Inactivation of Rat Liver RNA Polymerases I and II and Yeast RNA Polymerase I by Pyridoxal 5′-Phosphate. Evidence for the Participation of Lysyl Residues at the Active Site†

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ABSTRACT: Purified DNA-dependent RNA polymerase forms I (A) and II (B) from rat liver and form I from yeast are rapidly inactivated by pyridoxal 5′-phosphate at pH 8.0. The inhibition is relatively specific since pyridoxamine 5′-phosphate is not an inhibitor and pyridoxal is about 12 times less effective than pyridoxal 5′-phosphate. The inactivation is reversed by high concentrations of amines, and can be made irreversible by reduction with $NaBH_4$. Spectral analysis of the inhibited enzyme and its $NaBH_4$ reduction product indicates that a Schiff base forms between the aldehyde group of pyridoxal 5′-phosphate and one or more amino groups of the protein. N^{ϵ}-Pyridoxyllysine was identified as the only product in acid hydrolysates of the reduced yeast RNA polymerase I-pyridoxal 5′-phosphate complex. Complete inactivation of yeast polymerase I results in the incorporation of 3–4 mol of pyridoxal 5′-phosphate/1 mol of enzyme. DNA and nucleotide substrates partially protect the enzymes from inactivation. These results suggest that one or more lysyl amino groups are critical for the activity of animal RNA polymerases and show that pyridoxal 5′-phosphate is a suitable probe for studying the active sites of these enzymes. Comparison of the present results with those previously obtained with *Escherichia coli* RNA polymerase in this laboratory suggest a new degree of structural homology between eucaryotic and procaryotic RNA polymerases.

Knowledge about the amino acid composition and topography of active sites is important for understanding the nature of enzyme catalysis and substrate specificity. In recent years, a good deal of work has been carried out on the purification of animal RNA polymerases, especially those from rat liver and calf thymus (Roeder and Rutter, 1969, 1970; Kedinger et al., 1972; Gissinger and Chambon, 1972). Although the study of many of their properties and subunit structure is well-advanced (Chesterton and Butterworth, 1971; Weaver et al., 1971; Kedinger and Chambon, 1972; Schwartz and Roeder, 1974, 1975), little is known about their active sites and mechanism of action. Recently, Valenzuela et al. (1973) provided evidence for the presence of a metal ion at the active site of animal RNA polymerases, probably zinc by analogy with the *Escherichia coli* enzyme.

Searching for other groups at the active center of these enzymes, we have selected pyridoxal 5′-phosphate as a modifying agent. We have previously reported that this compound is a specific inhibitor of *E. coli* RNA polymerase, presumably by reacting with a critical amino group at the active center of the enzyme (Venegas et al., 1973; Bull et al., 1975). We now present results showing that pyridoxal 5′-phosphate is also a specific inhibitor of rat liver RNA polymerases I (A) and II (B) and yeast RNA polymerase I (A). The evidence presented suggests that pyridoxal 5′-phosphate inhibition results from the modification of one or more unique lysyl amino groups presumably located at the active site of the enzymes. A preliminary account of this work has been reported (Valenzuela et al., 1974).

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‡ Visiting scholar under a Technical Assistance Program between the governments of Belgium and Chile (1971–1973).

Materials and Methods

Materials. [^{3}H]UTP (20 Ci/mmol) and NaB^3H_4 (185 and 250 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. Nucleoside triphosphates, dithiothreitol, calf thymus DNA, and pyridoxal-HCl were purchased from Sigma Chem. Co., St. Louis, Mo. Pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate were generous gifts from Professors F. Marcus and N. Carvajal, respectively. DEAE-cellulose (DE-52) was a Whatman product, and DEAE-Sephadex A-25 was obtained from Pharmacia Fine Chemicals. Sucrose, glycerol, and ammonium sulfate were enzyme-grade reagents. Yeast cells were kindly provided by Red Star Yeast Co., Oakland, Calif.

