ACTION OF RIFAMYCIN ON RNA-POLYMERASE FROM SENSITIVE AND RESISTANT BACTERIA

Walter Wehrli, Fritz Knüsel and Matthys Staehelin

Research Laboratories of the Pharmaceutical Department of CIBA LIMITED, Basle, Switzerland

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The rifamycins inhibit specifically the DNA dependent RNA-polymerase reaction of Escherichia coli (Hartmann et al.,1967). We have shown that the enzyme itself interacts with the antibiotic and that RNA-polymerase isolated from rat liver is not inhibited by even high concentrations of the rifamycins(Wehrli et al.,1968). Mizuno and coworkers (1968) have obtained analogous results with streptovaricin, a substance chemically closely related to the rifamycins, as well as with the rifamycins themselves (Umezawa et al., 1968).

In the present work we have compared the RNA-polymerase of E. coli with that of Staphylococcus aureus. Wherease the growth of Staph.aur. is inhibited by a 1000 times smaller concentration of rifampicin than that required for inhibition of E.coli, the two enzymes differ by only a factor of 5-10 in their sensitivity towards the antibiotic. We have also tested the susceptability of RNA-polymerase from mutants of these two organisms selected for their resistance towards rifamycins. Whereas actinomycin C inhibits these enzymes to the same extent as the normal enzymes, rifamycin does not influence at all the activity of the two resistant enzymes.

Materials and Methods: CTP-H^3 was purchased from Schwarz Bio Research, Orangeburg, N.Y., unlabeled nucleotide triphosphate, calf thymus DNA and spermidine hydrochloride from Cal Biochem, Lucerne, Switzerland, and actinomycin C from Bayer, Leverkusen, Germany. The rifamycin derivative used in this work was rifampicin (Maggi et al., 1966).

RNA-polymerase was prepared from logarithmically growing E. coli ETH 2018, as described in a previous paper (Wehrli et al., 1968), RNA-polymerase from Staph.aur. SG 511 was isolated in the same way. Under standard conditions 1 mg protein incorporated 200-300 mμ moles of H^3-CMP in the case of E. coli, and 150-200 mμ moles of H^3-CMP in the case of Staph.aur. From both organisms, E. coli ETH 2018 and Staph.aur. SG 511, which had a minimal inhibitory concentration (MIC) of 5-10 and 0.007 μg rifampicin per ml cell culture respectively, resistant mutants have been selected by repeated subculturing with increasing concentrations of rifampicin. The resistant cells, which had a minimal inhibitory concentration of over 200 μg rifampicin per ml cell culture, were plated on agar in presence of 200 μg/ml rifampicin. One clone was isolated and grown in the culture medium containing no rifampicin for the production of the cells. Control experiments showed that the cells grown under those conditions were still resistant towards rifampicin. RNA-polymerase was isolated in the same way as that used for sensitive cells. Under standard conditions 1 mg protein incorporated 100-150 mμ moles of H^3-CMP in the case of resistant E. coli, and 60-100 mμ moles of H^3-CMP in the case of resistant Staph.aur.

Polymerase assay: The usual reaction mixture contained 20 µmoles Tris-HCl (pH 7.9); 2.5 µmoles β-mercaptoethanol; 2.0 µmoles $MgCl_2$; 20 µmoles NH_4Cl; 2 µmoles spermidine-HCl; 0.4 µmole ATP; 0.2 µmole GTP and UTP; 50 µg DNA (calf thymus); 0.05 µmole $[H^3]$CTP (specific activity 10 µC/µmole); and 20 µg protein, in a final volume of 0.25 ml. Incubation was carried out for 20 minutes at 37°. The reaction was stopped by placing the tubes in ice and by the addition of 0.5 ml 2 M HCl. The precipitate was collected on a glass filter disc (Whatman G F/C 2.4 cm diameter), washed with dilute HCl, dried and counted on a Packard Tricarb liquid scintillation counter.

