ON THE MECHANISM OF RIFAMPICIN INHIBITION OF DNA DEPENDENT RNA POLYMERASE FROM *Escherichia coli*: A NEW HYPOTHESIS OF THE DRUG ACTION

Aleš Cvekl, Květa Horská and Karel Šebesta

Institute of Organic Chemistry and Biochemistry
Czechoslovak Academy of Sciences, 166 10 Prague 6

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The influence of rifampicin on the temperature dependent synthesis of trinucleotides ApUpA and UpApU was studied. Whereas the elongation of dinucleotide ApU is stimulated to a great extent, the elongation of UpA is inhibited to about 50%. These results cannot be explained in terms of different models proposed for the mechanism of inhibition of RNA synthesis caused by rifampicin. According to our hypothesis rifampicin inhibits internal rearrangement of the RNA polymerase which occurs during the transition of the initiation complex to the stable elongation complex.

Structure, properties, and mode of action of rifampicin – a potent inhibitor of prokaryotic DNA dependent RNA polymerase – have been reviewed by different authors[1,2]. Rifampicin is known to form a tight non-covalent stoichiometric complex with RNA polymerase[3,4] and this fact results in the inhibition of high-molecular RNA synthesis[5]. Discovery of abortive initiation[6] brought further progress in the understanding of the inhibitory mechanism of rifampicin. McClure and Cech[7] suppose that the inhibiton of RNA synthesis caused by rifampicin is due to its steric blockage of dinucleotide translocation. On the other hand, according to Schulz and Zillig[8] rifampicin causes the destabilization of the ternary complex enzyme–DNA–short oligonucleotides during abortive initiation and thus interferes with elongation. Finally, Hartmann et al.[9] conclude that rifampicin influences the binding of reaction products, i.e. pyrophosphate and RNA. All these proposals concerning the mode of action of rifampicin are to a certain degree contradictory and none of them explains all aspects of this phenomenon.

In the present paper we have used a simple system of two similar reactions of the primed abortive initiation. We have found that rifampicin influences the synthesis of trinucleotides ApUpA and UpApU, respectively, on poly(dA-dT) template in a different manner. Using results presented in this paper as well as literary data enable us to formulate a new hypothesis concerning the mechanism of rifampicin inhibition.

EXPERIMENTAL

Substrates and enzyme: Dinucleotides (3'-5')ApU and UpA and DNA dependent RNA polymerase have been described in our recent paper[10].

Transcription reaction: The standard reaction mixture (15 μl) contained 40 mmol l^{-1} Tris-HCl (pH 7·9), 80 mmol l^{-1} KCl, 10 mmol l^{-1} $MgCl_2$, 0·1 mmol l^{-1} DTT, poly(dA-dT), holoenzyme, dinucleotide, and [α^{32}P]labelled ATP or UTP (Amersham, England) at concentrations given in Figs 1 and 2. RNA polymerase and template were incubated with/without rifampicin for 10 min. The molar concentration of rifampicin was 10 fold higher than that of holoenzyme which is sufficient in view of the respective binding constant[3,4]. The reactions were initiated by the addition of substrates and their time course was linear over the period studied (2—15 min). They were stopped by cooling to 0°C and by adding EDTA to a final concentration of 80 mmol . . l^{-1}. The mixtures were analyzed using paper electrophoresis in 0·05 mol l^{-1} sodium dihydrogen citrate[10].

RESULTS AND DISCUSSION

Rifampicin exhibits a dual effect on some reactions of abortive initiation; it stimulates at temperatures under 30°C whereas at 37°C an inhibitory effect has been observed[7,11,12]. To deal with this phenomenon we have measured systematically the temperature dependence of the initial velocity of trinucleotide synthesis (ApUpA and UpApU, respectively) in the temperature interval 24°C to 40°C with/without rifampicin in the reaction mixture as shown in Figs 1 and 2.

In the presence of rifampicin the synthesis of ApUpA is stimulated over the whole temperature interval although to a different extent. Sylvester and Cashel[13] observed the stimulation at 24°C and interpreted it as a destabilizing effect of rifampicin on the ternary complexes in agreement with Reisbig et al.[14]. On the contrary Oen and Wu[15] found an inhibitory effect of rifampicin on ApUpA synthesis but it was not described in detail.

Concerning the synthesis of the trinucleotide UpApU we found an inhibition caused by rifampicin over the whole temperature interval studied. This finding is in agreement with data published for the temperatures 24°C and 37°C only[13,15].

Nevertheless, this system of ApUpA and UpApU synthesis[13,15] was not used for discussion of the mode of rifampicin action. Reisbig et al.[14] observed directly a binding of rifampicin outside the catalytic centre of RNA polymerase, a fact which excludes the model of steric blockage proposed by McClure and Cech[7]. This led us to formulate a hypothesis explaining the mechanism of inhibition caused by rifampicin.

Rifampicin is neither substrate analogue or inhibitor of DNA binding nor inhibitor of the formation of the phosphodiester bond[16]. The inhibition of high-molecular RNA synthesis is a constant feature of rifampicin action regardless whether this drug affects the formation of one or more phosphodiester bonds[6—15]. The process of short oligonucleotide synthesis contributes to the conversion of the initiation complex to the stable elongation one which is resistant towards the action of rifampicin[17].

