THE TWO EFFECTS OF RIFAMPICIN ON THE RNA POLYMERASE REACTION

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SUMMARY

At 25 °C rifampicin strongly stimulates the synthesis of the dinucleotide pppA-U catalyzed by the DNA-dependent RNA polymerase from Escherichia coli. If the antibiotic is added to the enzyme during the synthesis of RNA the stimulatory effect on the dinucleotide synthesis is distinctly retarded as is its inhibitory action on RNA synthesis. It is proposed that this lag period is due to a retardation of the binding of rifampicin to RNA polymerase which is required for its action. Because of this slower binding rifampicin - although an inhibitor of RNA chain elongation - mimics the action of an inhibitor of RNA chain initiation.

Recently Johnston and McClure (1) made the important observation that the DNA-dependent RNA polymerase (EC 2.7.7.6) from Escherichia coli is able to catalyze in a DNA-dependent reaction the synthesis of large amounts of dinucleotide tetraphosphate. This reaction takes place even when the enzyme has been preincubated with rifampicin (1). In the presence of the antibiotic none of the longer oligo- or polyribonucleotides are formed. This result strongly suggests that rifampicin acts via the inhibition of RNA chain elongation after formation of the first phosphodiester bond. It immediately rules out any strong inhibition by rifampicin of the various steps of initiation of RNA synthesis such as the formation of the highly stable complex between RNA polymerase and DNA, the association of the substrate with the binary complex or the formation of the first phosphodiester bond (2 - 5). On the other hand it has been well established that rifampicin has no immediate effect on RNA chain elongation whereas

the initiation of synthesis of polyribonucleotides is completely blocked (6, 7). The latter result led to the common use of rifampicin as a specific inhibitor of the initiation of RNA synthesis by bacterial RNA polymerase. To reconcile these apparently contradictory findings we have reinvestigated the effect of rifampicin on the formation of dinucleotides and on RNA synthesis. Emphasis was placed on the influence of temperature on the action of the antibiotic since the various steps of RNA synthesis show a different dependence on temperature.

MATERIALS AND METHODS

DNA-dependent RNA polymerase holoenzyme has been prepared by the procedure of Burgess and Jendrisak (8). The affinity chromatography on DNA-cellulose has been substituted by chromatography on DNA-agarose as decribed by Schaller et al (9). The final preparation contained about 0.8 mole subunit sigma per mole of core enzyme. The composition of the reaction mixture for the synthesis of RNA and dinucleotide tetraphosphate was except when otherwise stated as described by Kerrich-Santo and Hartmann (2).

RESULTS

At 37 °C the synthesis of the dinucleotide pppA-U starts immediately when ATP and UTP are added to a preincubated mixture of RNA polymerase and an excess of T4 bacteriophage DNA. In agreement with the findings of Johnston and McClure (1) the rate of dinucleotide formation is reduced by 30 % if the enzyme had been preincubated with 2.4 μM rifampicin (Fig. 1). A shift of the temperature of incubation from 37 °C to 25 °C leads to a surprising result. At 25 °C the rate of synthesis of pppA-U is rather slow in the absence of the antibiotic. Upon preincubation of the enzyme with rifampicin, however, the rate of formation shows an eightfold increase (Fig. 1). Obviously rifampicin bound to the enzyme does not inhibit dinucleotide synthesis at this temperature. On the contrary, it acts as an efficient stimulant. The molecular

TABLE 2

SPECIFIC ACTIVITY OF ALKALINE PHOSPHATASE CALCULATED ON AMOUNT OF ALKALINE PHOSPHATASE PROTEIN IN GEL SCAN

Position in Cell Cycle	Area Under Alkaline Phosphatase Peak In Gel Scan*	Specific Activity of Plasma Membrane Alkaline Phosphatase	Specific Activity Alkaline Phosphatase/Area Under Alkaline Phosphatase Peak in Gel Scan
M	2.0	7.2	2.4
S	11.0	2.3	0.2
G_1	1.0	2.2	2.2

* Normalized by setting area under alkaline phosphatase peak in the G_1 phase gel scan equal to one

There is no significant difference in alkaline phosphatase activity among the M, G_1, and S cytosol fractions, or among the nuclear fractions. Furthermore, there is no significant difference ($P<0.2$) in alkaline phosphatase activity when the cytosol fractions from M, G_1, and S are compared to the corresponding nuclear fractions.

The next question was whether the increase in mitotic plasma membrane alkaline phosphatase activity was due to an increased amount of alkaline phosphatase protein in the membrane or whether it was due to modification of this protein near M, resulting in increased intrinsic activity. SDS polyacrylamide disc gel electrophoresis scanning patterns provided the answer. Figure 1 shows scanning patterns of 10% gels loaded with equal amounts of SDS solubilized plasma membrane proteins from either M, G_1, or S phase cells. Two cells for each phase were analyzed concurrently. One from each set was stained histochemically to locate the alkaline phosphatase activity, whereas the duplicate gel was stained with Coomassie Blue for protein. From the gel patterns, it can be seen that there was much more alkaline phosphatase protein in the S phase plasma membrane than in the G_1 and M phase plasma membranes (Fig. 1). The area under each alkaline phosphatase peak was calculated. Table 2 shows that the S phase plasma membrane contained 5.5 times as much alkaline phosphatase protein as the M phase (11/2) and 11 times that in the G_1 phase plasma membrane (11/1). The M phase plasma membrane contained two times as much

