YEAST RNA POLYMERASE III: A ZINC METALLOENZYME

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SUMMARY

Atomic absorption spectroscopy has been used to demonstrate that zinc is associated with yeast RNA polymerase III. The enzyme purified by DNA-Sepharose chromatography gives a single predominant protein band in polyacrylamide gel electrophoresis and contains 0.7 gram-atoms of zinc per 100,000 grams of protein. The zinc is tightly associated with the enzyme and cannot be removed by passing the protein through a column of Chelex-100 resin under conditions where free zinc is quantitatively removed. Inhibition by the chelating agent 1,10-phenanthroline demonstrates that the zinc is essential to the catalytic process. The enzyme is inhibited 50% at 0.1 mM and 100% at 1 mM 1,10-phenanthroline.

INTRODUCTION

Many DNA and RNA polymerases isolated from bacteria (1-3) and viruses (4-7) are zinc metalloenzymes. Valenzuela *et al.* (8) first reported that rat liver RNA polymerases I and II and sea urchin polymerases I, II, and III were inhibited by 1,10-phenanthroline, suggesting a functional role for a metal ion in eukaryotic RNA polymerases, but the presence of a metal ion was not directly demonstrated in these studies. More recently *Euglena gracilis* RNA polymerase II has been shown to contain 2.2 gram-atoms of catalytically essential zinc per mole of enzyme (9). Zinc has also been directly demonstrated to be present in yeast RNA polymerases I and II. RNA polymerase I was reported to contain 2.4 gram-atoms of zinc per mole (10), and RNA polymerase II, 0.98 gram-atoms per mole (11). Both yeast enzymes were inhibited by zinc chelating agents. In this communication we report that yeast RNA polymerase III is also a zinc metalloenzyme. Our enzyme preparation contains 0.7 gram-atoms of zinc per 100,000 grams of protein. Since the enzyme activity can be inhibited by a zinc chelating agent, it suggests that the zinc atoms

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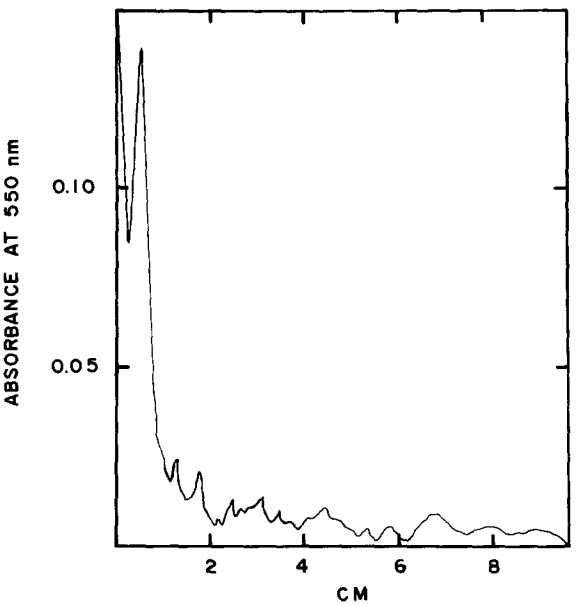

Fig. 1. Polyacrylamide gel electrophoresis of purified yeast RNA polymerase III. A 5 µg sample of enzyme purified through DNA- Sepharose chromatography was analyzed by electrophoresis in barbital buffer (system 6) in 5% polyacrylamide gels as described by Maurer (13). The system was modified to include 10% (v/v) glycerol and 5 mM 2-mercaptoethanol. After staining with Coomassie blue and destaining by diffusion, the gel was scanned at 550 nm on a Gilford spectrophotometer equipped with a Gilford linear transport device.

have a functional role in the catalytic process, or are situated near the catalytic site.

METHODS

Yeast RNA polymerase III was purified from a yeast nuclear lysate by a phosphocellulose batch process, chromatography on DEAE-Sephadex using a linear salt gradient to separate the multiple polymerases, and finally affinity chromatography on DNA-Sepharose. The buffer used contained 10 mM Tris-Cl (pH 7.9), 1 mM 2-mercaptoethanol, 0.1 mM EDTA, and 25% (v/v) glycerol. The purification will be fully described elsewhere (12). Purity of the final enzyme preparation is demonstrated by the single predominant protein band detected in polyacrylamide gel electrophoresis (Fig. 1).