Enzyme Purification. Yeast RNA polymerase I was prepared by a modification of the method of Buhler et al. (1974). The last two steps of this procedure are ion-filtration chromatography on DEAE-Sephadex and sucrose gradient centrifugation instead of DEAE-cellulose chromatography and glycerol gradient centrifugation. The enzyme obtained is homogeneous in acrylamide gel electrophoresis and has a specific activity of 300 units/mg of protein. One unit of activity is defined as 1 nmol of UMP incorporated per 10 min at 30° into RNA. The details of the purification procedure will be reported elsewhere (P. Valenzuela and W. J. Rutter, manuscript in preparation).

Rat liver RNA polymerases I and II were partially purified from isolated nuclei by a method which combines procedures of Roeder and Rutter (1969), Kedinger et al. (1972), Gissinger and Chambon (1972), and Kedinger and Chambon (1972). Enzyme I was purified by $(NH_4)_2SO_4$ fractionation, DEAE-cellulose chromatography, DEAE-Sephadex chromatography, and glycerol gradient centrifugation. Enzyme II was purified by $(NH_4)_2SO_4$ fractionation, DEAE-cellulose chromatography, and glycerol gradient centrifugation. The various steps employed are described in detail in the following paragraphs.

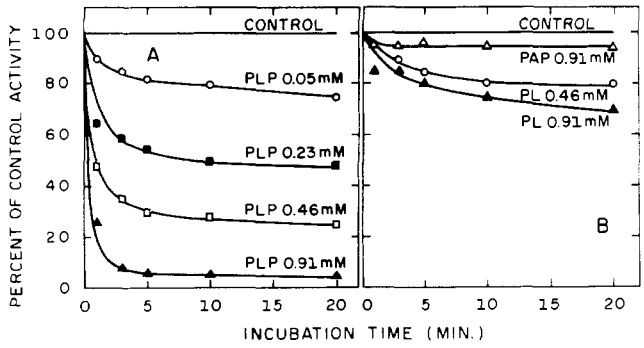

FIGURE 1: (A) Time-dependent inactivation of rat liver RNA polymerase I by different concentrations of pyridoxal 5′-phosphate. Enzyme (0.53 mg/ml) was incubated with increasing concentrations of pyridoxal 5′-phosphate at 30° in BGMED buffer (pH 7.9). Aliquots containing 5 μg of enzyme were taken at various times and assayed as described under Materials and Methods. 100% activity corresponds to the incorporation of 100 pmol of UMP into RNA. (B) Same experiment carried out with the analogs pyridoxamine 5′-phosphate (Δ - - - Δ) and pyridoxal (O - - - O; ▲ - - - ▲).

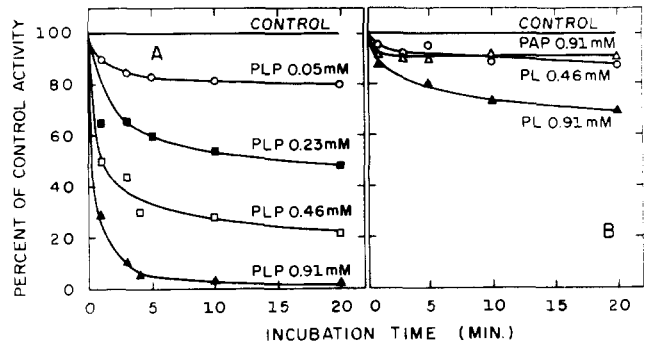

FIGURE 2: (A) Time-dependent inactivation of rat liver RNA polymerase II by different concentrations of pyridoxal 5′-phosphate. Enzyme (0.31 mg/ml) was incubated with increasing concentrations of pyridoxal 5′-phosphate at 30° in BGMED buffer (pH 7.9). Aliquots containing 3 μg of enzyme were taken at different times and assayed as described under Materials and Methods. 100% activity corresponds to the incorporation of 120 pmol of UMP into RNA. (B) Same experiment carried out with the analogs pyridoxamine 5′-phosphate (Δ - - - Δ) and pyridoxal (O - - - O; ▲ - - - ▲).

