# THE EFFECT OF RIFAMPICIN ON MITOCHONDRIAL RNA POLYMERASE FROM RAT LIVER

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## 1. Introduction

Mitochondria can carry out RNA synthesis by using their own DNA-dependent RNA polymerase. Properties of this enzyme, previously described, indicate that the mitochondrial enzyme is different from the same nuclear enzyme [1, 2]. It would be interesting to find a similarity between the mitochondrial and bacterial RNA polymerase in analogy to that reported for the mitochondrial protein synthesis machinery [3]. In this respect the investigation of the sensitivity of the mitochondrial RNA polymerase towards rifamycin and its derivatives seems particularly useful since it has been shown that these antibiotics are strong inhibitors of RNA synthesis in bacteria but not in nuclei of animal cells [4]. Shmerling [5] measuring the $^{14}$C-ATP incorporation in the acid insoluble material of either intact or swollen mitochondria from rat liver has reported that RNA synthesis is completely inhibited by rifamycin in both kinds of mitochondrial preparations. On the other hand, Wintersberger et al. [6] have found that rifampicin a highly potent rifamycin derivative even at concentrations as high as 50 $\mu$g/ml has no effect on mitochondrial RNA polymerase from yeast or rat liver. We have thus investigated the effect of rifampicin on RNA synthesis in isolated mitochondria and nuclei as well as in the presence of the solubilized mitochondrial enzyme from rat liver.

## 2. Materials and methods

Male albino rats weighing about 150 g and starved overnight were used in all experiments. Mitochondria were isolated from rat liver under sterile conditions as previously described [7] with special care taken to eliminate nuclear contamination. Swollen mitochondria were obtained by incubating intact mitochondria in a sterile test tube with 0.1 M sterile phosphate buffer, pH 7.4, for 15 min at 30° and then centrifuged. Pellets were collected in 0.25 M sterile sucrose. Nuclei were isolated and purified according to the procedure of Blobel and Potter [8]. To solubilize the enzyme, mitochondria were incubated in a hypotonic medium containing 10 mM tris-HCl, pH 7.4, 10 mM potassium phosphate buffer and 5 mM $\beta$-mercaptoethanol for 7 min at 0° and were shrunk by adding 0.5 mM final concentration of ATP and $MgCl_2$ and then sonicated at 1 A for 10 sec in an MSE sonicator. KCl was added to a final concentration of 0.15 M. The sonicate was centrifuged at 105,000 *g* for 45 min in a Spinco Model L Ultracentrifuge. The supernatant was used as the enzyme source. All procedures were carried out in the cold.

Highly purified *E.coli* RNA polymerase was purchased from Biopolymers Inc., New York.

RNA polymerase activity was measured as described before [9]. The incubation mixture contained in 0.1 ml: 4.4 $\mu$moles tris-HCl, pH 7.4; 0.25 $\mu$mole $MgCl_2$; 5 $\mu$moles KCl; 4 $\mu$g pyruvate kinase; 0.4 $\mu$mole phosphoenolpyruvate; 5.5 nmoles cold ATP, CTP and GTP, 4.5 nmoles $^3$H-UTP, sp. act. 2.2 Ci/mmole (The Radiochemical Centre, Amersham); final pH 7.4.


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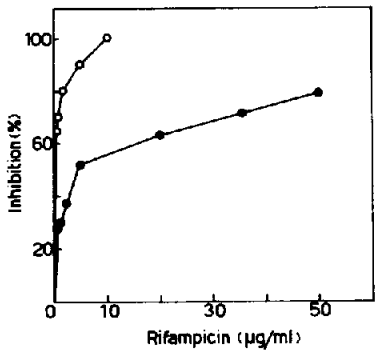


Fig. 1. Effect of rifampicin on mitochondrial and *E.coli* RNA polymerase. RNA polymerase was solubilized from rat liver mitochondria as reported in Material and methods. Highly purified *E.coli* RNA polymerase was purchased by Biopolimers Inc. 25 μg of rat liver mitochondrial RNA polymerase or 0.2 μg of *E.coli* RNA polymerase were incubated for 10 min at 30° in 100 μl of the reaction mixture reported in Material and methods. Rifampicin was added to the incubation mixture to reach the final concentrations shown in the figure. 100% solubilized mitochondrial activity was equivalent to 16 pmoles of UMP incorporated/mg prot./10 min, 100% of *E.coli* RNA polymerase activity was equivalent to the incorporation of 4 nmoles/mg prot./10 min. Zero time controls were already subtracted. ●——●: mitochondrial RNA polymerase; ○——○: *E.coli* RNA polymerase.