RNA polymerase activity was measured in a final volume of 50 µl. The standard conditions were 55 mM Tris-Cl (pH 7.9), 1 mM 2-mercaptoethanol, 1 mM $MnCl_2$, 5 mM KCl, 0.05 mM EDTA, 12.5% (v/v) glycerol, 100 µg/ml heat denatured calf thymus DNA, 0.1 mM each ATP, GTP, and CTP, and 0.05 mM [5-^{3}H]UTP (0.5 µCi per assay). After incubation at 30° for 20 min, the reaction was terminated by pipetting a 40 µl aliquot onto a disc of Whatman DE-81 ion-exchange paper. The discs were washed seven times for 5 min each with 5% disodium phosphate, twice in distilled water, twice in 95% ethanol, once in ether, and air dried. The radioactivity on the disc was determined in a Nuclear Chicago Mark II scintillation counter in toluene containing 4 grams per liter Omnifluor. One unit of activity corresponds to the incorporation of 1 nmole of UMP into product using the conditions given above.

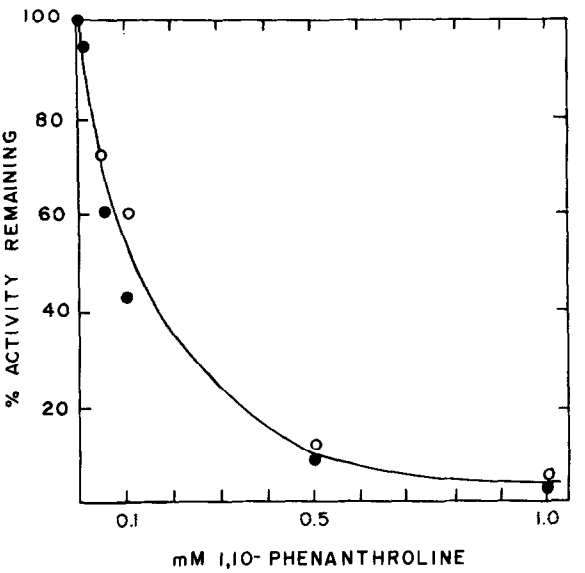

Fig. 2. Inhibition of yeast RNA polymerase III by 1,10-phenanthroline. The enzyme purified through DEAE-Sephadex chromatography was preincubated 20 min in the absence or presence of the indicated 1,10-phenanthroline concentration. Assay mix containing the same 1,10-phenanthroline concentration was used for measurement of polymerase activity as described in Methods. The enzyme activity in the absence of added 1,10-phenanthroline is defined as 100%. Normal assays at 1 mM $MnCl_2$ (o); assays at 1 mM $MnCl_2$ plus additional $MnCl_2$ equimolar to 1,10-phenanthroline (•).

Zinc was determined using an Instrumentation Laboratories Model 253 atomic absorption spectrophotometer. The protein sample (0.1 mg/ml) or a series of zinc standards prepared in the same buffer were aspirated directly into the flame of the instrument and the absorbance at 213.8 nm was recorded. The amount of zinc associated with the protein was determined from the standard curve.

RESULTS AND DISCUSSION

Inhibition of enzyme activity by a metal chelating agent provides indirect evidence that a metal ion is essential to the catalytic process. Zinc metalloenzymes are often quantitatively inhibited by low concentrations of 1,10-phenanthroline. As shown in Fig. 2 our yeast RNA polymerase III preparation purified through DEAE-Sephadex chromatography was inhibited more than 50% at 0.1 mM and nearly quantitatively at 1.0 mM 1,10-phenanthroline. Since this enzyme also requires Mn^{+2} for activity, the inhibition could have been due to chelation of the essential Mn^{+2}. In order to eliminate this possibility, the enzyme assays were conducted with additional Mn^{+2} added equimolar to the

TABLE I

Yeast RNA Polymerase III Activity and Zinc Content

Sample	Enzyme activity[a] (units/mg protein)	Zinc content[b] (g-atoms/100,000 g protein)
No treatment	6.9	0.67
After Chelex treatment	8.4	0.72

[a] A single enzyme activity measurement was used for calculation of the specific activity. Protein was determined as described by Lowry et al. (15) using bovine serum albumin as the standard.

[b] The zinc content before treatment is the average of 5 separate determinations (range 0.58-0.79). After the Chelex treatment only a single zinc analysis was performed due to the small amount of sample available.

1,10-phenanthroline concentration. These results are also shown in Fig. 2. Since there is no significant change in inhibition when additional Mn^{+2} is added, the inhibition must be due to chelation of the intrinsic zinc atom in the enzyme.

