Chemical Modification of Ribonucleic Acid Polymerase with N-Bromosuccinimide

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The mechanism of specific interaction between a protein and a nucleic acid is thought to reside partially in the arrangement and types of amino acid present in the protein. Attempts have been made to assign specific roles in this interaction to certain amino acids, for example, tryptophan and tyrosine are thought to be involved in unwinding the double helix of DNA (Brun et al., 1975), and in selecting between A-T- and G-C-rich regions (Gabbay et al., 1973).

We have used N-bromosuccinimide, a selective oxidizing agent for tryptophan (Spande & Witkop, 1967), in an attempt to elucidate the role of tryptophan in the activity of DNA-dependent RNA polymerase (EC 2.7.7.6) from Escherichia coli.

N-Bromosuccinimide completely inactivates RNA polymerase core enzyme in 25 molar excess, at pH 7.9 and 25°C. Changes in extinction at 280nm indicate that approximately two tryptophan residues are oxidized during the inactivation. A potential side reaction with N-bromosuccinimide is the oxidation of thiols. 5,5'-Dithiobis-(2-nitrobenzoic acid) titration was used to distinguish eight 'exposed' and 14 'buried' thiols in RNA polymerase. N-Bromosuccinimide oxidizes five 'exposed' thiols during the first 50% of the inactivation. No loss of 'buried' thiols could be detected. The activity of modified RNA polymerase could only be partially restored with dithiothreitol. No protection of activity against N-bromosuccinimide inactivation could be detected in the presence of DNA plus ATP plus GTP. There was no detectable cleavage of peptide bonds with N-bromosuccinimide, even when its concentration was increased 10-fold over that used to produce total loss of activity.

In an attempt to separate the roles of thiol and tryptophan oxidation in the inactivation, the surface thiol groups were reversibly protected with 5,5'-dithiobis-(2-nitrobenzoic acid). Ten thiol groups were protected, and after N-bromosuccinimide oxidation the protection was reversed with excess of dithiothreitol. The trinitrobenzoylated enzyme is completely inactivated with a 30-fold excess of N-bromosuccinimide. There is no loss of thiol groups over 80% of the activity loss. The modified enzyme has no activity on native and denatured DNA, and is not activated by the dinucleotide G-A. Far-u.v. circular dichroism was used to measure changes in secondary structure caused by N-bromosuccinimide oxidation. Native and modified enzyme have identical negative ellipticity at 220 nm, [θ]220 8.5 ± 0.3 m°·cm·dmol. Fluorescence-emission spectroscopy was used to establish that oxidation of tryptophan had occurred. The modified enzyme shows a 12% decrease in fluorescence emission relative to the native enzyme, with no shift in the emission maximum. Equilibrium dialysis was used to measure the enzyme's affinity for ATP in the presence of Mg2+. The dissociation constants were: modified enzyme $K_d$, 36 ± 4 μmol; native enzyme $K_d$, 9 ± 1 μmol. This decrease in binding affinity is not sufficient to account for complete inactivation of the enzyme.

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