Enzyme Solubilization and Removal of Chromatin. Nuclei from 1400 g of rat liver, prepared by the method of Widnell and Tata (1964), were lysed in 0.3 *M* $(NH_4)_2SO_4$ and sonicated for 5 min using a Bronwill Biosonik III sonicator, medium size probe. After dilution to 0.1 *M* $(NH_4)_2SO_4$ with TGMED (0.05 *M* Tris-HCl(pH 7.9), 30% glycerol, 0.005 *M* $MgCl_2$, 0.0005 *M* EDTA, and 0.0005 *M* dithiothreitol) and centrifugation (30 min at 44,000*g*), proteins were precipitated with solid $(NH_4)_2SO_4$ (58% saturation) at pH 8.0. Following centrifugation (30 min at 44,000*g*), the precipitate was dissolved in TGMED.

DEAE-Cellulose Chromatography. The solubilized enzyme solution was adjusted to 0.05 *M* $(NH_4)_2SO_4$ and loaded onto a DEAE-cellulose column (350 ml). Enzymes I and II were separated by stepwise elution as described by Mandel and Chambon (1971) using TGMED containing 0.05, 0.12, 0.14, 0.16, and 0.30 *M* $(NH_4)_2SO_4$, successively. Fractions containing enzyme I, which eluted at 0.12 *M* $(NH_4)_2SO_4$ were pooled, precipitated with solid $(NH_4)_2SO_4$ at pH 8.0, centrifuged and dissolved in TGMED. The same procedure was followed for fractions containing enzyme II which eluted at 0.3 *M* $(NH_4)_2SO_4$.

DEAE-Sephadex Chromatography. A method developed by Goldberg, Perriard, and Rutter (personal communication) using the principles of ion-filtration chromatography (Kirkegaard et al., 1972) was followed. Enzyme I from the previous step was adjusted to 0.3 *M* $(NH_4)_2SO_4$ and loaded onto a 3 × 40 cm DEAE-Sephadex A-25 column previously equilibrated with TGMED–0.1 *M* $(NH_4)_2SO_4$. The column was then eluted with TGMED–0.3 *M* $(NH_4)_2SO_4$. The tubes with highest activity were pooled and precipitated with solid $(NH_4)_2SO_4$ at pH 8.0.

Glycerol Density Gradient Centrifugation. Enzyme I from DEAE-Sephadex chromatography or enzyme II from DEAE-Sephadex chromatography was subjected to centrifugation on a 15–30% glycerol gradient described by Kedinger and Chambon (1972). The buffer used was BGMED which contained 0.02 *M* barbital-HCl (pH 7.9) instead of 0.05 *M* Tris-HCl (pH 7.9). The active fractions were pooled, adjusted to 50% glycerol, and stored at −80° until used. With this procedure, rat liver enzyme I with a specific activity of 20 units/mg of protein and rat liver enzyme II with 38 units/mg of protein were obtained.

Enzyme Assay. RNA polymerases were assayed in a final volume of 0.06 ml. The standard reaction mixture contained 20 m*M* barbital-HCl (pH 7.9); 12.5% glycerol; 2.5 m*M* $MgCl_2$; 1.6 m*M* $MnCl_2$; 6 m*M* NaF; 0.6 m*M* each ATP, GTP, and CTP; 0.02 m*M* UTP; 0.4 μCi of [^{3}H]-UTP; 0.3 m*M* dithiothreitol; 0.05 m*M* EDTA; 5 m*M* 2-mercaptoethanol; and 15 μg of native calf thymus DNA. Rat liver and yeast enzyme I were assayed at 0.04 *M* $(NH_4)_2SO_4$ and enzyme II at 0.12 *M* $(NH_4)_2SO_4$. After incubation for 5 or 10 min at 37°, 0.05 ml of the reaction mixture was spotted directly onto Whatman DE-81 filter paper discs. The filters were washed several times with 5% Na_2HPO_4, twice with water, and twice with ethanol and dried. Their radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation counter. Ultraviolet and visible spectra of the polymerase preparations were recorded using a Cary 118 C double beam spectrophotometer.

