ARE ALL NUCLEOTIDYL TRANSFERASES METALLOENZYMES?

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SUMMARY

Previous studies (1,2) have shown that DNA polymerases (sea urchin and E. coli I) and E. coli RNA polymerases are zinc metalloproteins. We report here that DNA-dependent RNA polymerases I and II from rat liver, I, II and III from sea urchin,and RNA-dependent DNA polymerase (reverse transcriptase) from Rous sarcoma virus are inhibited by pre-incubation with the chelating agent, o-phenanthroline. These enzymes are not affected by pre-incubation with the related compound, m-phenanthroline, which is not a metal chelating agent. The data suggest that, in addition to Mg^{++} or Mn^{++}, the enzymes require a tightly bound metal ion (possibly zinc) for catalytic activity. The DNA-terminal transferase is also inhibited by o-phenanthroline but not m-phenanthroline (3). On the other hand, we found that tRNA-nucleotidyl transferases from Rous sarcoma virions and yeast are not affected by either o- or m-phenanthroline. These observations suggest that many but not all nucleotidyl transferases are metalloenzymes.

Chang and Bollum (3) reported that DNA-terminal nucleotidyl transferase was inhibited by the metal chelating agent o-phenanthroline, and they raised the possibility that a tightly bound divalent metal ion might be involved in nucleotide polymerizing reactions. Subsequently, Slater et al (1) demonstrated that the DNA polymerase and the nuclear RNA polymerase from sea urchins (S. franciscanus) were inhibited by o-phenanthroline but were unaffected by the related compound, m-phenanthroline, which is not a metal chelator. In addition, these workers demonstrated that the E. coli enzyme contains approximately 2 gram-atoms zinc per molecule and the sea urchin enzyme contains approximately 4 gram-atoms of zinc per molecule. More recently, Scrutton et al (2) have shown that the E. coli DNA dependent RNA polymerase is also specifically inhibited by o- but not m-phenanthroline and contains approximately 2 gram-atoms of zinc per molecule.

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In this paper, we have investigated the effects of o- and m-phenanthroline on the activity of the DNA dependent RNA polymerases from rat liver and sea urchin and the RNA dependent DNA polymerase from Rous sarcoma virus (RSV). The inhibition,which we observed,of the polymerases by o-phenanthroline suggests that a divalent metal ion (perhaps zinc) is required for enzymatic activity. In contrast, the tRNA-nucleotidyl transferase from RSV virions and yeast are unaffected by o- or m-phenanthroline. This experiment gives no evidence for, but does not rule out, divalent ion requirement in these enzymes as well.

The effects of o- and m-phenanthroline on the activity of RNA polymerase are shown in Figure 1 (rat liver RNA polymerases) and Figure 2 (sea urchin

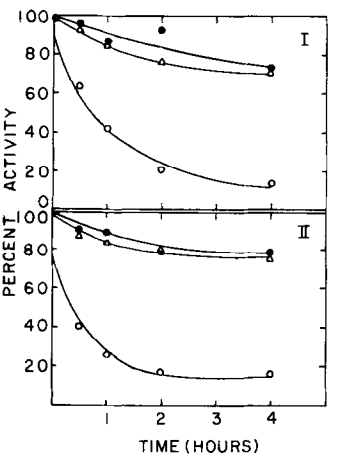
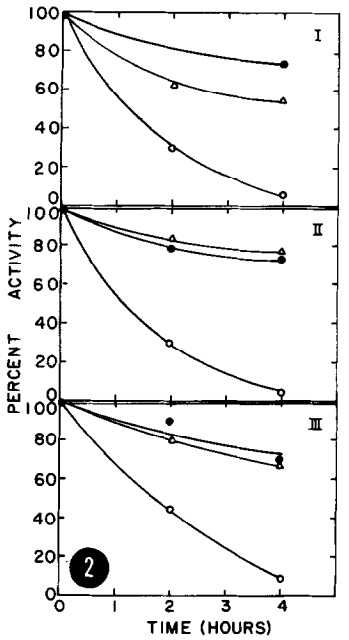


FIGURE 1. Effect of o- and m-phenanthroline on the activity of rat liver RNA polymerases. The rat liver RNA polymerases were prepared and assayed according to the procedure of Roeder and Rutter (7) as modified by M. Goldberg and J-C Perriard (personal communication). They were incubated at 25°C in 25% glycerol, 50 mM Tris HCl, pH 7.9, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.04% β-mercaptoethanol (TGMED) and 10% DMSO. The phenanthroline concentration was 1 mM. At the times indicated, aliquots were removed and assayed. ●——●: control; Δ——Δ: m-phenanthroline; O——O: o-phenanthroline.

