Vol. 98, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1981 Pages 261-267

# ON THE INHIBITION OF YEAST RNA POLYMERASES A AND B BY tRNA AND α-AMANITIN.

Michèle Sawadogo

Service de Biochimie, Département de Biologie,
C.E.N. - Saclay, 91191 Gif-sur-Yvette, Cedex, France.

Received November 17,1980

SUMMARY : Like α-amanitin, tRNA binds reversibly to yeast RNA polymerases A or B, in a 1:1 stoechiometry and inhibits enzyme B preferentially. Kinetic studies showed that the binding sites for the two inhibitors are completely independent. The dissociation constant of the enzyme-inhibitor complexes was determined. Only in the case of tRNA, it varied with the nature and concentration of the template. The stimulation factor $P_{37}$ does not interfere with the binding of both inhibitors.

## INTRODUCTION

The three different forms of RNA polymerases in the eukaryotic cell can be distinguished by their sensitivity to α-amanitin. Studies with mammalian RNA polymerase B, which is most sensitive (1, 2) showed that the toxin inhibits RNA chain elongation (2), by binding in a 1:1 stoechiometry (3) to the second largest subunit of the enzyme (4). In yeast, enzyme B is less sensitive (5) and, at variance with the mammalian enzyme, enzyme A can be inhibited by high concentrations of the toxin (6). The removal of two subunits enhances its sensitivity to the inhibitor (6). The mechanism by which α-amanitin blocks RNA chain elongation is not known. While studying the inhibition of yeast RNA polymerases A and B by different compounds, I found a striking analogy in the response of the enzymes to α-amanitin and tRNA. Therefore I investigated the possibility that tRNA and α-amanitin, although very different in chemical structure, could bind at the same site on RNA polymerase.

0006-291X/81/010261-07$01.00/0

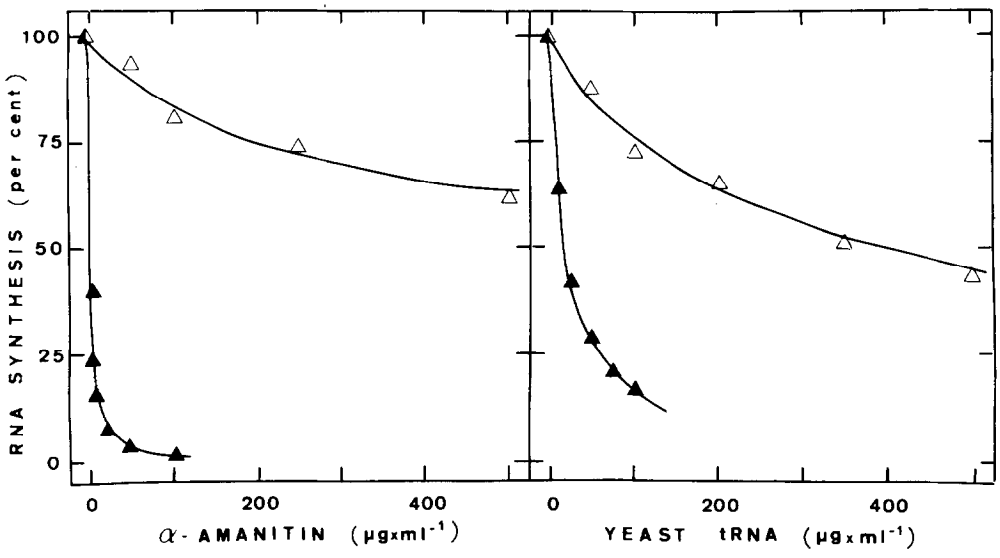


Figure 1 : Inhibition of yeast RNA polymerases A and B by tRNA and α-amanitin.
Standard reaction mixtures (100 µl) contained α-amanitin and yeast tRNA as indicated. RNA synthesis, was initiated by the addition of yeast RNA polymerase A (Δ-Δ) or B (▲-▲) at the final concentration of 10 µg/ml, and measured after 15 min incubation at 30°C. Control [$^3$H] UMP incorporation in the absence of inhibitor was 0.26 and 0.47 nmole with RNA polymerases A and B, respectively.

