SELECTIVE INHIBITION BY ZINC OF RNA SYNTHESIS INITIATION IN THE RNA POLYMERASE I REACTION

Yoshikuni NAGAMINE, Den'ichi MIZUNO and Shunji NATORI
Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received 10 November 1978
Revised version received 25 December 1978

1. Introduction

RNA polymerase of both eukaryotic and prokaryotic cells is known to contain tightly bound zinc [1–5]. Removal of this zinc from eukaryotic RNA polymerase I or II by treatment with chelating agents resulted in almost complete loss of catalytic activity [6]. The zinc in *E. coli* RNA polymerase was found to be combined with the β' subunit [7] and on its replacement by cobalt, transcription of the lactose operon in vitro became less sensitive to cyclic AMP and catabolite gene activator protein [8]. Thus, zinc is an intrinsic component of RNA polymerase and may have essential roles in the regulation of transcription.

We have reported a convenient method for measuring the effects of divalent cations on the initiation and elongation steps of RNA synthesis separately [9]. In the present work we used this method to examine the effect of externally added zinc on the RNA polymerase I reaction in vitro, finding that externally added zinc selectively inhibits the initiation of RNA synthesis but has no effect on elongation of the RNA chain.

2. Materials and methods

2.1. *Preparation and assay of RNA polymerase I*

RNA polymerase I was partially purified from the nucleoli of Ehrlich ascites tumor cells by the method in [10]. The enzyme was stable when kept at −80°C in the presence of 50% glycerol and was completely insensitive to 40 μg/ml of α-amanitin. The specific activity of the enzyme was 2000 units/mg protein, when 1 unit of enzyme was defined as the amount catalyzing incorporation of 1 pmol UMP in 1 min under standard assay conditions in the presence of 1 mM $MnCl_2$. The standard assay mixture contained, in 0.25 ml total vol.: 40 mM Tris–HCl (pH 7.9); 0.25 mM ATP, GTP and CTP; 0.025 mM UTP; 1 μCi [^{3}H]UTP (20 Ci/mmol); 50 mM $(NH_4)_2SO_4$; 2 μg/ml of α-amanitin; 10% glycerol; 20 μg/ml Ehrlich ascites tumor DNA, and RNA polymerase I. After incubation for 5–10 min at 37°C, the radioactivity incorporated into the acid-insoluble fraction was measured.

2.2. *Assay of the effect of $ZnSO_4$ on the initiation and elongation of RNA synthesis*

As reported before, $MnCl_2$ is a better effector than $MgCl_2$ for the initiation step of RNA synthesis and either $MnCl_2$ or $MgCl_2$ is necessary for elongation of the RNA chain [9]. In studies on the effect of zinc on elongation of the RNA chain, RNA synthesis was initiated in the presence of $MnCl_2$; after 5 min preincubation, AF/013(*O*-octyloxime of 3-formylrifamycin SV), various concentrations of $ZnSO_4$ and [^{3}H]UTP were added to the reaction mixture, the concentration of $MnCl_2$ or $MgCl_2$ was adjusted to the optimum for RNA chain elongation, and incorporation of [^{3}H]UTP into RNA in 5 min was measured. In studies on the effect of zinc on the initiation of RNA synthesis, RNA synthesis was initiated in the presence of either $MnCl_2$ or $MgCl_2$ and various concentrations of $ZnSO_4$; after 5 min preincubation, the concentration of $ZnSO_4$ was adjusted to 0.08 mM, which does not affect RNA chain elongation and

that of $MnCl_2$ or $MgCl_2$ was adjusted to the optimum for RNA chain elongation; then AF/013 and [^{3}H]UTP were added to the reaction mixture and incorporation of [^{3}H]UTP into RNA in 5 min was measured. The details of the experiments are given in fig.2,3 legends.

3. Results

Addition of zinc to the mixture for assay of RNA polymerase I markedly inhibited RNA synthesis. Either 1 mM $MnCl_2$ or 4.8 mM $MgCl_2$ is known to be necessary for optimum RNA synthesis [9]. As shown in fig.1, $ZnSO_4$ inhibited RNA synthesis in the presence of both these divalent cations, though the inhibition was usually greater in the presence of $MgCl_2$ than in the presence of $MnCl_2$.

The reaction of RNA polymerase I consists of three distinct steps, namely initiation, elongation and termination. Thus there are at least three possible ways in which zinc could be inhibitory: by reduction of the initiation frequency, decrease in the elongation rate, or increase in the termination frequency. We have developed a convenient method for examining the effects of divalent cations on the initiation and elongation steps separately [9]. Using this method we examined the effect of zinc on the elongation and initiation steps of the RNA polymerase I reaction. When zinc was added to the reaction mixture after RNA synthesis had been initiated, it had little effect on RNA synthesis irrespective of the divalent cations in the medium, as shown in fig.2. The result indicates that zinc did not inhibit elongation of the RNA chain and also that it did not enhance the frequency of termination. Thus, its inhibitory effect on over-all RNA synthesis must be on the initiation step.

