INHIBITORY EFFECTS OF RIFAMPICIN AND SOME DERIVATIVES ON RIBONUCLEASE H (HYBRIDASE) FROM RAT LIVER

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1. Introduction

In the course of our studies on the purification and characteristics of ribonuclease H [1, 2] from rat liver (W. Roewekamp, W. Schmid, B. Benecke and C.E. Sekeris, manuscript in preparation) and its role on hormonal control of transcription, we have observed that rifampicin and especially some of its derivatives exert a marked inhibitory action on this nuclease. Recently Mölling et al. [3] have shown that a hybridase connected with the virion of avian myeloblastosis virus is likewise inhibited by a rifampicin derivative.

2. Materials and methods

2.1. *Ribonuclease H (hybridase)*

This was prepared from rat liver cytosol by a method to be published in detail elsewhere (W. Roewekamp, W. Schmid, B. Benecke and C.E. Sekeris, manuscript in preparation). Essentially the method consists in preparation of a 70,000 *g* supernatant fraction from rat liver, DEAE-32 cellulose chromatography, ammonium sulfate precipitation, hydroxylapatite and phosphocellulose chromatography. Chromatography on phosphocellulose leads to the appearance of two active fractions as shown in fig. 1.

2.2. *Assay of hybridase*

The assay is based on the ability of hybridase to degrade the RNA moiety of DNA–RNA hybrids [1, 2]. DNA–RNA hybrid of which the RNA moiety is radioactively labelled, was prepared as described below and incubated with hybridase in the presence of Mn^{2+} ions (0.6 mM) in a final volume of 150 μl for 60 min at 37°. Ten μl of the inhibitor, dissolved in ethylene glycol–ethanol 1:1, were added at zero time, the controls receiving the same amount of the solvent. At the end of the incubation period aliquots were brought on filter paper discs and the amount of remaining precipitable activity was measured by dropping the filter papers in 5% perchloric acid and then washing with 5% PCA, ethanol and ether as described previously [4]. The filter papers were then placed in scintillation vials filled with T-Fluor (5 g PPO, 0.2 g dimethyl-POPOP in 1 l toluol) and counted in a Mark I Nuclear Chicago liquid scintillation counter. The radioactivity remaining on the filters is reversely proportional to hybridase activity.

2.3. *Preparation of DNA–RNA hybrid*

Heat denatured rat liver DNA was transcribed either with rat liver RNA polymerase B or with *E. coli* RNA polymerase. The incubation mixture was as described in [5], the incubation time was 60 min at 37°. At the end of the incubation period the mixture was treated with ribonuclease (5 μg/ml) to degrade free RNA. This treatment is not necessary when the DNA is transcribed by rat liver polymerase B which leads to an almost total (over 95%) formation of hybrids. 0.5% SDS was then added and the mixture submitted to chromatography on Sephadex G-75 equilibrated with 0.065 M Tris-HCl buffer pH 7.9. The fractions containing the DNA–RNA hybrids were collected and pooled. The rifampicin derivatives tested were: AF 05, AF 013 and PR 19.

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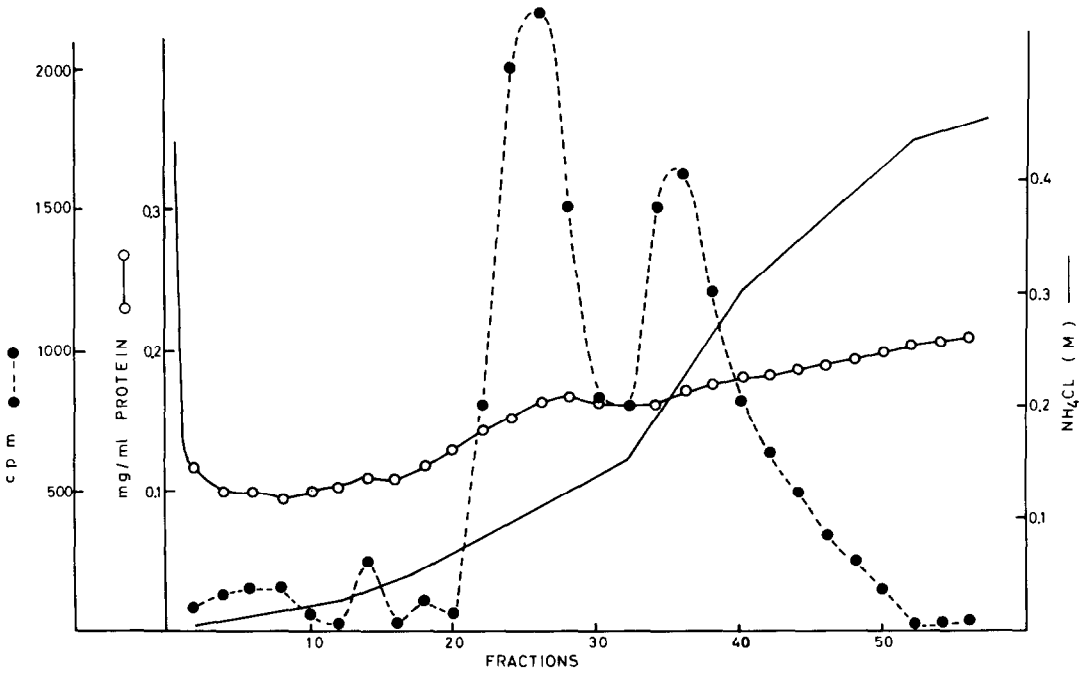