Identification of N^{ϵ}-Pyridoxyllysine after Reaction of RNA Polymerase with Pyridoxal 5′-Phosphate. Yeast RNA polymerase (2 mg) in 0.025 *M* barbital (pH 7.9); 10% glycerol; 0.005 *M* $MgCl_2$; 0.020 *M* 2-mercaptoethanol; and 0.0005 *M* EDTA was incubated for 30 min at 37° with 2×10^{-4} *M* pyridoxal 5′-phosphate and then treated with 0.001 *M* NaB^3H_4 (250 Ci/mol) at 0°. Following dialysis against several changes of H_2O, the sample was hydrolyzed in a sealed tube in the presence of 6 *N* HCl for 24 hr at 110°. After removal of HCl by repetitive low pressure rotatory evaporation, an aliquot was applied to a sheet of Whatman 3MM paper and subjected to high voltage electrophoresis in pyridine–acetic acid–water (1:10:89, v/v) for 90 min at 2700 V. Another aliquot was subjected to paper chromatography in butanol–pyridine–acetic acid–water (30:30:6:24, v/v) (Shapiro et al., 1968). After drying, the radioactivity was located by counting small squares of the chromatogram in a toluene based liquid scintillation cocktail. The mobility of the radioactive derivative was compared with that of synthetic pyridoxyllysine prepared from poly-L-lysine hydrobromide by the method of Schnackerz and Noltman (1971).

Measurement of Pyridoxal 5′-Phosphate Incorporation. The extent of pyridoxal 5′-phosphate incorporation was

[1] Abbreviation used is: TGMED, 0.05 *M* Tris-HCl (pH 7.9), 30% glycerol, 0.005 *M* $MgCl_2$, 0.0005 *M* EDTA, and 0.0005 *M* dithiothreitol.

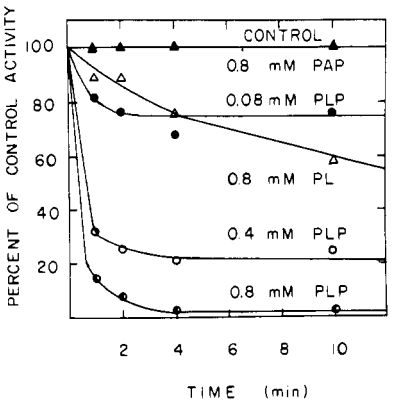

FIGURE 3: Time-dependent inactivation of yeast RNA polymerase I by different concentrations of pyridoxal 5′-phosphate. The enzyme concentration was 0.25 mg/ml and the other conditions were as in Figures 1 and 2. 100% activity corresponds to the incorporation of 500 pmol of UMP into RNA/2.5 μg of polymerase. The triangles correspond to experiments carried out with the analogs pyridoxamine 5′-phosphate (▲ - - - ▲) and pyridoxal (△ - - - △).

measured by a procedure based on the quantitative retention of RNA polymerase on nitrocellulose filters (Krakow and Goolsby, 1971; Hinkle and Chamberlin, 1972). Yeast RNA polymerase I (1.6×10^{-6} *M*) was incubated with pyridoxal 5′-phosphate at concentrations ranging from 0.02 to 0.4 m*M* for 20 min at 30° in buffer BGMED (pH 7.9). Then, 1 m*M* NaB^3H_4 was added and the mixtures were incubated for an additional 5 min at 0°. After quenching with an excess of cold $NaBH_4$, the solutions were filtered through nitrocellulose filters which were later washed extensively with 1 m*M* $NaBH_4$. After drying, the filters were counted by liquid scintillation in Beckman filter-solv solution. Using NaB^3H_4 of a specific activity of 185 Ci/mol, 1 mol of pyridoxal 5′-phosphate incorporated per mol of enzyme retained 8500 cpm in the filter. An enzyme molecular weight of 500,000 was used in the calculations (Buhler et al., 1974).

Results and Discussion

Pyridoxal 5′-phosphate has been used as a probe for phosphate binding sites of several enzymes (Anderson et al., 1966; Rippa et al., 1967; Shapiro et al., 1968; Marcus and Hubert, 1968; Johnson and Deal, 1970: Schnackerz and Noltman, 1971). The highly selective reaction between this reagent and only a few amino groups of several enzymes makes of this compound an extremely useful tool for the identification of amino groups participating in catalysis. In this report, pyridoxal 5′-phosphate has been used to extend to mammalian RNA polymerases our finding that an amino group may function at the active center of the *E. coli* enzyme (Venegas et al., 1973).