## MATERIAL AND METHODS

Standard transcription mixtures (100 µl) for RNA polymerase A contained 70 mM Tris-HCl pH 8, 5 mM $MgCl_2$, 1 mM $MnCl_2$ 1 mM dithiothreitol, 1 mM each of ATP, GTP and CTP, 0.5 mM [$^3$H] UTP (20 cpm/pmol) and 2.5 µg of alkali-denatured calf thymus DNA. For RNA polymerase B, the conditions were : 70 mM Tris-HCl pH 8, 2 mM $MnCl_2$, 1 mM dithiothreitol, 25 mM ammonium sulfate, 0.4 mM each of ATP, GTP and CTP, 0.2 mM [$^3$H] UTP (20 cpm/pmol) and 2.5 µg of denatured calf thymus DNA. Yeast tRNA and α-amanitin were added as indicated. Transcriptions were initiated, at 30°C, by the addition of 1 µg of RNA polymerase. After 15 min of incubation at 30°C the RNA synthesized was collected by the usual acid-precipitation technique.

Yeast RNA polymerase A (6), RNA polymerase B and $P_{37}$ factor (7), were prepared as previously described. Yeast tRNA and α-amanitin were purchased from Boehringer (Mannheim).

## RESULTS

The inhibition of RNA polymerases A and B by tRNA and α-amanitin is shown in Fig. 1. Remarkably, in both cases, the B enzyme was much more sensitive to the inhibitor than enzyme A,

and the dose response curves for the two inhibitors presented striking analogies. Therefore I investigated the possibility that tRNA and α-amanitin, although very different in chemical structure, would bind to RNA polymerases at the same site. Assuming that one molecule of inhibitor (I) binds reversibly to each RNA polymerase molecule (E), the complex being inactive, which has been shown to be the case for the complex of α-amanitin and calf thymus RNA polymerase B (3), then the dissociation constant K of the enzyme-inhibitor complex (EI) is given by equation (1) (below), provided that $I_o$, the initial inhibitor concentration, is very much higher than the enzyme concentration. Under conditions where RNA synthesis (S) is proportional to the RNA polymerase concentration, equation (2) is obtained, where $S_o$ is the RNA synthesis in the absence of inhibitor.

$$K = \frac{[E] \cdot I_o}{[EI]} \quad (1) \qquad \frac{S_o}{S} = 1 + \frac{I_o}{K} \quad (2)$$

Plotting the results of Fig. 1 according to equation (2) thus gives an estimate for the dissociation constant of α-amanitin for yeast RNA polymerase A of $8.6\times10^{-4}$ M. For yeast RNA polymerase B, the value obtained was $1.1\times10^{-6}$ M, which was in good agreement with the results of Dezélée et al. (5) and Schultz and Hall (8) indicating that yeast RNA polymerase B is $10^3$ times less sensitive to the amatoxin than mammalian RNA polymerases B (Fig. 2).

Similar linear curves were also obtained when the same graphic representation was applied to the inhibition data by yeast tRNA. Therefore, tRNA also appears to act by binding reversibly, in a 1:1 stoechiometry, to RNA polymerase A or B. To investigate whether the two inhibitors bound to the RNA polymerase at the same site, transcriptions were performed in the presence of va-