Fig. 1. Phosphocellulose chromatography of hybridase from rat liver. The enzyme preparation in 0.065 M Tris-HCl buffer, pH 7.9 containing 1 mM mercaptoethanol and 20% v/v glycerol was passed through a phosphocellulose column, precycled and equilibrated with the same buffer. The column was washed and then eluted with a 0–0.4 M NH_4Cl linear gradient in the above mentioned buffer. Fractions of 15 ml were collected and assayed for hybridase activity as described in Methods.
(●–●–●): Hybridase activity; (○–○–○): protein, mg/ml; (———): (M) NH_4Cl.

3. Results and discussion

As mentioned in Methods two peaks of hybridase activity appear after phosphocellulose chromatography, which we have tentatively called A and B (see fig. 1). Due to the fact that we are still not aware of the interrelationship between the two activities we have tested the action of rifampicin and its derivatives separately on them. The derivatives tested are AF 05, AF 013 and PR 19. The first two derivatives have been shown to inhibit the DNA-dependent RNA polymerases of eukaryotes [6, 7, and P. Chambon, personal communication]. It is evident from figs. 2 and 3 that both hybridase activities A and B are inhibited by the above mentioned substances. However AF 013 is the most potent of the inhibitors, completely inhibiting activity A at a concentration of 200 μg/ml and activity B up to 80% at the same concentration. PR 19 shows 50% inhibition of activity A and 30% of activity B at a concentration of 200 μg/ml, whereas rifampicin and AF 05 show only a very weak inhibitory action. The finding that rifampicin and its derivatives inhibit hybridase activity points to possible common structural features between the nuclease and other enzymes inhibited by these substances such as the DNA-dependent RNA polymerases [6, 7] and the reverse transcriptases [8]. In this context it is of interest that hybridase has been found connected with the virion of avian myeloblastosis virus [3], very probably being a component of the reverse transcriptase and involved in DNA synthesis catalysed by this enzyme. Both DNA polymerizing activity and the hybridase are inhibited by rifampicin derivatives.

Recently we have detected hybridase activity in the integument of blowfly larvae [9]. This enzyme is likewise inhibited by AF 013.

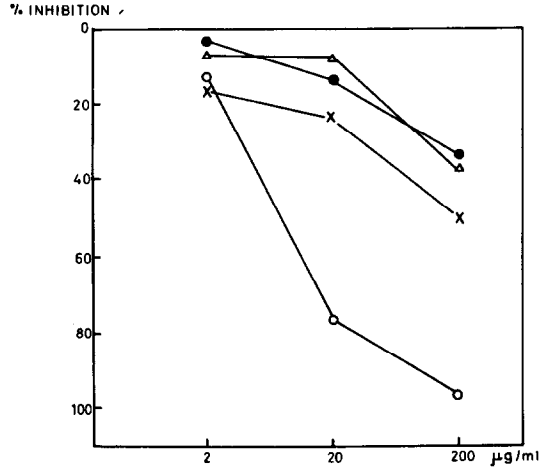

Fig. 2. Inhibitory action of rifampicin and derivatives on hybridase (activity A) fractions 22–28, (see fig. 1).
(●–●–●): rifampicin;
(△–△–△): AF 05;
(○–○–○): AF 013;
(x–x–x): PR 19.

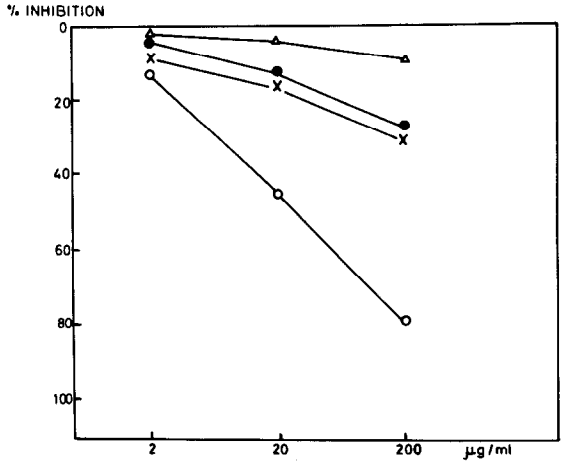

Fig. 3. Inhibitory action of rifampicin and derivatives on hybridase (activity B) fractions 32–40 (see fig. 1).
(●–●–●): rifampicin;
(△–△–△): AF 05;
(○–○–○): AF 013;
(x–x–x): PR 19.

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