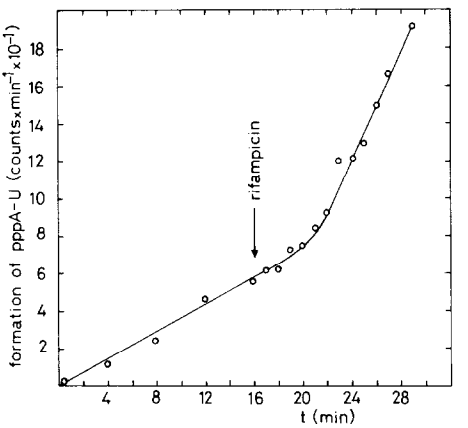

Fig. 2. Stimulation of synthesis of the dinucleotide pppA-U at 25 °C by addition of rifampicin. The experimental conditions were the same as described in Fig. 1 for the incubation without rifampicin except that rifampicin (2 μg/ml) was added 16 min after the start of pppA-U synthesis.

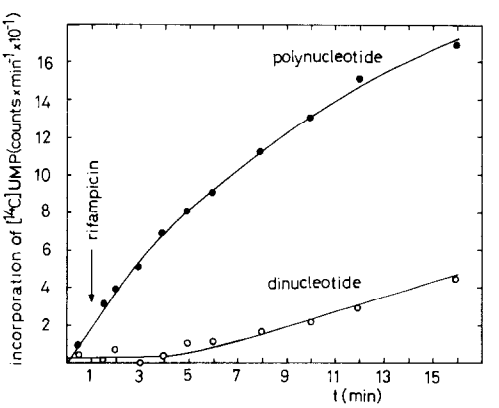

Fig. 3. Delay of action of rifampicin when added to an incubation mixture synthesizing RNA. The experimental conditions were the same as described in Fig. 1 for the incubation without rifampicin except that 1.2 mM of each CTP and GTP were present in addition to ATP and [^{14}C]UTP. 1 min after start of RNA synthesis rifampicin (2 μg/ml) was added. The polynucleotide formed was measured by the radioactivity remaining on the starting line of the chromatogram.

teresting to note, however, that at first the rate continues unchanged for almost 4 min before the higher catalytic activity of the complex between enzyme and antibiotic is observed. To exclude the possibility that the distinct delay of action is due to the

particular use of ATP and UTP as sole substrate the experiment was repeated using all four ribonucleoside triphosphates (Fig. 3). At the beginning of the incubation, when rifampicin is still absent, almost no dinucleotides are formed whereas polyribonucleotides are synthesized. This has to be expected in the presence of a complete set of substrates. After addition of rifampicin the synthesis of polyribonucleotides continues unchanged for 4 min in agreement with the earlier observation of the delayed effect of rifampicin on RNA chain elongation (6). Simultaneously dinucleotide formation does not increase. After the lag period the rate of synthesis of polyribonucleotides decreases significantly. At the same time dinucleotide formation rises (Fig. 3). Since a portion of the RNA polymerase molecules is still involved in RNA chain elongation a smaller rate of dinucleotide formation is found than that observed after preincubation of the enzyme with antibiotic when no polynucleotides can be made. Analogous observations are made when rifampicin, together with the substrate, is added to a preincubated mixture of RNA polymerase and DNA (data not shown).

DISCUSSION

After an incubation of RNA polymerase with rifampicin for less than 5 s no catalytic activity is observed (2). Why, then, is the action of rifampicin so slow on RNA polymerase already engaged in catalytic activity? The direct correlation between the inhibition of RNA polymerase and the binding of rifampicin to it points to the binding as being a prerequisite for the inhibitory action (10). If so, the lag period in the action of the antibiotic (Figs. 2 and 3) may be caused by a reduction of the rate of binding of rifampicin to the enzyme. This hypothesis is strongly supported by recent measurements of the rate constants of the association of

rifampicin with RNA polymerase (11). At 37°C DNA reduces the bimolecular rate constant from 1.2×10^6 M^{-1} s^{-1} to 3×10^3 M^{-1} s^{-1}, suggesting a strong protecting effect of the template on RNA polymerase against the attack of the antibiotic. Possibly the reaction product and the substrate increases this effect. This hypothesis easily explains the apparently contradictory observations mentioned in the introduction. Accordingly, the mode of action of rifampicin may be described as follows: by binding to RNA polymerase rifampicin acts as a powerful inhibitor of RNA chain elongation. After its binding to the enzyme oligo- and polyribonucleotides can no longer be formed. The presence of DNA and, possibly, of other components of the reaction, drastically reduces the rate of binding of the antibiotic to the enzyme. Therefore at low concentration rifampicin acts much more slowly on RNA polymerase engaged in catalytic activity than on free enzyme. This leads to the apparent mode of action of rifampicin as an inhibitor of RNA chain initiation (6). At high concentrations rifampicin overcomes the protecting effect of DNA and the other components of the reaction on RNA polymerase due to the concentration dependence of the rate of the bimolecular reaction of association. A rapid inhibition of the RNA chain elongation is the result (12, 13).

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