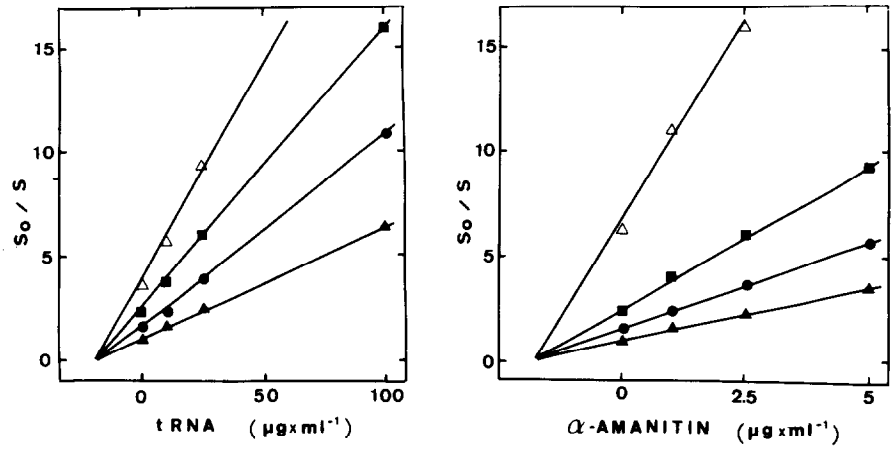


Figure 2 : Combined inhibition of yeast RNA polymerase B by α-amanitin and tRNA.
Transcription mixtures (100 µl) contained 10 µg/ml of yeast RNA polymerase B and varying concentrations of the inhibitors. Incubation time was 15 min. The results are plotted following equation 3. Left pannel : Effect of increasing concentrations of tRNA in the absence (▲-▲) or presence of α-amanitin at the concentration of 1 µg/ml (●-●), 2.5 µg/ml (■-■) or 5 µg/ml (Δ-Δ). Right pannel : Effect of increasing concentrations of α-amanitin in the absence (▲-▲) or presence of tRNA at the concentration of 10 µg/ml (●-●), 25 µg/ml (■-■) or 100 µg/ml (Δ-Δ).

rying concentrations of both compounds (Fig. 2). Indeed, in the case of two inhibitors ($I_1$ and $I_2$) added simultaneously, it is possible to derive equation (3) if the two inhibitor binding sites are totally independent, and equation (4), if the two inhibitors bind to the same enzyme site.

$$\frac{S_o}{S} = 1 + \frac{I_1}{K_1} + \frac{I_2}{K_2} + \frac{I_1 \cdot I_2}{K_1 \cdot K_2} \quad (3) \qquad \frac{S_o}{S} = 1 + \frac{I_1}{K_1} + \frac{I_2}{K_2} \quad (4)$$

Intermediate cases, where the binding of one inhibitor would modify the affinity of the enzyme for a second inhibitor binding at a different site, are also possible. However, in the case of tRNA and α-amanitin on yeast RNA polymerase B, the results were in complete agreement with the theoretical situation described by equation (3) (Fig. 2). Therefore, the binding sites of the two inhibitors are different. The binding of one inhibitor has

no effect on the affinity of the RNA polymerase for the second one.

This conclusion was reinforced by studying the effect of increasing concentrations of DNA on the inhibition. The nature and the concentration of the template were without effect on the measured dissociation constant for α-amanitin (not shown), a result in accordance with those of Lindell et al. (1) and Cochet-Meilhac and Chambon (3) who found a similar binding of the toxin to free or DNA bound mammalian RNA polymerase B. On the contrary, the apparent dissociation constant for tRNA was found to be smaller when native DNA, instead of denatured DNA, was used as template. This suggested the existence of a competition between tRNA and DNA at the level of the template binding site of RNA polymerase B, since the affinity of the yeast enzyme B for denatured DNA is much higher than its affinity for native DNA (9). Accordingly, the apparent dissociation constant of tRNA for RNA polymerase B increases linearly as a function of the denatured DNA concentration (Fig. 3). By extrapolating the curve to zero, one can estimate the true dissociation constant of tRNA for yeast RNA polymerase B to be 3.7 µg/ml ($1.5\times10^{-7}$ M).

Recently, the yeast protein $P_{37}$ was shown to interact with RNA polymerase B and stimulate RNA synthesis in vitro (7). Under conditions where RNA polymerase B is complexed with $P_{37}$, the sensitivity of the enzyme to α-amanitin remained unchanged (result not shown). On the other hand, there was an apparent increase in its affinity for tRNA, instead of a decrease as expected if the two effectors competed for the same site. The explanation could be that tRNA binds $P_{37}$, thereby preventing the stimulation of RNA synthesis by the factor.