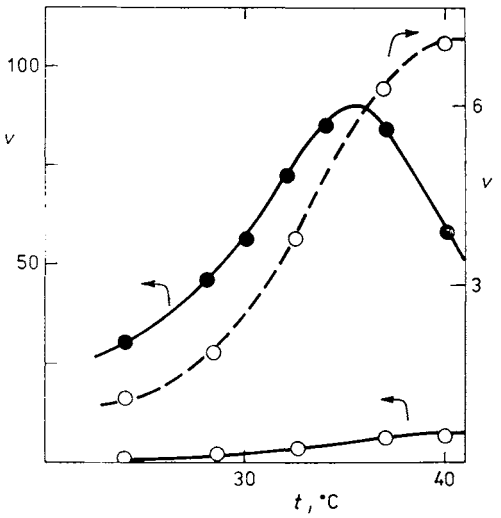

FIG. 1

Temperature dependence of initial velocity of ApUpA synthesis. The reactions (final volume of 15 μl) were run at standard salt conditions with 2 μg poly(dA-dT), 4·4 μg holoenzyme and 0·2 mmol l^{-1} ApU, 0·05 mmol l^{-1} [α^{32}P]ATP (2 . 10^3 Bq/nmol i.e. 10^5 CPM per assay) in the absence (○) and presence (●) of 5·5 . 10^{-3} mg/ml rifampicin; *v* initial velocity in pmol min^{-1}

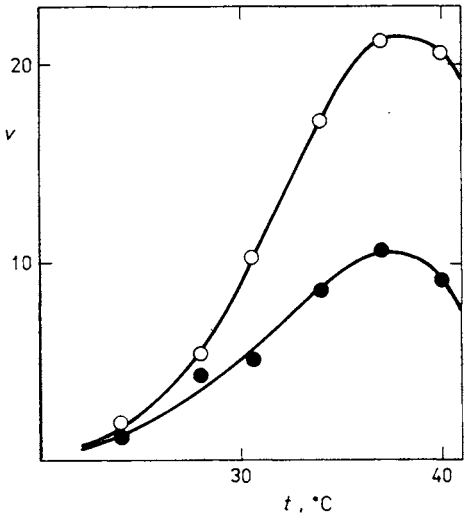

FIG. 2

Temperature dependence of initial velocity of UpApU synthesis. The reactions (final volume of 15 μl) were run at standard salt conditions with 1 μg poly(dA-dT), 0·5 μg holoenzyme and 0·05 mmol l^{-1} UpA, 0·015 mmol l^{-1} [α^{32}P]UTP (6·7 . 10^3 Bq/nmol i.e. 10^5 CPM per assay) in the absence (○) and presence (●) of 0·625 . 10^{-3} mg/ml rifampicin; *v* see legend of Fig. 1

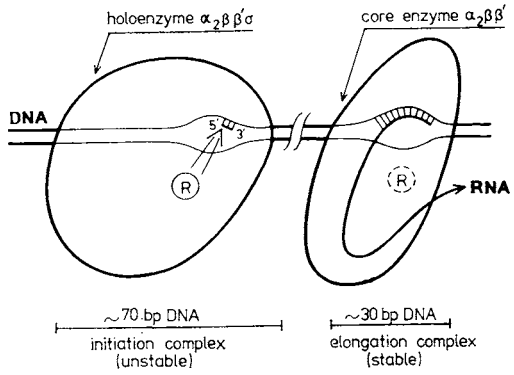

FIG. 3

Proposed model of the inhibition mechanism of rifampicin. Rifampicin R allosterically inhibits the conversion between the initiation to elongation complex which induces inhibitoin of high-molecular RNA synthesis

However, rifampicin affects the abortive synthesis of oligonucleotides in a different manner[6-9,11,13,15,18-20] and at least in some cases in a temperature dependent manner[7,12]. It seems necessary to separate the effects of rifampicin on abortive synthesis which by themselves are not capable to provoke the inhibition of high-molecular RNA synthesis from its key influence on RNA synthesis. We suggest a simple model of action of rifampicin which is in agreement with the majority of reported data.

We presume that the drug inhibits internal rearrangement of the initiation complex to the elongation one (Fig. 3). Besides this inhibitory effect on high-molecular RNA synthesis, rifampicin influences also the kinetic constants for abortive initiation. This side effect is responsible for the stimulation of ApUpA synthesis (Fig. 1); which is due to the decreased stability of the corresponding ternary complex[8,13,14]. The inhibition of UpApU synthesis (Fig. 2) is probably caused by its effect on binding of initiation dinucleotide to its binding site[7].

As to the localization of rifampicin binding site it has been established spectroscopically that rifampicin binds outside the catalytic centre of the RNA polymerase[14,21] at least 3·7 nm from the initiation binding site[21]. The conformational change of RNA polymerase in the presence of rifampicin has been confirmed fluorometrically[3,4,22].

The inhibitory effect of rifampicin is due to its influence of the dramatic structural, functional, and conformational change of RNA polymerase during the formation of a stable elongation complex[17] (Fig. 3). This complex is characterized by a decreased number of DNA base pairs involved in direct interaction between the enzyme and DNA[23]. The stability of this complex includes its resistance towards rifampicin[17].

At present the most important support for the proposed model is the detailed functional analysis of the formation of stable complexes on the lac UV5 promoter[23] and A_1 promoter in phage T7 (ref.[20]). During transcription of the lac UV5 promoter in presence of rifampicin only dinucleotides and trinucleotides are formed, although their synthesis is lower than in the absence of the drug[18]. However, if the synthesis of defined short oligonucleotides is allowed to proceed with subsequent addition of rifampicin and respective NTPs, the resistance towards rifampicin is completed only after the oligonucleotide chain has attained the length of 9–16 nucleotides[23].

The inhibitory action of rifampicin on RNA synthesis cannot be therefore described in terms of the kinetic constants for the formation of short oligonucleotides during the abortive and productive initiation but in terms of structural and functional properties of the complexes arising during the phase of stable elongation complex formation.

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