Results and Discussion: Fig. 1 shows the degree of inhibition of RNA-polymerase from E. coli ETH 2018. 0.2 µg rifampicin per ml reaction mixture inhibits the incorporation of H^3-CMP to 90%. In contrast, RNA-polymerase from E. coli resistant to rifampicin is not inhibited significantly by a concentration of 200 µg per ml reaction mixture. The difference in sensitivity between normal and rifampicin resistant E. coli is therefore at least 10,000 fold. Higher concentrations of rifampicin inhibit the resistant polymerase to some extent, but this effect is not due to the specific action of rifampicin since rifarubin (Bickel et al., 1966), a rifamycin derivative which does not inhibit the growth of E. coli and is inactive towards normal E. coli RNA-polymerase up to concentrations of 20 µg/ml, shows the same inhibition of normal and resistant enzyme at high concentrations. As a comparison actinomycin C was tested with RNA-polymerase from normal and rifampicin resistant E. coli.

Both enzymes were inhibited to exactly the same degree (Fig.1).

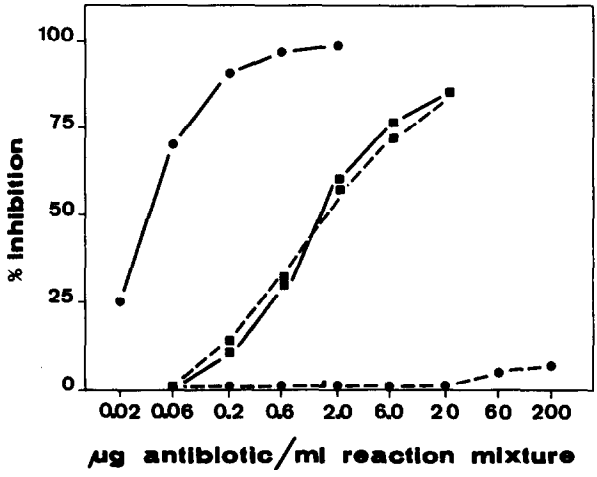

Fig. 1 Inhibition of RNA-polymerase isolated from E. coli ETH 2018 (———) and from a rifampicin resistant strain of E. coli (— — —) by rifampicin (●) and actinomycin C (■). Assay conditions as described in Methods.

Since E. coli cells are relatively insensitive towards rifampicin (MIC = 10 µg/ml), we also tested Staph.aur. (MIC = 0.002 µg/ml). The RNA-polymerase from this organism is 5-10 times more sensitive towards rifampicin (Fig. 2), i.e. 0.02 µg rifampicin per ml inhibit the incorporation of H^3-CMP to 90 %. But again RNA-polymerase isolated from Staph.aur. resistant towards rifampicin was not affected by concentrations of antibiotic as high as 60 µg/ml.

In the case of E. coli the selection of resistant cells was carried out three times and each time the mutation resulting in cells resistant towards rifampicin also yielded an RNA-polymerase resistant towards the antibiotic. Preliminary experiments with the resistant enzyme have not shown other signi-

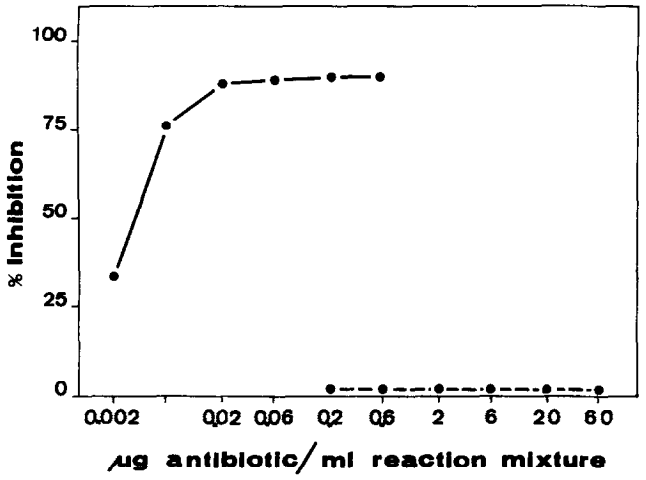

Fig. 2 Inhibition of RNA polymerase isolated from Staph.aur. 14 (———) and from a rifampicin resistant strain of Staph.aur. (– – –) by rifampicin. Assay conditions as described in Methods.

ficant differences in its properties. The finding of a bacterial enzyme which is not influenced by rifamycin together with the fact that actinomycin inhibits both normal and resistant enzymes in exactly the same way yields further proof that the rifamycins interact directly with the enzyme.

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