A RIFAMPICIN RESISTENT RNA-POLYMERASE FROM *E. COLI* ALTERED IN THE β-SUBUNIT

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1. Introduction

Rifamycin [1], streptovaricin [2] and their derivatives inhibit the function of DNA-dependent RNA-polymerase of prokaryotes and viruses. As shown by Wehrli et al. [3] the rifamycin derivative rifampicin forms a stable complex with RNA-polymerase from *E. coli*. Sippel and Hartmann [1] and diMauro et al. [4] demonstrated that this antibiotic inhibits neither the primary binding of polymerase to DNA nor chain elongation but is largely reduced in activity when a complex of DNA, enzyme, and purine ribonucleosidetriphosphate has been formed prior to its addition [4]. Therefore, rifampicin appears to act on an initiation step which precedes the formation of the first internucleotide bond [5]. DiMauro et al. have also demonstrated that the "minimal enzyme", consisting of subunits α, β and β' [6], and not the initiation factor σ [7] is the target of inhibitory action.

The question as to which of the subunits of minimal enzyme is the point of attack of the antibiotic has not yet been answered, mainly because it has not been possible to separate native subunits; furthermore, denatured subunits which are isolable, have lost their capacity to interact with the compound. In this communication evidence is presented indicating that rifampicin may interact with subunit β, which therefore should play a role in initiation of RNA synthesis.

2. Materials and methods

DNA-dependent RNA-polymerases were isolated from one rifampicin sensitive and five rifampicin resistent *E. coli* strains as described previously [8, 8a] with two variations: Elution of enzyme from the DEAE column was performed with a buffer containing 0.05 M instead of 0.01 Tris, otherwise identical to the TMA-buffer used previously. The final steps were two consecutive gradient centrifugations in Rotor SW 27 of the Beckman Spinco Model L centrifuge; the first through a linear gradient of 10% (w/v) sucrose + 5% (v/v) glycerol in TMA to 35% sucrose + 10% glycerol in TMA for 20 hr at 27,000 rpm; the second through the same gradient, but with 0.5 M NH_4Cl added and for 40 hr at 27,000 rpm.

Strain 1 is derived from *E. coli* B/4, strain 2 from *E. coli* CR 63, strain 3 and 5 from *E. coli* B, strain 4 (ETH 2018) from a K12 strain. Strain 6, *E. coli* K12 Hfr AB, is rifampicin sensitive. Strains 1–3 are "spontaneous" mutants isolated from agar plates containing 100 μg/ml rifampicin. Strains 4 and 5 were kindly supplied by W.Wehrli and K.Knüsel, Ciba, Basle.

Polyacrylamide gel electrophoresis was performed in gels containing 7,5% acrylamide, 0,1% sodium-dodecylsulphate, 6 M urea and 0,4 M tris, pH 9.5 [9].

Electrophoresis on cellulose acetate sheets (cellogel strips 4 × 17 cm for analytical electrophoresis, Chemetron, Milano) was performed in a moist chamber on a refrigerated (0°C) Teflon-surface (Holzel-Technik, 825 Dorfen, Germany) for 1–2.5 hr in an electric field of about 60 Volts/cm not exceeding a current of 25 mA per strip. The strips were thoroughly equilibrated with buffer (0,5 M boric acid, 0,01 M EDTA, 0,01 M β-mercaptoethanol,

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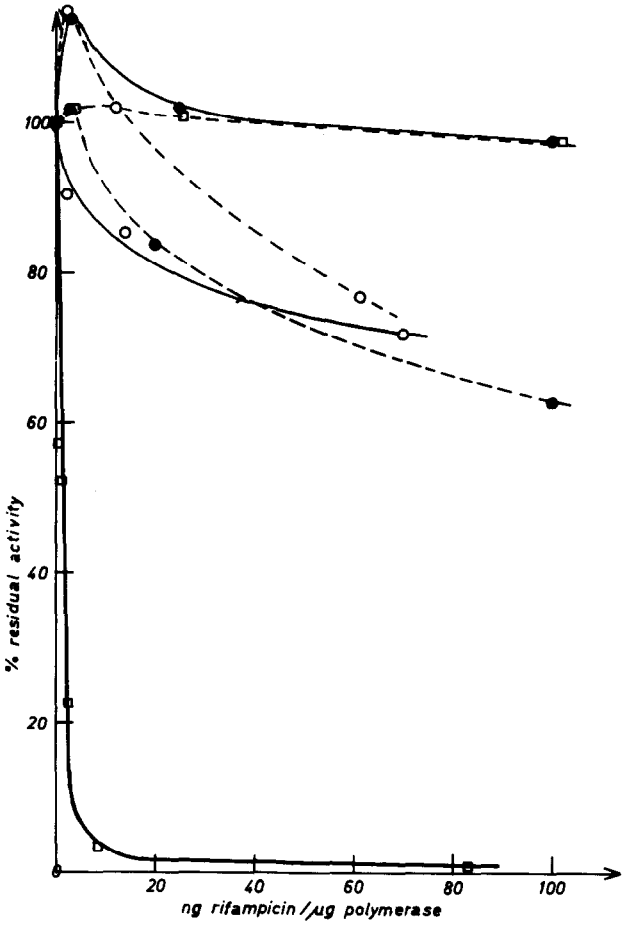

Fig. 1. Inhibitory effect of rifampicin on highly purified RNA-polymerases from strain 1–6. Assays were performed as described previously [8] but with the addition of 10^{-3} M β-mercaptoaethanol. The specific activity of the enzyme from strain 6 was 96 mu/mg. (1 mu = 1 mμ M of ATP incorporated per minute under standard conditions [8a]. o——o Enzyme from strain 1; •- - -• strain 2; o- - -o strain 3; •——• strain 4; □- - -□ strain 5; □——□ strain 6.

