RIFAMPICIN INHIBITION OF POLY(dT)·POLY(A)-PRIMED POLY(A) SYNTHESIS BY HeLa AMP POLYNUCLEOTIDYLEXOTRANSFERASE

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1. Introduction

AMP polynucleotidylexotransferase from corn seedlings [1], an enzyme catalyzing the sequential addition of AMP moieties to the 3′ hydroxyl terminus of an oligonucleotide primer to form poly(A) [2], is inhibited by rifampicin and rifampicin derivatives with modified aminopiperazine side chains [3]. We report here a similar sensitivity of the tRNA-primed exotransferase partially purified from HeLa S_3 cells [4]. The aminopiperazine derivatives of rifampicin are known to preferentially inhibit and thus bind RNA tumor viral reverse transcriptase as compared with their inhibition of RNA and DNA-dependent polymerases [5].

2. Methods and materials

AMP polynucleotidylexotransferase was prepared from a post-ribosomal supernatant of an homogenate of exponentially growing HeLa S_3 cells by ammonium sulfate precipitation and DEAE-cellulose chromatography as described previously [4]. The activity was determined by measuring the accumulation of acid-insoluble radioactivity from labeled ATP. A reaction mixture (0.1 ml) containing 7 μmoles Tris (pH 8.8), 1 μmole dithiothreitol, 0.12 μmole [8-^{14}C]ATP (1.5 mCi/mmole), 10 μg bovine serum albumin, 0.1 μmole $MnCl_2$, 6.2 μg enzyme and the appropriate primer was incubated at 30°C. Aliquots were removed after 30, 60, and 90 min, delivered to filter paper disks and the total AMP incorporated into acid-insoluble material determined as described previously [2]. With tRNA as primer the enzyme catalyzed the addition of 47 nmoles AMP/mg/min under these conditions.

The synthetic oligomers were purchased from PL Biochemicals, Inc., Milwaukee, Wisc., the tRNA of *Escherichia coli* and the ATP from Schwarz-Mann, Orangeburg, N.Y. The rifampicin and aminopiperazine derivatives were generously donated by Gruppo Lepetit spa, Milan.

3. Results

The sensitivity of the HeLa exotransferase to rifampicin SV and aminopiperazine derivatives of rifampicin is similar to that reported for several RNA tumor virus reverse transcriptases by Gurgo et al. [5]. The 2,5-demethyl-4-*N*-benzyl demethyl rifampicin is most effective (50% inhibition at 160 μg/ml) inhibitor of the tRNA-primed HeLa exotransferase (fig. 1, panel A). The level of sensitivity of the exotransferase to rifampicin SV is similar to that of the DNA-dependent RNA polymerase of Ehrlich ascites carcinoma cells reported by Umezawa et al. [6].

In distinguishing reverse transcriptase of an RNA tumor virus from eukaryotic DNA polymerases, $(dT)_{10}$·poly(A) is a preferred synthetic duplex as compared with $(dT)_{10}$·poly(dA) [7]. The HeLa exotransferase exhibits a similar preference for $(dT)_{10}$·poly(A). See table 1. Of further interest is the inhibition of the low level of AMP incorporation in the absence of added primer upon the addition of $(dT)_{10}$·poly(A) or poly$(dT)_{10}$. The inhibition of the exotransferase by deoxyoligomers appears to be the antithesis of the inhibition of the Rauscher leukemia virus reverse transcriptase by polyribonucleotides reported by Tuominen and Kenney [8]. The $(dT)_{10}$·poly(A) primed reaction with the HeLa exotransferase exhibits essentially the

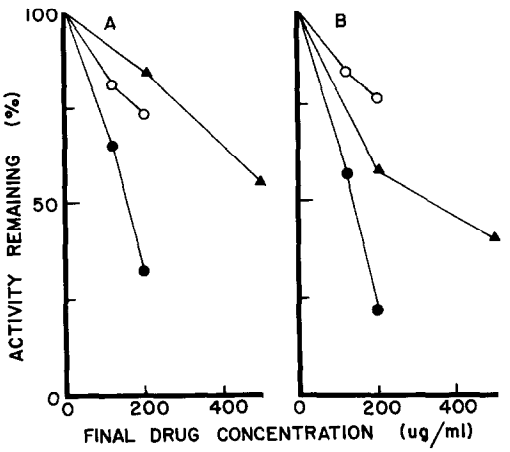

Fig. 1. Inhibition of HeLa exotransferase by rifampicin and aminopiperazine derivatives. Reaction mixtures as described in Methods and materials but containing: 0.5 μg dimethylsulfoxide, the indicated amount of rifampicin SV (▲—▲—▲), *N*-dimethyl rifampicin AF/AP (○—○—○) or 2,5-dimethyl-4-*N*-benzyl demethyl rifampicin AF/ABDMP (●—●—●) and 0.41 A_{260} *E. coli* tRNA (panel A) or 0.05 A_{260} (dT·poly(A) as primer (panel B) were incubated at 30° C. In the absence of drug, 0.7, 3.1 and 7.0 nmoles AMP were incorporated into acid-insoluble material with tRNA in 30, 60 and 90 min, respectively. 1.5, 4.5 and 8.8 nmoles AMP were incorporated in the $(dT)_{10}$·poly(A)-primed reaction in 30, 60 and 90 min, respectively. The percent activity remaining at each time point was within 1.5% of the mean of all three time points with a given concentration of a given drug.

Table 1
Effect of various primers on the level of AMP incorporated into acid-insoluble material by HeLa exotransferase.

Primer	Amount added (A_{260})	Poly(A) synthesized (nmole AMP)
tRNA	0.21	7.4
$(dT)_{10}$·poly(A)	0.05	9.8
$(dT)_{10}$·poly(A)	0.25	17.3
$(dT)_{10}$·poly(dA)	0.25	0.5
Poly(A)	0.09	18.5
Poly$(dT)_{10}$	0.25	0.2
None	–	0.9

Reaction mixtures as described in Methods and materials containing the indicated oligomers were incubated at 30° C for 90 min. The total nmoles of radioactive AMP incorporated into acid-insoluble material was determined on filter paper disks as described previously [2]. 2466 cpm was equivalent to nmole AMP.

same sensitivity to the aminopiperazine derivatives of rifampicin as the tRNA-primed reaction (cf. panel B with panel A in fig. 1). However, the reaction primed with the synthetic duplex is more sensitive to rifampicin SV than the reaction primed with the double stranded tRNA.

4. Discussion

These somewhat surprising observations indicate that a non-template requiring, ribonucleotide polymerizing activity (AMP polynucleotidylexotransferase) can be inhibited by drugs, heretofore thought specific for a template-requiring, deoxyribonucleotide polymerizing enzyme (reverse transcriptase). Furthermore, the dual primer–template function of $(dT)_{10}$·poly(A), although true for the reverse transcriptase reaction is not requisite for its preferential use by an enzyme adding AMP moieties to the primer poly(A) portion of the duplex. Alternatively, the AMP polynucleotidylexotransferase may be among the polypeptide components of a functional reverse transcriptase [9] and those associated with eukaryotic mRNA's [10].

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