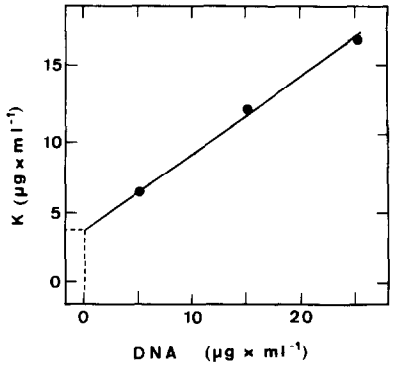


Figure 3 : Determination of the dissociation constant of tRNA for yeast RNA polymerase B.
The apparent dissociation constants of tRNA for yeast RNA polymerase B were determined at different concentrations of denatured DNA template, using the graphic representation shown in Fig. 2, then plotted as a function of DNA concentration. This representation allows one to estimate the true dissociation constant at zero DNA concentration.

## DISCUSSION

The kinetic studies show a striking analogy in the response of RNA polymerases A and B to α-amanitin and tRNA. In both cases the B enzyme is the most sensitive to the inhibitor. The mechanism of inhibition by tRNA is not known but one could imagine that it binds to the RNA product site or to the template site on the enzymes. On the other hand, α-amanitin is an inhibitor of RNA chain elongation and there is the possibility that the toxin binds close to the RNA product site, thereby preventing the translocation process. These considerations led me to investigate whether the two inhibitors compete for the same binding site. By means of simple kinetic studies, this hypothesis was not substantiated. On the contrary, the results argue in favor of completely independent binding sites for the two inhibitors. This conclusion was further reinforced by the fact that tRNA, but not α-amanitin, interferes with the binding of the template.

Another effector which influences the *in vitro* activity of yeast RNA polymerases A and B is the yeast protein $P_{37}$. This

protein probably binds at the level of subunit $AB_{23}$ which is one of the common polypeptides of A and B enzymes (10). Since $P_{37}$ stimulates RNA chain elongation (M. Sawadogo, unpublished result) it was of interest to see whether it interfered or not with the inhibition by α-amanitin or tRNA. In fact, the inhibition remained unchanged when RNA polymerase B was complexed with $P_{37}$.

All these results indicate that the yeast RNA polymerases can bind, at the same time, to at least three different effectors producing independent effects. This finding could well reflect the complex regulation for which the RNA polymerases behave as target in the nucleus.

REFERENCES

(1) Lindell, T.J., Weinberg, F., Morris, P.W., Roeder, R.G. and Rutter, W.J. (1970) Science, 170, 447-449.
(2) Kedinger, C., Gniazdowski, M., Mandel, J.L., Gissinger, F., and Chambon, P. (1970) Biochem. Biophys. Res. Commun. 38, 165-171.
(3) Cochet-Meilhac, M. and Chambon, P. (1974) Biochim. Biophys. Acta 353, 160-184.
(4) Brodner, O.G. and Wieland, T. (1976) Biochemistry 15, 3480-3484.
(5) Dezélée, S., Sentenac, A. and Fromageot, P. (1970) FEBS lett. 7, 220-222.
(6) Huet, J., Buhler, J.M., Sentenac, A. and Fromageot, P. (1975) Proc. Natl. Acad. Sci. USA 72, 3034-3038.
(7) Sawadogo, M., Sentenac, A. and Fromageot, P. (1980) J. Biol. Chem. 255, 12-15.
(8) Schultz, L.D. and Hall, B.D. (1976) Proc. Nat. Acad. Sci. USA 73, 1029-1033.
(9) Dezélée, S., Sentenac, A. and Fromageot, P. (1972) FEBS lett. 21, 1-6.
(10) Sawadogo, M., Huet, J. and Fromageot, P. (1980) Biochem. Biophys. Res. Commun. 96, 258-264.