The reaction was started by adding either mitochondria or nuclei or solubilized enzyme at the concentrations given in the legends of the table and figures. After incubation for 10 min at 30°, the reaction was stopped by adding 0.05 ml of a saturated solution of sodium pyrophosphate and 10 ml of cold 5% trichloroacetic acid, cooled in ice and treated further as described earlier. DNAase, electrophoretically purified, was purchased from Worthington, Freehold, New Jersey. Rifampicin was kindly donated by Lepetit, Milano, Italy.

## 3. Results and discussion

The results summarized in table 1 show that rifampicin, at the two concentrations tested, inhibits $^3$H-UTP incorporation into RNA of swollen mitochondria, whereas it is without effect with isolated nuclei. The enzyme activity of intact mitochondria even if very low is completely unaffected by the antibiotic. The

Table 1

Effect of rifampicin on $^3$H-UTP incorporation into RNA of rat liver nuclei and mitochondria. RNA polymerase activity was assayed as described in Material and methods. Concentration of proteins in 0.1 ml of standard incubation mixture were: 125 μg for intact mitochondria, 40 μg for either swollen mitochondria or nuclei. Rifampicin was added to the incubation mixture to reach the final concentrations reported in the table. Data reported were obtained by subtracting zero time values.

| | Inhibitor (μg/ml) | Enzyme activity (pmoles/mg prot./10 min) | Inhibition (%) |
|---|---|---|---|
| Intact mitos | 0 | 1.5 | |
| | 10 | 1.5 | 0 |
| | 100 | 1.5 | 0 |
| Swollen mitos | 0 | 14 | |
| | 10 | 7.8 | 44 |
| | 50 | 3.1 | 78 |
| Nuclei | 0 | 42 | |
| | 10 | 42 | 0 |
| | 50 | 40 | 5 |

lack of inhibition in intact mitochondria is probably due to the impermeability of rat liver mitochondrial membrane to rifampicin as is the case for actinomycin D [2].

Our results do not confirm the data of Shmerling that in intact mitochondria RNA synthesis is inhibited by rifamycin. This could be explained as assuming that the intact mitochondrial preparation is already partially swollen and therefore not completely impermeable to the antibiotic. It is worth stressing that he does not state whether mitochondria were germ-free and this seems particularly important [9]. Furthermore the drug concentration used is very low in respect to the amount of proteins in the incubation mixture. In fact we have found that the percentage inhibition decreases with increasing amounts of of mitochondrial protein (see also in fig. 2, experiments with solubilized enzyme). This dependence is expected if rifampicin inhibits bacterial RNA polymerase by interfering directly with the enzyme protein and not with the template [4].

To investigate the effect of various concentrations of rifampicin on mitochondrial RNA polymerase, we solubilized the enzyme by sonicating the mitochondrial preparation. After centrifugation of the sonicated extract, the supernatant was used as enzyme source.

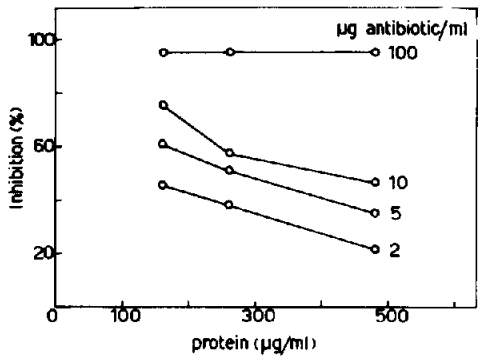


Fig. 2. Inhibition by rifampicin of mitochondrial RNA polymerase at various enzyme concentrations. Experimental conditions as reported in fig. 1.

In this system, RNA synthesis is not dependent on externally added DNA because the mitochondrial DNA is present in the extract and probably saturates the enzyme. Addition of DNAase indeed (50 µg/ml) completely inhibits the reaction. 5 µg/ml rifampicin inhibited mitochondrial enzyme activity by 50% whereas in the same conditions purified *E.coli* RNA polymerase was almost completely inhibited (fig. 1).

Inhibition by rifampicin is dependent on the amount of the mitochondrial RNA polymerase in the assay mixture (fig. 2). 100 µg/ml of rifampicin completely inhibits mitochondrial RNA polymerase even at higher enzymic protein concentration.

In conclusion, our results demonstrate that the mitochondrial RNA polymerase from rat liver is sensitive to rifampicin. The mitochondrial enzyme thus resembles the bacterial polymerase and differs from the nuclear one. The lack of inhibition in the presence of intact mitochondria indicates that mitochondrial membrane is impermeable to the antibiotic and suggests that probably the mitochondrial polymerase *in vivo* may not be affected by rifampicin.

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