As shown in Figures 1–3, preincubation of rat liver RNA polymerases I and II and yeast enzyme I with an excess of pyridoxal 5′-phosphate results in a rapid inactivation of the enzymes. The degree of inhibition observed at equilibrium is dependent on the concentration of the inhibitor. The enzymes are also inhibited by pyridoxal; however, the data from Figures 1 and 2 show that pyridoxal 5′-phosphate is about 12 times more effective. Pyridoxamine 5′-phosphate is only slightly inhibitory.

The effect of pyridoxal 5′-phosphate is reversible. Substantial recovery of activity could be achieved by dilution or by the addition of amines which would be expected to bind the aldehyde. Figure 4 shows that 0.05 *M* ethylenediamine,

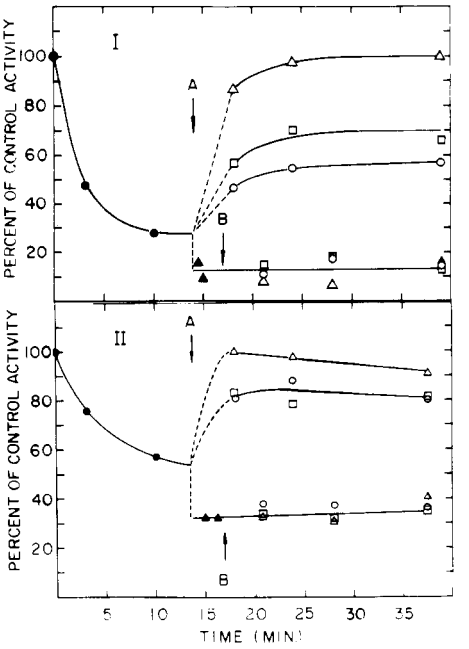

FIGURE 4: Effect of amines and $NaBH_4$ on the inhibition of rat liver RNA polymerases I and II by pyridoxal 5′-phosphate. Enzymes (ca. 0.38 mg/ml) were incubated with 0.4 m*M* pyridoxal 5′-phosphate in BGMED (pH 7.9). At the time indicated by the arrow A, one-half of the sample was treated with 50 m*M* ethylenediamine (△ - - - △), 50 m*M* lysine (□ - - - □), or 50 m*M* Tris (○ - - - ○). The other half of the sample was treated with 1.5 m*M* $NaBH_4$. This sample was treated later, arrow B, with 50 m*M* ethylenediamine (△ - - - △), 50 m*M* lysine (□ - - - □), or 50 m*M* Tris (○ - - - ○). Control tubes contained BGMED instead of pyridoxal 5′-phosphate.

0.05 *M* lysine, or 0.05 *M* Tris when added to rat liver enzyme I–pyridoxal 5′-phosphate and enzyme II–pyridoxal 5′-phosphate mixture results in complete reactivation of the enzymes. Similar results (not shown) were obtained with yeast enzyme I.

The effect of reducing the enzyme–inhibitor complex with $NaBH_4$ was studied using rat liver forms I and II. The results are shown in Figure 4. After reduction, the enzyme is inhibited by an additional 20% and it is no longer reactivated by the addition of amines. In the absence of pyridoxal 5′-phosphate the same concentration of $NaBH_4$ has no effect on enzyme activity. Similar results were obtained with yeast polymerase I. These findings suggest that the inactivation by pyridoxal 5′-phosphate is due to the reversible formation of a Schiff base with one or more amino groups of the enzyme. High concentrations of amines would compete with the enzyme for pyridoxal 5′-phosphate and lessen the inhibition. If the Schiff base is reduced with $NaBH_4$ to a more stable covalent bond amines are no longer effective in reactivating the enzyme.

Further evidence for the formation of a Schiff base between inhibitor and enzyme is provided by spectrophotometric analysis. As shown in Figure 5, the addition of pyridoxal 5′-phosphate to a solution containing yeast RNA polymerase I results in characteristic spectral changes. The differential spectrum shows two positive absorption bands at 335 and 435 nm. The absorption spectrum of the reduced enzyme–pyridoxal 5′-phosphate complex shows a band with a maximum at 325 nm as expected for N^{ϵ}-pyridoxal 5′-phosphate lysine (Fischer et al., 1963). This experiment was also carried out with the rat liver enzymes. Similar results were obtained. However, since these enzymes are not pure, the results are not as significant.

