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 NaBH4. Spectral analysis of the inhibited enzyme and its NaBH4 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. Nepsilon-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 Eschericha coli RNA polymerase in this laboratory suggest a new degree of structural homology between eucaryotic and procaryotic RNA polymerases.