Vol. 81, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 30, 1978 Pages 662-666

SUBUNITS OF YEAST RNA POLYMERASE I INVOLVED IN INTERACTIONS WITH DNA AND NUCLEOTIDES

Pablo Valenzuela, Paulina Bull, Josefina Zaldivar,
Alejandro Venegas and Joseph Martial

Received February 13, 1978

Laboratorio de Bioquimica
Departamento de Biologia Celular I.CC.BB.
Universidad Catolica
Casilla 114-D
Santiago, Chile

ABSTRACT

Reaction of yeast RNA polymerase I with pyridoxal 5'-phosphate and sodium borohydride under conditions which inactivate the enzyme results in the specific binding of pyridoxal 5'-phosphate to subunits of 185,000, 137,000, 48,000 and 36,000 daltons. Nucleotides, which protect the enzyme from inactivation specifically inhibit the binding of pyridoxal 5'-phosphate to subunits of 185,000 and 137,000 daltons. DNA which also protects the enzyme from inactivation by pyridoxal 5'-phosphate prevents the binding of the reagent to the four polypeptides.

These results suggest that subunits of 185,000 and 137,000 are involved in interactions with both nucleotides and DNA presumably of the type leading to initiation and/or polymerization and that subunits of 48,000 and 36,000 daltons also bind to DNA but this interaction is not strictly required for polymerase activity.

INTRODUCTION

Yeast nuclei as well as nuclei from other eucaryotes contain three RNA polymerases which can be distinguished by their different structure, enzymatic properties and cellular function (1-3). The enzymes have been purified as complex structures composed of several polypeptides of different molecular weights (3-7). Each enzyme is characterized by two unique large subunits and several smaller subunits some of them common to two or three of the enzymes (8,9). Nothing is known about the functional reason for such complex structures as well as about the role of each of the polypeptide components. This knowledge is fundamental to fully understand the role of the enzyme in specific transcription.

0006-291X/78/0812-0662$01.00/0

In the present communication we report the use of pyridoxal 5'-phosphate as chemical probe to study the putative subunits of yeast RNA polymerase I (or A). We have previously shown that RNA polymerases including yeast RNA polymerase I are rapidly inhibited by pyridoxal 5'-phosphate through the formation of a Schiff base with lysyl amino groups presumably located at the active site of the enzymes (10-12). In the present study we have identified the subunits of yeast polymerase I which react with pyridoxal 5'-phosphate and measured the effect of DNA and nucleotides on the reactivity of these subunits.

MATERIALS AND METHODS

The source of nucleotides, isotopes and other reagents used is the same as described previously (10-12). RNA polymerase I from yeast (Saccharomyces cerevisiae) was purified to apparent homogeneity and assayed as described before (4,7).

To identify the enzyme polypeptides which react with pyridoxal 5'-phosphate polymerase I (1.5 mg/ml) was incubated with 0.1mM pyridoxal 5'-phosphate for 20 min at 30°C. Then the reaction mixture was chilled, made 1.5mM in [^{3}H]$NaBH_4$ and incubated 5 min at 0°C. After heating for 5 min, to destroy unreacted [^{3}H]$NaBH_4$, the protein was precipitated with cold 10% TCA and subunits separated by gel electrophoresis.

Twelve percent acrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate was carried out in 12x15 cm x 1.5 mm slab gels. Buffers and solutions were prepared according to Laemmli (13). The gels were fixed by shaking them in 50% isopropanol-10% trichloroacetic acid and stained with 0.1% Coomassie blue in 25% isopropanol-10% trichloroacetic acid at 23°C. Gels were destained at 23°C with 10% acetic acid.

Radioactivity in each enzyme subunit was detected after electrophoresis by fluorography (14). The gel was soaked in dimethyl sulfoxide for 60 min, immersed in 20% PPO in dimethyl sulfoxide for 3 hr at 23°C washed with water, dried over filter paper and autoradiographed with RP Royal X-Omat (Kodak) film for 20 days. Stained gels and films were scanned at 550 nm with a linear transport device attached to a spectrophotometer.