Next, the effect of zinc on the initiation of RNA synthesis was examined. As shown in fig.3, addition

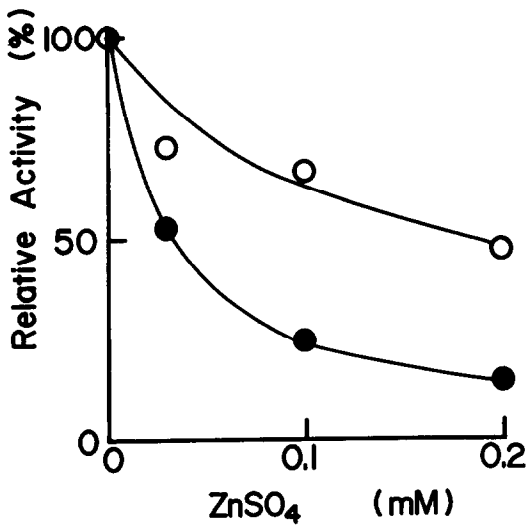

Fig.1. Effect of $ZnSO_4$ on over-all RNA synthesis. RNA synthesis was achieved with 12 units of partially-purified RNA polymerase I in the presence of 4.8 mM $MgCl_2$ (•) or 1 mM $MnCl_2$ (○) and various amounts of $ZnSO_4$ under the standard conditions. The reaction was terminated after incubation for 5 min. The amounts of UMP incorporated in the absence of $ZnSO_4$ were 70 pmol with $MgCl_2$ and 120 pmol with $MnCl_2$.

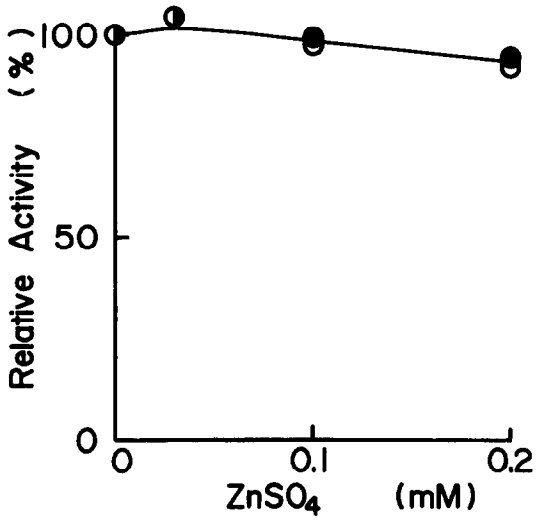

Fig.2. Effect of $ZnSO_4$ on RNA elongation. RNA synthesis was initiated using 12 units of partially-purified RNA polymerase I and 40 μg/ml of DNA in the presence of 0.5 mM $MnCl_2$ in 50 μl standard reaction mixture without [^{3}H]UTP. After 5 min preincubation at 37°C, the mixture was diluted 5-fold without changing the concentrations of any of the ingredients except $MnCl_2$, RNA polymerase I and DNA. $MnCl_2$ and $MgCl_2$ were adjusted to 1 mM and 4.8 mM, respectively, and then 10 μg/ml of AF/013, [^{3}H]UTP and various concentrations of $ZnSO_4$ were added. The incorporation of [^{3}H]UTP into RNA during further incubation for 5 min at 37°C was measured. The incorporations (100%) with $MnCl_2$ and $MgCl_2$ were 46 pmol and 48 pmol, respectively. (○——○) Effect of $ZnSO_4$ with 0.5 mM $MnCl_2$; (•——•) effect of $ZnSO_4$ with 4.8 mM $MgCl_2$.