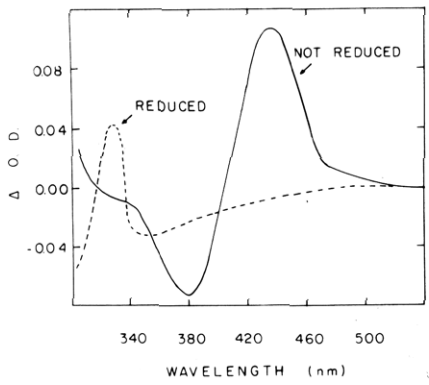

FIGURE 5: Difference spectra of reduced and nonreduced complexes of yeast RNA polymerase I with pyridoxal 5′-phosphate. Enzyme (0.4 mg/ml) was incubated with 0.2 m*M* pyridoxal 5′-phosphate for 10 min at 30° in BGMED (pH 7.9). The reference cuvet contained 0.2 m*M* pyridoxal 5′-phosphate in the same buffer. Reduced polymerase–pyridoxal 5′-phosphate complex was obtained by using 0.6 m*M* $NaBH_4$. The reference cuvet contained a sample treated identically except that pyridoxal 5′-phosphate was omitted.

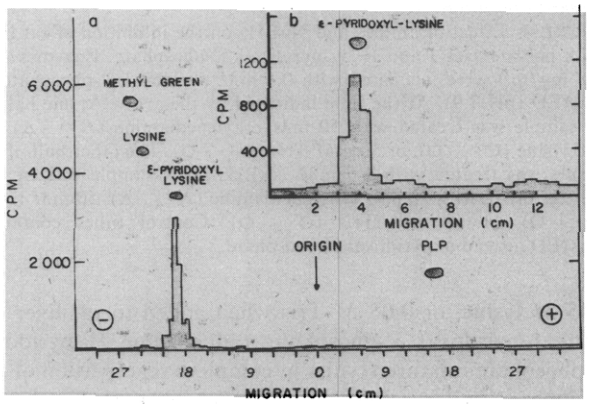

FIGURE 6: Identification of N^{ϵ}-pyridoxyllysine after acid hydrolysis of the reduced yeast RNA polymerase I–pyridoxal 5′-phosphate complex. (a) High voltage electrophoresis in pyridine–acetic acid–water (1:10:89, v/v) (pH 3.5). (b) Paper chromatography in butanol–pyridine–acetic acid–water (30:30:6:24, v/v). The conditions are described under Materials and Methods.

A positive identification of an N^{ϵ}-pyridoxyllysine derivative was made by reducing the yeast enzyme I–inhibitor complex with NaB^3H_4. After exhaustive dialysis, the enzyme was hydrolyzed and a sample subjected to a high voltage paper electrophoresis and paper chromatography. The results are shown in Figure 6. Only one radioactive spot was found. In both systems its migration was identical with that of synthetic N^{ϵ}-pyridoxyllysine prepared in this laboratory. The R_f obtained using paper chromatography agrees well with the value reported in the literature for N^{ϵ}-pyridoxyllysine (Shapiro et al., 1968). These results show that the group which reacts with pyridoxal 5′-phosphate in yeast RNA polymerase I is an ϵ-amino group of a lysine residue. The same has been found for other enzymes which are inactivated by pyridoxal 5′-phosphate (Shapiro et al., 1968; Schnackerz and Noltman, 1971; Anderson et al., 1966; Rippa et al., 1967; Marcus and Hubert, 1968) including *E. coli* RNA polymerase (Bull et al., 1975).

By measuring the incorporation of tritium to the Schiff base double bond it is possible to estimate the number of pyridoxal 5′-phosphate molecules incorporated per mole of enzyme. In Figure 7, the results obtained at different pyridoxal 5′-phosphate concentrations have been related to the extent of enzyme inactivation. The plot is linear to about 30% residual activity. At this point 100% inactivation extrapolates to 3 mol of pyridoxal 5′-phosphate linked per mol of enzyme. Then, a deviation from this line is observed and complete inactivation extrapolates to 4–5 mol of inhibitor attached. These data show that at pH 7.9 the inactivation by pyridoxal 5′-phosphate is due to a highly selective reaction of the inhibitor with only a few lysine residues since yeast RNA polymerase I contains more than 200 lysine residues (P. Valenzuela, unpublished results). The results also indicate that pyridoxal 5′-phosphate may be a suitable probe to study some of the still unknown functions of the several subunits of eucaryotic RNA polymerases. We are currently investigating which subunit(s) react with pyridoxal 5′-phosphate in yeast RNA polymerase I.