RESULTS AND DISCUSSION

Yeast RNA polymerase I has been purified to apparent homogeneity as a complex multimeric protein containing polypeptides with molecular weights 185,000, 137,000, 48,000, 44,000, 41,000, 36,000, 28,000, 24,000, 20,000, 14,500, 12,300 and 9,000 (4,5,8). Figures 1A and 2A show the polypeptide composition of RNA polymerase I isolated from yeast as shown by 12% acrylamide gels. When the enzyme is treated with 0.1mM pyridoxal phosphate (a concentration which we have shown previously to rapidly inhibit the enzyme (12) and the formed Schiff base is then reduced with [^{3}H]$NaBH_4$, the label is found mainly associated with four of the polymerase subunits, the 185,000 (I_{185}), 137,000 (I_{137}), 48,000 (I_{48})

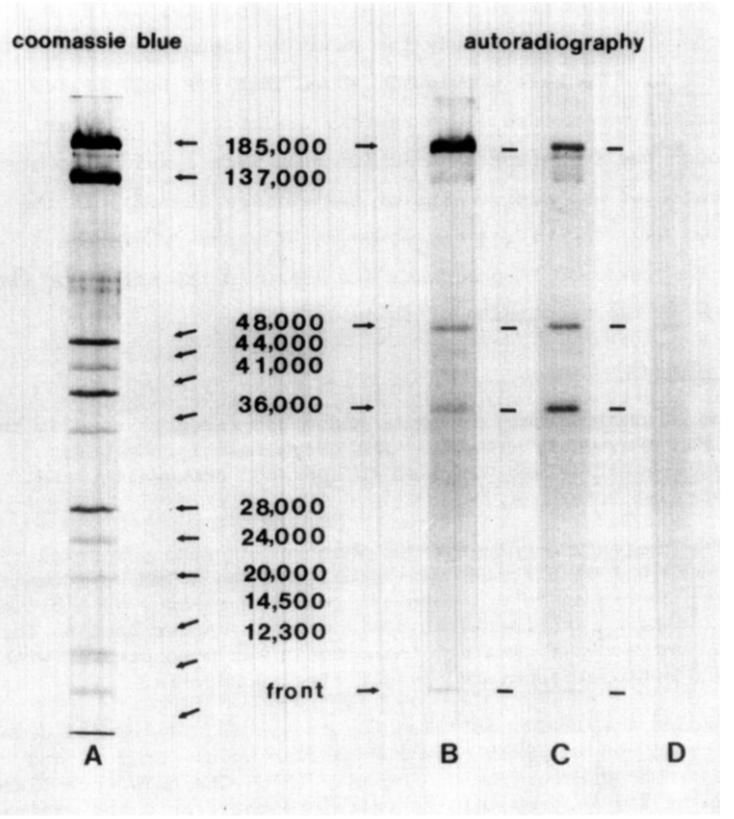

FIGURE 1. Labeling pattern of polypeptide components of yeast RNA polymerase I after reaction with pyridoxal 5'-phosphate and reduction with [^{3}H]$NaBH_4$. A) Coomassie blue staining of unreacted enzyme; B) Autoradiography of enzyme subunits after treatment with pyridoxal 5'-phosphate and [^{3}H]$NaBH_4$; C) Autoradiography of enzyme subunits of enzyme treated as in B, in the presence of 1mM ATP, CTP and GTP; D) Autoradiography of enzyme treated as in B, in the presence of 0.3 mg/ml of native calf thymus DNA.

and 36,000 (I_{36}) dalton polypeptides. These results are shown in Figures 1B and 2B. They indicate that pyridoxal 5'-phosphate reacts preferentially with amino groups in polypeptides I_{185}, I_{137}, I_{48} and I_{36} and suggest that reaction with one of these subunits is the reason for the inactivation of the enzyme. Huet et al. have shown that although polypeptides I_{48} and I_{36} influence transcription of native DNA, they are not strictly required for enzyme activity (15). Therefore it is likely that the inactivation of polymerase I by pyridoxal 5'-phosphate is due to the reaction of the inhibitor with subunits I_{185} and/or I_{137}.

This hypothesis is further substantiated by the results obtained when the reaction with pyridoxal 5'-phosphate is carried out in the

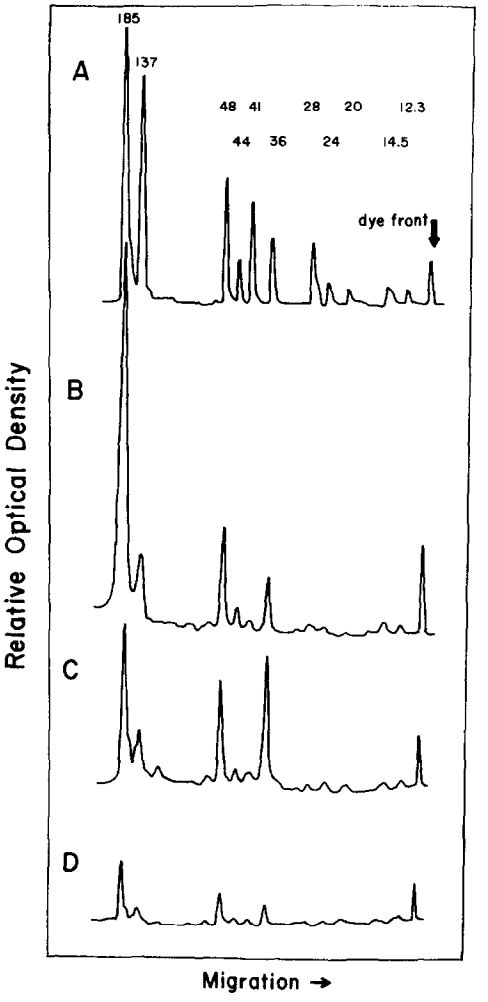

FIGURE 2. Densitometer tracings of the stained gels and autoradiograms of Figure 1. A through D have the same meaning as Figure 1. Numbers indicate the molecular weight $x10^{-3}$.

