RIFAMYCIN INSENSITIVITY OF RNA SYNTHESIS IN YEAST

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1. Introduction

Rifamycin and its derivatives are strong inhibitors of RNA synthesis in bacteria but not in the nuclei of animal cells [1–3]. It has been shown that the antibiotic binds to the bacterial DNA-dependent RNA polymerase, thereby interfering with the process of chain intiation [4–6]. The sensitivity or insensitivity of RNA synthesis towards rifamycin is therefore a property of the respective enzymes. This distinguishes rifamycin from other antibiotic inhibitors of RNA synthesis, such as actinomycin, which are known to react with the DNA template rather than with the polymerase.

In this communication we report studies on the effect of rifampicin, a highly potent rifamycin derivative, on the synthesis of RNA catalyzed by cellular extracts as well as by a partially purified preparation of DNA-dependent RNA polymerase from the yeast, *Saccharomyces cerevisiae.* In addition, we have examined the influence of the antibiotic on the RNA synthesis in isolated and highly purified yeast mitochondria. Rifamycin was found to be without effect on these reactions, even at concentrations as high as 50 μg/ml.

2. Materials and methods

The haploid, wild type strain D273-10 B and a locally isolated diploid strain of *S. cerevisiae* was used. Cells were grown to late log phase in a medium containing 0.5% peptone, 0.3% yeast extract and 1% glucose. They were disrupted mechanically and the mitochondria were collected by centrifugation and purified by flotation in urografin gradients as described earlier [7, 8]. Since yeast nuclei are destroyed during homogenization, the mitochondria-free cell extract contains most of the "nuclear" RNA polymerase activity in a soluble form. The enzyme was partially purified by fractionation with protamine sulfate and ammonium sulfate according to the procedure of Frederick, Maitra and Hurwitz [9].

E. coli B was grown in nutrient broth and extracts were prepared as described by Maitra and Hurwitz [10]. A highly purified preparation of RNA polymerase from *E. coli* K12 was kindly donated by Dr. H. Sterbach, Gottingen.

The reaction mixture for the determination of RNA polymerase activity contained: 50 mM tris HCl (pH 7.5); 1 mM EDTA, 2 mM β-mercaptoethanol, 50 μM each of ATP, GTP, CTP and UTP, 2 μCi/ml ^{3}H-UTP (Schwarz BioResearch Inc., specific activity 2.0 Ci/mmole), 4.2 mM phosphoenolpyruvate, 100 μg/ml pyruvate kinase, 100 μg/ml calf thymus DNA (native or denatured, as indicated), 10 mM $MgCl_2$ or 1.7 mM $MnCl_2$ and enzyme. Incubation mixtures of 1.0 ml were used for the estimation of RNA polymerase activity in mitochondria or in crude cell extracts. After incubation for 15 min at 35°C, samples were cooled in ice, precipitated with 5% TCA and processed further as described earlier [7] (method A). For the purified enzyme preparations a simpler procedure was used: mixtures of a total volume of 0.125 ml were prepared and incubated as above. 100 μl aliquots were then removed and applied to discs (25 mm in diameter) of Whatman 3MM filter paper which were further treated according to the procedure of Bollum [11] (method B).

Samples of rifampicin were kindly donated by Ciba Ltd., Basel.

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3. Results and discussion

Fig. 1 summarizes our results. As a control, we have employed the DNA-dependent RNA polymerase from *E. coli*. This enzyme is inhibited very strongly by rifampicin, being nearly completely inactive in the presence of 1 μg/ml of the antibiotic (curve a). Curve b shows the effect of rifampicin on the polymerase activity measured in the crude cellular extract of *E. coli*. Here RNA synthesis is completely independent of externally added DNA apparently because the enzyme is still saturated with cellular (*E. coli*) DNA. Nevertheless, an inhibition of about 70% is achieved by the addition of rifampicin. This indicates that also in this case the synthesis of new RNA chains is initiated throughout the incubation time of our assay.