Various compounds were tested for their ability to protect the enzyme from inactivation by pyridoxal 5′-phosphate. The results obtained with yeast RNA polymerase I are shown in Table I. It can be seen that nucleotides alone

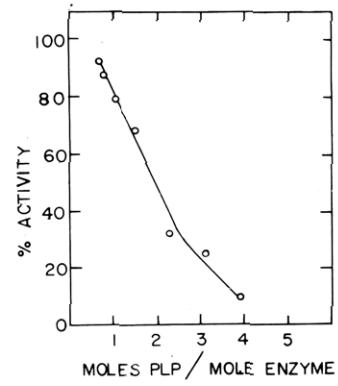

FIGURE 7: Enzyme activity as a function of the extent of pyridoxal 5′-phosphate incorporation. Yeast RNA polymerase I (0.8 mg/ml) was incubated with various concentrations of pyridoxal 5′-phosphate at pH 7.9, reduced with NaB^3H_4 and analyzed for incorporated radioactivity as described under Materials and Methods. Identically prepared aliquots, treated with cold $NaBH_4$, were analyzed for enzyme activity. 100% activity corresponds to the incorporation of 1.5 nmol of UMP into RNA/8 μg of polymerase.

Table I: Effect of Nucleotides and DNA on the Inactivation of Yeast RNA Polymerase I by Pyridoxal 5′-Phosphate.[a]

	Percent of Control Activity	Percent Protection
0 m*M* pyridoxal 5′-phosphate (PLP) (control)	100	
0.2 m*M* PLP	50	
0.2 m*M* PLP; 1.0 m*M* ATP	65	30
0.2 m*M* PLP; 1.0 m*M* GTP	65	30
0.2 m*M* PLP; 1.0 m*M* CTP	60	20
0.2 m*M* PLP; 1.0 m*M* (total) ATP, GTP and CTP	90	80
0.2 m*M* PLP; 0.16 mg/ml of DNA	80	60
0.2 m*M* PLP; 0.16 mg/ml of DNA; 1.0 m*M* (total) ATP, GTP, and CTP	95	90

[a] Enzyme (0.4 mg/ml) was incubated for 5 min with nucleotides or DNA and then another 10 min with pyridoxal 5′-phosphate at 30° in BGMED buffer (pH 7.9). After this time period, aliquots were assayed for activity as indicated under Materials and Methods. 100% activity corresponds to the incorporation of 750 pmol of UMP into RNA per 4 μg of polymerase. Control tubes contained BGMED buffer instead of pyridoxal 5′-phosphate.

protect the enzyme when present at high concentrations (1 mM is about 200 times K_m). ATP and GTP are more effective than CTP and interestingly, a mixture of ATP, GTP, and CTP is even more effective. DNA also protects the enzyme from inactivation. Results similar to those presented in Table I were found with rat liver enzymes I and II. The protection afforded by DNA and nucleotides suggest that the sites of pyridoxal 5′-phosphate attachment are near to both the template and nucleotide binding sites.

This study using mammalian and yeast enzymes and our previous results using the *E. coli* enzyme (Venegas et al., 1973; Bull et al., 1975) suggest that procaryotic and eucaryotic RNA polymerases are mechanistically similar. Structural and functional similarities between these RNA polymerases have been found. Enzymes from both sources have high molecular weights of about 400,000–500,000 and are composed of multiple subunits. The number and molecular weight of subunits from rat liver and yeast polymerases show an overall structural resemblance to those from the *E. coli* enzyme (Chambon, 1974). In addition to the previously reported effect of metal chelating agents on the activity of rat liver and sea urchin enzymes (Valenzuela et al., 1973), our findings with pyridoxal 5′-phosphate add a new degree of structural similarity between procaryotic and eucaryotic RNA polymerases: the active site.