6 M urea, pH adjusted to 9.5 with NaOH then placed onto the surface of the chamber, the penetrable (dull) side facing upwards and carefully blotted with filter paper, removing excess moisture but avoiding air bubbles to form between sheet and surface. The samples (about 1–2 μl per cm, 5–30 mg of protein/ml) were applied with a soft tipped polyethylene pipette. The bands were stained with 0.5% (w/v)

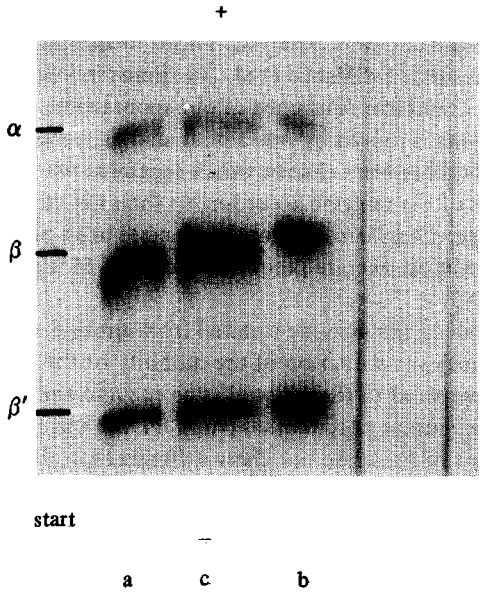

Fig. 2. Pattern of RNA-polymerase subunits obtained by electrophoresis on cellulose acetate sheets (cellogel). 2 hr, 1100 volts, 20 mA. (a) Enzyme from strain 5; (b) enzyme from strain 6; (c) a mixture of enzymes from strain 5 and 6.

Amidoschwarz in 45% methanol + 45% water + 10% glacial acetic acid for 10 min; excess dye was removed by successive washes with the same solvent.

3. Results and discussion

RNA-polymerase isolated from each of the six straines named in Materials and Methods had specific activities of the same order of magnitude and were more than 90% pure as judged from the patterns obtained by electrophoresis on cellulose acetate sheets and in polyacrylamide gels. The effect of rifampicin on the activities of the enzymes is shown in fig. 1. While the enzyme from strain 6 is inhibited to 50% by 1 ng of rifampicin/μg enzyme, the same amount of antibiotic has no inhibitory effect on the enzymes isolated from strains 1–5. Even large excess of rifampicin (1–2 μg per assay containing 5–20 μg of enzyme) did not cause significant inhibition.

In polyacrylamide gel electrophoresis where

mobility is correlated to molecular weight [10] no difference between the subunit patterns of rifampicin sensitive "wild type" enzyme and the five mutant enzymes could be observed. This is in agreement with the results of diMauro et al. [4]. Since it is probable that rifampicin resistence of our spontaneous mutants is caused by one amino acid exchange a method has been chosen which separates the subunits of the enzyme according to their net electrical charge. Therefore, electrophoresis has been performed on cellulose acetate sheet (cellogel) in 6 M urea at pH 9.5.

Indeed the enzyme isolated from strain 5 shows a significant reduction of the mobility of the β-band as compared to that of wild type enzyme and all other mutant polymerases (fig. 2). Different B and K strains of *E coli* do not show differences in their RNA-polymerase subunit patterns. This excludes the possibility that the observed change is due to a strain difference.

Since strain 5 arose spontaneously, it is probable that it is the result of only one mutational event in one of the structural genes for polymerase. Hence the structural change of the β-subunit is probably the cause of rifampicin resistence. Final proof is expected from the analysis of further mutants and from contransduction experiments. The unaltered subunit patterns of strain 1–4 can be due to amino acid exchanges not leading to net charge alterations.

The stoichiometry of complex formation between enzymes and rifampicin yields close to one antibiotic binding site per 13s particle [11]. This argues for the involvement of only one of the enzyme subunits. Our experiments suggest β (of which one is present per 13s particle), as the site for the action of rifampicin and therefore as the subunit involved in the formation of the initiation complex between DNA, enzyme and purineribonucleosidetriphosphate.

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References

[1] A.Sippel and G.Hartmann, Biochim. Biophys. Acta 157 (1968) 218.
[2] S.Mizuno, H.Yamazaki, K.Nitta and H.Umezawa, Biochim. Biophys. Acta 157 (1968) 322.
[3] W.Wehrli, F.Knüsel, K.Schmid and M.Staehelin, Proc. Natl. Acad. Sci., U.S. 61 (1968) 667.
[4] E.diMauro, L.Snyder, P.Marino, A.Lamberti, A.Coppo and G.P.Tocchini-Valentini, Nature 222 (1969) 533.
[5] D.Anthony, E.Zeszotek and Goldthwait, Proc. Natl. Acad. Sci. U.S. 56 (1966) 1026.
[6] W.Seifert, P.Qasba, G.Walter, P.Palm, M.Schachner and W.Zillig, Europ. J. Biochem. 9 (1969) 319.
[7] R.R.Burgess, A.A.Travers, J.J.Dunn and K.F.E.Bautz, Nature 221 (1969) 43.
[8] W.Zillig, E.Fuchs and R.L.Millette, in: Procedures in Nucleic Acid Research, G.L.Cantoni and D.R.Davies, eds. (Harper and Row, New York, 1966) 323.
[8a] E.Fuchs, R.L.Millette, W.Zillig and G.Walter, European J. Biochem. 3 (1967) 183.
[9] L.Ornstein, Annals N.Y. Acad. Sci. 121 (1964) 321.
[10] A.L.Shapiro, E.Vinuela and J.V.Maizel, Biochem. Biophys. Res. Commun. 28 (1967) 815.
[11] W.Wehrli, pers. commun.