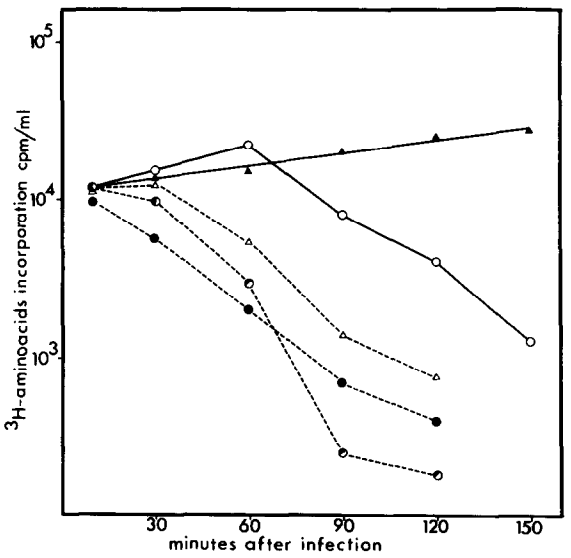

Fig.2. Aliquots of an actively growing *Pseudonomas* culture were placed in separate flasks; at the indicated times 0.3 μg/ml of rifampicin was added. Incorporation of aminoacids was done as described in Materials and methods. ▲ normal cells, △ cells + rifampicin added at time zero, ○ infected cells, ● cells treated with rifampicin 15 min before infection and ◐ cells treated with rifampicin 20 min after infection.

diminished to a half of that obtained in the control cells. It is important to point out here that after rifampicin treatment, incorporation of [^{3}H]uracil by infected cells is about one third that of rifampicin treated uninfected cells. This result suggests that at 30 min post-infection there is a net viral RNA synthesis, which is more sensitive to the drug than the host RNA synthesis.

To study the effect of rifampicin on protein synthesis, experiments on incorporation of ^{3}H-labelled aminoacids in uninfected and infected cells were carried out. Fig.2 shows that when rifampicin is added to actively growing *Pseudomonas*, 15 min before infection, protein synthesis 30 min post infection has been decreased to a third of that in untreated infected cells. This suggests that under these conditions the antibiotic affects viral protein synthesis more than protein synthesis in uninfected cells. These results are comparable with the data presented for RNA synthesis. Protein synthesis at 120 min post-infection, when almost 60% of the total mature virus has been made in this minimal medium, is decreased to 10% of that in untreated infected cells if rifampicin has been added 15 min prior to infection. If the drug is added 20 min post-infection a 20-fold decrease in protein synthesis occurs, showing that at this time there is present a polymerase sensitive to the drug. These results correlate well with those presented in Table 1, since the production of mature phage is affected by the antibiotic when this is added even 60 min after infection.

From the results presented here it is concluded that for the formation of mature PM2 phage, an intact polymerase system is needed from the beginning of infection. Up to now it has not been known whether this is of bacterial or viral origin.

It has been reported but not confirmed that purified PM2 possess a RNA polymerase activity [3], from the experiments just described it would seem reasonable to assume that the enzyme suggested as present in mature phage is not capable of transcribing the necessary information for the synthesis of the phage in the presence of rifampicin. It is also possible that the phage utilizes the host polymerase which has been demonstrated here to be very sensitive to rifampicin. Furthermore, these results show unequivocally that, during PM2 replication there is no synthesis of a rifampicin-resistant polymerase as occurs in bacteriophage T_7 [10].

The data just presented are compatible with the idea that bacteriophage PM2 uses the host polymerase early in infection and that probably later in infection a viral-coded polymerase, with a greater sensitivity to inhibition by rifampicin than the host polymerase is responsible for the phage maturation.

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