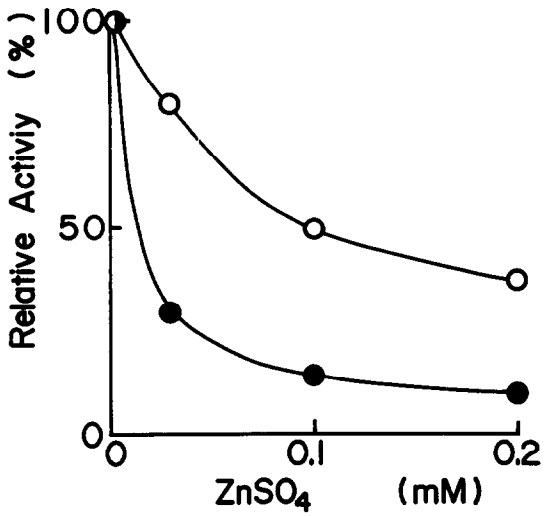

Fig.3. Effect of $ZnSO_4$ on initiation of RNA synthesis. 12 units of partially-purified RNA polymerase I was incubated in the presence of 4.8 mM $MgCl_2$ or 1.25 mM $MnCl_2$ and various concentrations of $ZnSO_4$ in 100 μl of standard reaction mixture without [^{3}H]UTP. After 5 min preincubation at 37°C, the reaction mixture was diluted 2.5-fold without changing the concentrations of any of the ingredients except divalent cations and RNA polymerase I. The concentrations of $MgCl_2$ and $MnCl_2$ were adjusted to 4.8 mM and 0.5 mM, respectively, and that of $ZnSO_4$ was adjusted to 0.08 mM, a concentration that did not affect chain elongation. Then 10 μg/ml of AF/013 and [^{3}H]UTP was added and the incorporation of [^{3}H]UTP into RNA during further incubation for 5 min at 37°C was measured. The incorporations (100%) in the presence of $MnCl_2$ and $MgCl_2$ were 75 pmol and 15 pmol, respectively. (○——○) Effect of $ZnSO_4$ with 1.25 mM $MnCl_2$; (●——●) effect of $ZnSO_4$ with 4.8 mM $MgCl_2$.

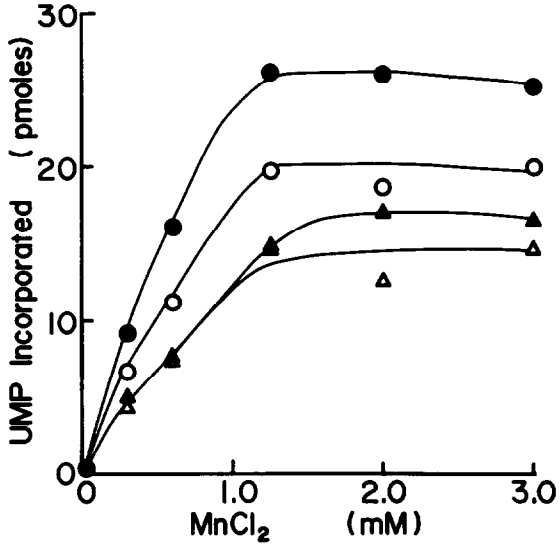

Fig.4. Effect of $MnCl_2$ on the inhibitory effect of $ZnSO_4$. RNA synthesis was initiated in the presence of increasing amounts of $MnCl_2$ with a fixed amount of $ZnSO_4$. After 1 min preincubation, the reaction mixture was diluted 5-fold without changing the concentrations of any of the ingredients except divalent cations and RNA polymerase I. $MnCl_2$ and $ZnSO_4$ were adjusted to 0.6 mM and 0.04 mM, respectively, and then 10 μg/ml of AF/013 and [^{3}H]UTP into RNA during further incubation for 5 min at 37°C was measured. Concentration of $ZnSO_4$: (●) 0 mM; (○) 0.06 mM; (▲) 0.12 mM; (△) 0.2 mM.

of $ZnSO_4$ at the initiation step greatly decreased the amount of RNA polymerase I engaged in RNA synthesis. $ZnSO_2$ inhibited initiation more in the presence of $MgCl_2$ than in the presence of $MnCl_2$, which is consistent with its inhibitory effect on over-all RNA synthesis with these divalent cations shown in fig.1. Thus, zinc seems to reduce the frequency of initiation, resulting in apparent inhibition of over-all RNA synthesis.

Zinc might inhibit the initiation step of RNA synthesis by competing with $MnCl_2$ or $MgCl_2$ for effector sites on RNA polymerase I. However, this possibility was excluded by the finding that increasing amounts of $MnCl_2$, which is a better effector in initiation than $MgCl_2$, did not prevent the inhibitory effect of $ZnSO_4$, as shown in fig.4.

$ZnSO_4$ was reported to enhance the melting of double stranded DNA [11]. The inhibitory effect of zinc might be due to increase in the single strand region on template DNA, where an abortive complex could be formed. However, this is unlikely because, as shown in fig.5, the inhibitory effect of zinc on over-all RNA synthesis was detected with both native and heat denatured DNA as template.