RNA polymerases). The concentration dependence of the inhibition by o- and m-phenanthroline of RSV reverse transcriptase and rat liver RNA polymerase II is shown in Figure 3. Other experiments have shown that the inhibition



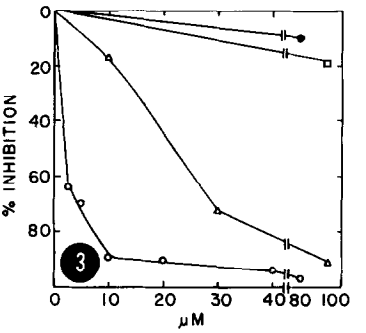


FIGURE 2. Effect of o- and m-phenanthroline on the activity of sea urchin RNA polymerases. The sea urchin RNA polymerases (the generous gift of Dr. J-C. Perriard) were prepared and assayed according to the procedure of Roeder and Rutter (8). They were incubated in TGMED+10% DMSO at 4°C. The phenanthroline concentration was 10 mM. At times indicated aliquots were removed and assayed. ●——●: control; Δ——Δ: m-phenanthroline; O——O: o-phenanthroline.

FIGURE 3. Concentration dependence of the effect of o- and m-phenanthroline on RSV reverse transcriptase and rat liver RNA polymerase II. RSV reverse transcriptase was prepared and assayed according to the method of Levinson et al (9). It was incubated at 37°C for 30 min and assayed at 37°C for 30 min. The RNA polymerase was incubated in TGME (no β-mercaptoethanol)+1% DMSO for 60 min at 37°C and assayed for 10 min at 37°C. The percent inhibition is relative to a sample incubated under identical conditions without inhibitor. RSV reverse transcriptase: Θ——Θ o-phenanthroline; ●——● m-phenanthroline. RNA polymerase II: Δ——Δ o-phenanthroline; □——□ m-phenanthroline.

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increases with increasing temperature of the pre-incubation and decreases with increasing concentration of β-mercaptoethanol in the pre-incubation. For example, pre-incubation of rat liver RNA polymerase I in 1 mM o-phenanthroline for 30 min at 4°C and 25°C resulted in 15% and 42% inhibition, respectively. Further, pre-incubation of rat liver RNA polymerase II at 37°C for 60 min in 100 mM o-phenanthroline containing 0%, 0.03% and 0.1% β-mercaptoethanol

resulted in 91%, 83% and 42% inhibition respectively. In addition, we found that rat liver RNA polymerase I is more sensitive to o-phenanthroline (72% inhibition) when transcribing native calf thymus DNA than when transcribing polydeoxycytidine (30% inhibition) but RNA polymerase II is equally sensitive on both templates (90% inhibition).

The tRNA-nucleotidyl transferases from Rous virus and from yeast appear to be insensitive to either o- or m-phenanthroline under all conditions tested (Table 1). These results are consistent with the report

TABLE I

Effect of o- and m-phenanthroline on tRNA-nucleotidyl Transferases from RSV and Yeast

	CPM Incorporated		
	Control	m-phenan.	o-phenan.
RSV (2 hr incubation)	3400	3800	3400
Yeast (30 min incubation)	21,000	20,000	18,000

The RSV enzyme was prepared and assayed according to Faras et al. (10) and the yeast enzyme according to Morris and Herbert (11). The enzymes were incubated prior to assay at 37°C in the presence of 40 µM phenanthroline (RSV) or 400 µM phenanthroline (yeast) for the times shown.

(4) that N-methyl Isatin β thiosemicarbazone (IBT), another chelating agent, also inhibits RSV reverse transcriptase and rat liver RNA polymerase II, but not RSV or yeast tRNA-nucleotidyl transferase. With both agents the reverse transcriptase is 4-10X more sensitive to the chelator than RNA polymerase. Mercaptoethanol also interferes with inhihition by both the o-phenanthroline and N-methyl IBT. Other chelating agents are effective at relatively higher concentrations, i.e., at least 10 mM EDTA, 8-hydroxy-

quinoline or 8-hydroxyquinoline-5-sulfonic acid are required for significant inhibition.

The inhibition by o-phenanthroline is probably related to its metal chelating properties, since similar inhibition is not produced by the non-chelating analog m-phenathroline.

Zinc has been found in stoichiometric quantities in E. coli RNA and DNA polymerases which suggests that zinc is the metal ion involved. On the basis of the above observations, it seems likely that many if not all DNA and RNA polymerases and nucleotidyl transferases from both pro- and eukaryotes might be metalloenzymes. The metal ion might interact with the polynucleotidyl primer to enhance the nucleophilicity of the reacting 3'-hydroxyl group. The chelating agent might inhibit by binding to the metal ion and blocking the active site, by removing the indigenous metal ion or by facilitating exchange with another ineffective metal ion. It is already known that these enzymes require in addition magnesium or manganese ions which likely interact with the nucleotide substrates.

The present observations are relevant to reports (5,6) that zinc is required for DNA synthesis in cell division. However, the involvement of zinc in many, if not all nucleotidyl transferases and many other proteins precludes a specific interpretation of the effect.

ACKNOWLEDGEMENTS

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