presence of 1mM nucleotides (condition which we have previously found protects 60 to 80% of the enzyme activity (12)), the amount of pyridoxal 5'-phosphate attached to the subunit I_{185} decreases to about one-fifth, however, the labeling of polypeptides I_{137} and I_{48} has not changed and that of I_{36} is slightly increased (Figures 1C and 2C). These results confirm that binding of PLP to polypeptides I_{48} and I_{36} does not result in enzyme inhibition and strongly suggest that the subunit I_{185} has a nucleotide binding site involved in enzyme activity. The data do not allow us to discard a role of the subunit I_{137}, since it is also labeled

by pyridoxal 5'-phosphate although to a considerable less extent.

We have previously reported that DNA also protects yeast RNA polymerase I from inactivation by pyridoxal 5'-phosphate (12). When the reaction with pyridoxal 5'-phosphate is done in the presence of DNA (Figures 1D and 2D), the label of subunits I_{185}, I_{137}, I_{48} and I_{36} is significantly decreased indicating that the binding of DNA to the enzyme protects these polypeptides from PLP attachment and suggesting that these subunits are involved in enzyme-template interactions.

Our results indicating interaction between subunits I_{48} and I_{36} with DNA are in agreement with the findings of Huet et al. (15) which indicate that these two more loosely bound subunits of yeast polymerase I improve transcription of double stranded DNA versus polyd(AT) and are consistent with the suggestion that these polypeptides may have a subtle role at the level of enzyme-DNA interactions (15).

ACKNOWLEDGEMENTS

We wish to thank Leslie Spector for typing the manuscript. This research was supported in part by funds from the Fondo de Investigacines, Universidad Catolica de Chile, the Organization of American States and the PNDU/UNESCO Program RLA 75/047. This paper is dedicated to the memory of our friend and colleague, Professor Enrique Beytia.

REFERENCES

1. Chambon, P. (1975) Ann. Rev. Biochem. 44, 613-638.
2. Roeder, R.G. (1976) in RNA Polymerase, Losick, R. and Chamberlin, M., Eds., pp. 285-329, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. Valenzuela, P., Hager, G.L., Weinberg, F. and Rutter, W.J. (1976) Proc. Nat. Acad. Sci. USA 73, 1024-1028.
4. Valenzuela, P., Weinberg, F., Bell, G.I. and Rutter, W.J. (1976) J. Biol. Chem. 251, 1464-1470.
5. Buhler, J.M., Sentenac, A. and Fromageot, P. (1974) J. Biol. Chem. 249, 5963-5970.
6. Dezelee, S., Wyers, F., Sentenac, A. and Fromageot, P. (1976) Eur. J. Biochem. 65, 543-552.
7. Valenzuela, P., Bell, G.I., Weinberg, F. and Rutter, W.J. (1977) in Methods in Cell Biology, Chromosomal Protein Research, Vol. 18, Stein, G.S., Stein, J.L. and Kleinsmith, L.J., Eds, Academic Press, New York, in press.
8. Buhler, J.M., Dezelee, S., Huet, J., Iborra, F. and Sentenac, A. (1976) J. Biol. Chem. 251, 1712-1717.
9. Valenzuela, P., Bell, G.I., Weinberg, F. and Rutter, W.J. (1976) Biochem. Biophys. Res. Commun. 71, 1319-1325.
10. Venegas, A., Martial, J. and Valenzuela, P. (1973) Biochem. Biophys. Res. Commun. 55, 1053-1059.
11. Bull, P., Zaldivar, J., Venegas, A., Martial, J. and Valenzuela, P. (1975) Biochem. Biophys. Res. Commun. 64, 1152-1159.
12. Martial, J., Zaldivar, J., Bull, P., Venegas, A. and Valenzuela, P. (1975) Biochemistry 14, 4907-4911.
13. Laemmli, U.K. (1970) Nature 227, 680-685.
14. Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83-
15. Huet, J., Buhler, J.M., Sentenac, A. and Fromageot, P. (1975) Proc. Nat. Acad. Sci. USA 72, 3034-3038.