The enzyme preparation purified through DNA-Sepharose chromatography was analyzed directly by atomic absorption spectroscopy to determine its zinc content. As shown in Table I the sample contained 0.67 gram-atoms of zinc per 100,000 grams of protein. We wanted to rule out the possibility that zinc was a contaminant in the buffer or only loosely and non-specifically bound to the enzyme. Therefore, an enzyme sample was passed through a 0.5 ml column of Chelex-100 resin and was subsequently analyzed for zinc. Control experiments indicated that the resin could quantitatively remove at least 100 times more free zinc than that detected in the protein sample. Neither the zinc content nor the RNA polymerase activity was reduced by the Chelex treatment (Table I). Since only a single predominant protein band is detected by polyacrylamide gel electrophoresis, the zinc must be associated with yeast RNA polymerase III.

These collective results clearly establish that zinc is tightly associated

with the enzyme and strongly suggest that inhibition by 1,10-phenanthroline is due to the formation of a ternary complex rather than by removal of the zinc atom from the protein. This needs experimental verification by dialyzing the enzyme against 1,10-phenanthroline to determine if the zinc content changes. Further studies are also necessary to determine if any of the substrates interact directly with the zinc atom during catalysis.

The zinc-protein stoichiometry cannot be accurately determined as we do not know the molecular weight of the enzyme. Using a value of 620,000, the calculated minimum molecular weight of yeast RNA polymerase III based on a reported subunit composition (14), the protein would contain 4.3 gram-atoms of zinc per mole. This value is much higher than previously reported for any RNA polymerase and is certainly suspect. We believe that the molecular weight of the enzyme purified by our procedure is nearer to 350,000 (12). Using this value the enzyme would contain 2.5 gram-atoms of zinc per mole. This value compares favorably with that reported by Auld *et al.* (10) for yeast RNA polymerase I (2.4 gram-atoms of zinc per 650,000 grams of protein), but is more than twice that reported by Lattke and Weser (11) for yeast RNA polymerase II (1 gram-atom of zinc per 460,000 grams of protein). The exact stoichiometric relationship between zinc and yeast RNA polymerase III must await a reliable measurement of the molecular weight and zinc content on the same sample. Nevertheless it has now been established that all the yeast nuclear RNA polymerases are zinc metalloenzymes. The original question posed by Valenzuela *et al.* (8) whether all nucleotidyl transferases are metalloenzymes appears to be one enzyme closer to an affirmative answer.

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REFERENCES

1. Slater, J. P., Mildvan, A. S., and Loeb, L. A. (1971) Biochem. Biophys. Res. Commun. 44, 37-43.
2. Springgate, C. F., Mildvan, A. S., Abramson, R., Engle, J. L., and Loeb, L. A. (1973) J. Biol. Chem. 248, 5987-5993.
3. Scrutton, M. C., Wu, W. C., and Goldthwait, D. A. (1971) Proc. Nat. Acad. Sci. USA 68, 2497-2501.
4. Coleman, J. E. (1974) Biochem. Biophys. Res. Commun. 60, 641-648.
5. Auld, D. S., Kawaguchi, H., Livingston, D. M., and Vallee, B. L. (1974) Proc. Nat. Acad. Sci. USA 71, 2091-2095.
6. Poiesz, B. J., Seal, G., and Loeb, L. A. (1974) Proc. Nat. Acad. Sci. USA 71, 4892-4896.
7. Auld, D. S., Kawaguchi, H., Livingston, D. M., and Vallee, B. L. (1975) Biochem. Biophys. Res. Commun. 62, 296-302.
8. Valenzuela, P., Morris, R. W., Faras, A., Levinson, W., and Rutter, W. J. (1973) Biochem. Biophys. Res. Commun. 53, 1036-1041.
9. Falchuk, K. H., Mazus, B., Ulpino, L. and Vallee, B. L. (1976) Biochemistry 15, 4468-4475.
10. Auld, D. S., Atsuya, I., Campino, C., and Valenzuela, P. (1976) Biochem. Biophys. Res. Commun. 69, 548-554.
11. Lattke, H., and Weser, U. (1976) FEBS Lett. 65, 288-292.
12. Wandzilak, T. M. and Benson, R. W., in preparation.
13. Maurer, H. R. (1971) Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis, deGruyter, New York.
14. Valenzuela, P., Hager, G. L., Weinberg, F., and Rutter, W. J. (1976) Proc. Nat. Acad. Sci. USA 73, 1024-1028.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.