4. Discussion

This paper reports evidence that zinc selectively inhibits the initiation step of RNA synthesis catalyzed by RNA polymerase I. This inhibition by zinc depends on the presence of divalent cations as effectors of initiation. Zinc caused > 80% inhibition with

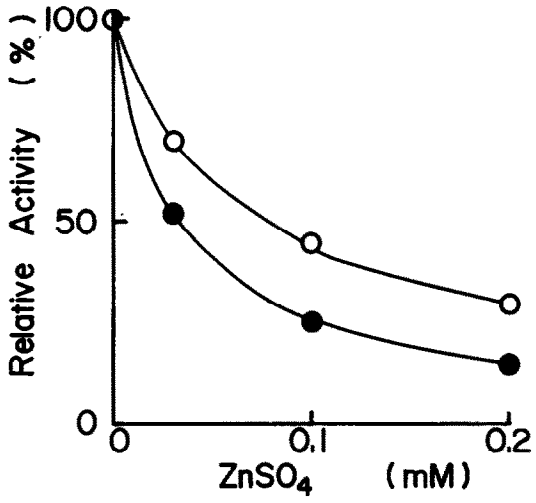

Fig.5. Effect of $ZnSO_4$ on RNA synthesis with native and denatured DNA. RNA synthesis was carried out using 12 units of partially-purified RNA polymerase I in the presence of 4.8 mM $MgCl_2$ and various concentrations of $ZnSO_4$ with native DNA (○) or heat-denatured DNA (●). The reaction was terminated after incubation for 5 min at 37°C. The incorporations of UMP (100%) were 70 pmol and 28 pmol, respectively, with native and heat-denatured DNA.

$MgCl_2$ as effector, but much less inhibition with $MnCl_2$, which is better effector of initiation than $MgCl_2$. This selective and almost complete inhibition of RNA synthesis in the presence of $MgCl_2$ indicates that zinc could be used to discriminate initiated and uninitiated complexes in the RNA polymerase I reaction.

The inhibitory effect of zinc was not due to its competition with manganese (or possibly magnesium) for the effector site, because increasing amounts of $MnCl_2$ did not prevent the inhibitory effect of a fixed amount of $ZnSO_4$. Moreover since it was found that zinc inhibited RNA synthesis irrespective of the nature of the template DNA, its inhibitory effect was probably not due to its interaction with DNA. Thus the inhibition may be due to the interaction of zinc with RNA polymerase I itself.

RNA polymerase I consists of several subunits, and it is uncertain which subunit is the target for externally added zinc. However, a catalytic subunit can be excluded, because zinc did not change the elongation rate of the RNA chain when added to reaction mixture in which RNA synthesis had already started. Since the assay of RNA chain initiation reported here reflects the efficiency of RNA polymerase I binding to template DNA, the target of zinc is likely to be a subunit that is involved in template-binding, such as β' of *E. coli* RNA polymerase [12].

Acknowledgements

We thank Dr S. Mitsuhashi, Gunma University and Dr T. Oda, Okayama University, for kindly providing AF/013. This work was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (no. 258055).

References

[1] Scrutton, M. C., Wu, C. W. and Goldthwart, D. A. (1971) Proc. Natl. Acad. Sci. USA 68, 2497–2501.
[2] Halling, S. M., Sanchez-Anzaldo, F. J., Fukuda, R., Doi, R. H. and Meares, C. F. (1977) Biochemistry 16, 2880–2884.
[3] Auld, D. S., Atsuya, I., Campino, C. and Valenzuela, P. (1976) Biochem. Biophys. Res. Commun. 69, 548–554.
[4] Falchuk, K. H., Mazus, B., Ulpino, L. and Vallee, B. L. (1976) Biochemistry 15, 4468–4475.
[5] Wandzilak, T. W. and Benson, R. W. (1978) Biochemistry 17, 426–431.
[6] Valenzuela, P., Morris, R. W., Faras, A., Levinson, W. and Rutter, W. J. (1973) Biochem. Biophys. Res. Commun. 53, 1036–1041.
[7] Wu, C.-W., Speckhard, D. C. and Wu, F. Y.-H. (1977) Fed. Proc. Fed. Am. Soc. Exp. Biol. 36, 882 abstr.
[8] Speckhard, D. C., Wu, F. Y.-H. and Wu, C.-W. (1977) Biochemistry 16, 5228–5234.
[9] Nagamine, Y., Mizuno, D. and Natori, S. (1978) Biochim. Biophys. Acta 519, 440–446.
[10] Roeder, R. G. and Rutter, W. J. (1970) Proc. Natl. Acad. Sci. USA 65, 675–682.
[11] Hossenlopp, D. C., Oudet, P. and Chambon, P. (1974) Eur. J. Biochem. 41, 397–411.
[12] Zillig, W., Palm, P. and Heil, A. (1976) in: RNA polymerase (Losick, R. and Chamberlin, M. eds) pp. 101–125, Cold Spring Harbor Laboratory, New York.