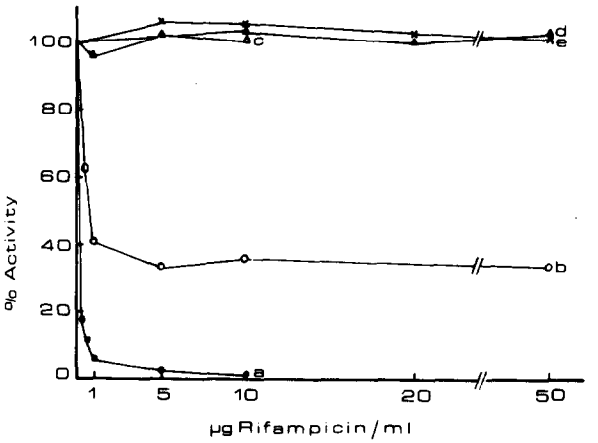

Fig. 1. Effect of rifampicin on the synthesis of RNA catalyzed by RNA polymerases from *E. coli* and yeast. RNA polymerase activity was measured as described in *Materials and methods*. Rifampicin was added to the incubation mixtures to yield the final concentrations shown in the abscissa. For the particular experiments reported here, the following conditions were used: Purified *E. coli* RNA polymerase (curve a): Determinations were done according to method B. Assay mixtures contained native DNA as template, Mg^{2+} as divalent cation and 2 μg enzyme. 100% activity was equivalent to 64 pmoles UMP incorporated. *E. coli* extract: (curve b): Determinations were done according to method B. No DNA was added, assay mixtures contained Mg^{2+} and 130 μg protein. 100% activity was equivalent to 85 pmoles UMP incorporated. Partially purified yeast RNA polymerase (curve c): Determinations were done according to method B. Assay mixtures contained denatured DNA as template, Mn^{2+} as divalent cation and 40 μg enzyme. 100% activity was equivalent to 119 pmoles UMP incorporated. Yeast extract (curve d): Determinations were done according to method A. Assay mixtures contained denatured DNA, Mn^{2+} and 1.9 mg protein. 100% activity was equivalent to 175 pmoles UMP incorporated. Yeast mitochondria (curve e): Determinations were done according to nethod A. No DNA was added because mitochondrial DNA serves as template in intact mitochondria [7]. Assay mixtures contained Mg^{2+} and 2.3 mg protein. 100% activity was equivalent to 116 pmole UMP incorporated.

The yeast RNA polymerase, in contrast, is not inhibited by rifampicin at concentrations as high as 50 μg/ml, regardless of whether a partially purified enzyme preparation (curve c) or the crude cell extract (curve d) is used. Enzyme activity is in both cases completely dependent on externally added DNA, denatured DNA being much more effective in promoting RNA synthesis than native DNA. Rifampicin insentivity, however, is not affected by the kind of DNA used as template, nor by the replacement of Mn^{2+} by Mg^{2+} in the assay mixture. A preincubation of the enzyme preparation with rifampicin (50 μg/ml) for 2 min in the absence of the triphosphates does not reduce the activity of the polymerase.

A second point of interest concerned the synthesis of RNA in isolated and highly purified yeast mitochondria. We found that this process again was not affected by rifampicin (curve e). We consider the possibility that rifampicin cannot penetrate the mitochondrial membrane unlikely because the mitochondria used were highly swollen. This is evident from the fact, that they are, for instance, permeable to actinomycin, D, which at a concentration of 50 μg/ml caused a 90% inhibition of RNA synthesis. In spite of numerous trials, we have not yet been able to solubilize the mitochondrial RNA polymerase. The enzyme appears to be firmly bound to the inner membrane and severe destruction of this membrane destroys the enzyme activity. After mild mechanical distintegration of mitochrondria with an Ultra-Turrax homogenizer, a variable amount of RNA polymerase activity is still measurable. No inhibition of UMP incorporation is observed upon addition of rifampicin to such a preparation. Even a preincubation of mitochondria with 50 μg/ml rifampicin prior to the addition of the triphosphates does not reduce their RNA synthesizing activity.

Experiments with rat liver mitochondria which had been swollen by treatment with phosphate or with digitonin [12] gave identical results; no inhibition of RNA synthesis is obtained.

Our experiments are consistent with but do not prove the assumption that the mitochondrial RNA polymerase is not sensitive to rifampicin. Even if factors other than the insensitivity of the mitochondrial enzyme are responsible for our observations, the mere fact that RNA synthesis in isolated mitochondria, in contrast to bacterial RNA synthesis, is not affected by rifampicin, is of interest. In this respect the rifamycins obviously contrast to other antibiotics, such as chloramphenicol or erythromycin, which interfere with bacterial protein synthesis. Although these substances have no effect on cytoplasmic protein synthesis in eukaryotic cells, they do block the corresponding processes in the mitochondria [13–15]. For the use of rifamycin as a drug it may therefore be important to known that RNA synthesis in both, nuclei and mitochondria, is not inhibited by this antibiotic.

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