Acknowledgments

The authors are indebted to Dr. P. Chambon from Strasbourg; to Drs. W. Goldberg, J. C. Perriard, and W. J. Rutter from San Francisco for their encouragement and for providing details of their purification techniques prior to publication, and to Drs. M. Bender and L. Rall for their critical reading of this manuscript.

References

Anderson, B. M., Anderson, C. D., and Churchich, J. E. (1966), *Biochemistry 5,* 2893.

Buhler, J. M., Sentenac, A., and Fromageot, P. (1974), *J. Biol. Chem. 249,* 5963.

Bull, P., Zaldivar, J., Venegas, A., Martial, J., and Valenzuela, P. (1975), *Biochem. Biophys. Res. Commun. 64,* 1152.

Chambon, P. (1970), *Enzymes, 3rd Ed. 10,* 261–331.

Chesterton, G. J., and Butterworth, P. H. W. (1971), *FEBS Lett. 15,* 181.

Fischer, E. H., Forrey, A. W., Hedrick, J. L., Hughes, R. C., Kent, A. B., and Krebs, E. G. (1963), in Chemical and Biological Aspects of Pyridoxal Catalysis, Snell, E. E., Fasella, P. M., Braunstein, A., and Rossi-Fanelli, A., Ed., New York, N.Y., Pergamon Press, p 554.

Gissinger, F., and Chambon, P. (1972), *Eur. J. Biochem. 28,* 277.

Goldberg, M., Perriard, J. C., Hager, G., Hallick, R. E., and Rutter, W. J. (1973), in Control of Transcription, Biswas, B. B., Mandal, R. K., Stevens, A., and Cohn, W. E., Ed., New York, N.Y., Plenum Press, p 241.

Hinkle, D. C., and Chamberlin, M. J. (1972), *J. Mol. Biol. 70,* 157.

Johnson, G. S., and Deal, W. C. (1970), *J. Biol. Chem. 245,* 238.

Kedinger, C., and Chambon, P. (1972), *Eur. J. Biochem. 28,* 283.

Kedinger, C., Gissinger, F., Gniazdowski, M., Mandel, J. L., and Chambon, P. (1972), *Eur. J. Biochem. 28,* 269.

Kirkegaard, L. H., Johnson, T. J. A., and Bock, R. M. (1972), *Anal. Biochem. 50,* 122.

Krakow, J. A., and Goolsby, S. P. (1971), *Biochem. Biophys. Res. Commun. 44,* 453.

Mandel, J. L., and Chambon, P. (1971), *FEBS Lett. 15,* 175.

Marcus, F., and Hubert, E. (1968), *J. Biol. Chem. 243,* 4923.

Rippa, M., Spanio, L., and Pontremoli, S. (1967), *Arch. Biochem. Biophys. 118,* 48.

Roeder, R. G., and Rutter, W. J. (1969), *Nature (London) 224,* 234.

Roeder, R. G., and Rutter, W. J. (1970), *Proc. Natl. Acad. Sci. U.S.A. 65,* 675.

Schnackerz, K. D., and Noltman, E. A. (1971), *Biochemistry 10,* 4837.

Schwartz, L. B., and Roeder, R. G. (1974), *J. Biol. Chem. 249,* 5898.

Schwartz, L. B., and Roeder, R. G. (1975), *J. Biol. Chem. 250,* 3221.

Shapiro, S., Enser, M., Pugh, E., and Horecker, B. L. (1968), *Arch. Biochem. Biophys. 128,* 554.

Valenzuela, P., Bull, P., Martial, J., Venegas, A., Zaldivar, J., and Tellez, R. (1974), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 33,* 1417.

Valenzuela, P., Morris, R. W., Faras, A., Levinson, W., and Rutter, W. J. (1973), *Biochem. Biophys. Res. Commun. 53,* 1036.

Venegas, A., Martial, J., and Valenzuela, P. (1973), *Biochem. Biophys. Res. Commun. 55,* 1053.

Weaver, R. F., Blatti, S. P., and Rutter, W. J. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 2994.

Widnell, C. C., and Tata, J. R. (1964), *Biochem. J. 92,* 313.