INHIBITION OF RNA CHAIN INITIATION IN E. coli CORE RNA POLYMERASE WITH 2-HYDROXY-5-NITROBENZYL BROMIDE

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SUMMARY: The modification of E. coli core RNA polymerase with 2-hydroxy-5-nitrobenzyl bromide (Koshland's Reagent) resulted in the benzylation of 6 out of 13 cysteines, and 10 out of 20 tryptophans in the polymerase, and occurred with an 8% decrease in its $[\theta]_{220}$. The modification resulted in a maximal inhibition of 60% of the RNA chains on both calf thymus and micrococcal DNA templates. γ-^{32}P-ATP studies showed the inhibition occurred at RNA chain initiation. This study raises the possibility that the modified core polymerase may synthesize specific RNA(s).

INTRODUCTION

During the last ten years, since its discovery, much work has been done on the characterization of the E. coli DNA-dependent RNA polymerase (1). Yet, probably due to the size and complexity of its subunits, and to their unknown primary and secondary structure, the molecular basis of RNA chain initiation and template-directed nucleotide triphosphate (NTP) polymerization remains mostly unknown. The closest well-defined model nucleic acid enzyme to RNA polymerase is pancreatic RNase (2). A study of this enzyme only suggests the types of amino acid residues and sequences that might participate in NTP pyrophosphorolysis and polymerization, but suggests very little about the molecular basis of RNA chain initiation or template-directed polymerization.

Our efforts over the last few years have been aimed at learning more about the molecular basis of the sequential steps in the RNA polymerase reaction (DNA binding, RNA chain initiation, and polymerization) by using selective amino acid modifications. Previous modification studies in other laboratories have shown the involvement of polymerase lysines (3, 4) in both NTP and DNA binding (on the β and β' subunits), and of histidines (on

the β subunit) (3), in phosphorolysis and polymerization, as is the case with pancreatic RNase (2). Although the displaceable sigma subunit seems to be involved in proper RNA chain initiation (5), so too are the core polymerase subunits, as indicated by their modification during T4 phage infection (6). The requirement for different RNA polymerases for the synthesis of different RNA molecules has also been found in eukaryotic systems (7). In this paper we describe the selective inhibition of certain RNA chain initiations by benzylation of the cysteines and tryptophans in core E. coli RNA polymerase with 2-hydroxyl-5-nitrobenzyl bromide.

METHODS AND MATERIALS

Core RNA polymerase (460 units/mg) was isolated from frozen "3/4 log, enriched medium, E. coli β cells" (Grain Processing, Muscatine, Iowa) according to the DEAE-cellulose, phosphocellulose, agarose procedure of Burgess (8). The polymerase preparations (100 μg) gave the characteristic β, β', α disc gel electrophoresis patterns (8) with negligible contaminating protein. Acetone solutions of 2-hydroxy-5-nitrobenzyl bromide (Koshland's Reagent), 2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine (BNPS-skatole), and of acetyl imidazole were added to the polymerase in 0.1 M Tris pH = 7.5 at the cited molar ratios. Iodoacetic acid and diethyl oxydiformate were added in aqueous solutions. Maximal inactivation with 2-hydroxy-5-nitrobenzyl bromide was found by titration studies to occur at 20/1 [modifier]/[polymerase], but 100/1 molar ratios were routinely used in all other experiments. DNA binding experiments were performed on B-6 nitrocellulose filters (Schleicher and Schuell, Inc.) according to Hinkle and Chamberlin (5). All binding experiments were performed at saturating DNA concentrations ([1]/[1] ratio polymerase (2 μg)/DNA (1 μg)(1-2 x 10^3 cpm/μg)). The ^{32}P-end-labelled DNA for these experiments was prepared as previously described (9). The γ^{32}P-ATP for this labelling and for the RNA chain initiation studies was prepared according to the procedure of Glynn and Chappell (10).

RNA chain initiation was followed by γ-^{32}P-ATP incorporation (11).

RNA polymerization activity was followed by the incorporation of ^{3}H-CTP (1 mc/mM) into 5% TCA precipitable material (12). 50 µg of polymerase to 100 µg of DNA template was used in both types of assays. The cysteic acid content of performic acid oxidized (13) 6 N HCl polymerase hydrolysates was performed on a Beckman-Spinco Amino Acid Analyzer Model 120 C. The number of modified tryptophans was determined by N-bromosuccinimide titration (14). CD measurements were performed on a Cary 60 recording spectrophotometer equipped with a Model 6001 circular dichroism attachment.

RESULTS AND DISCUSSION

The inactivation curve for the core RNA polymerase with 2-hydroxy-5-nitrobenzyl bromide is shown in Figure 1. A maximal 60% inactivation of

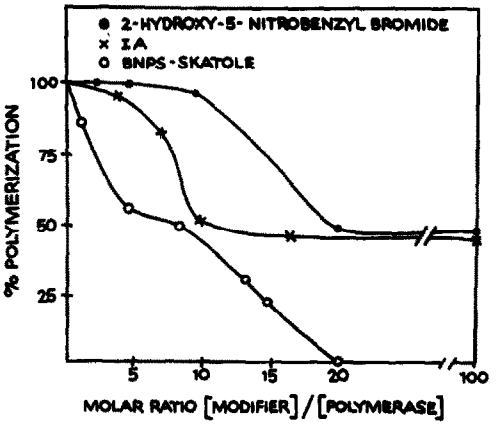

Figure 1. Inactivation of E. coli core RNA polymerase by alkylating (2-hydroxy-5-nitrobenzyl bromide, and iodoacetic acid (IA)) and oxidizing (BNPS-skalote) agents.

polymerization of ^{3}H-CTP into RNA was achieved at a [20]/[1] ratio. No further inactivation was observed when the molar ratio was increased 10^{5}-fold. The modification occurred with an 8% decrease in the $[\theta]_{220}$ of the polymerase enzyme. Similar decreases in $[\theta]_{220}$ have been found to occur in pCMB and in 2 M urea (12) with complete inactivation of the enzyme. At a 100/1 ratio of 2-hydroxy-5-nitrobenzyl bromide/polymerase 10 out of 20

TABLE I

FUNCTIONAL SPECIFICITY OF THE

2-HYDROXY-5-NITROBENZYL BROMIDE MODIFICATION

TEMPLATE	POLYMERASE FUNCTION Percent Activity*		
	DNA Binding**	Initiation***	Polymerization
Calf Thymus DNA	100	40	40
Micrococcus lysodeikticus DNA	100	40	40

*Compared to equal amounts of unmodified polymerase.
**Performed at saturation levels of DNA, i.e. 1/1 [polymerase]/[^{32}P-labelled DNA]
***Performed according to procedure of Hurwitz et al. (11).

tryptophans and 6 out of 13 cysteines were modified. From the present information it cannot be ascertained whether the effect on activity is due to a change in conformation, modification of certain cysteines, or a modification of certain tryptophans. A maximal 60% inactivation of polymerization activity has also been found with three other alkylation agents. The titration curve for one of these, iodoacetic acid, is also shown in Figure 1. N-acetylimidazole and diethyl oxydiformate showed a similar 60% maximal inactivation. By contrast, another tryptophan and cysteine modifier, the mild oxidizing agent BNPS-skatole (2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine) (15), completely inactivated the polymerase at a 20/1 molar ratio (Fig. 1).

In order to determine whether the benzylation of core RNA polymerase effected polymerization or a preceding step in the reaction sequence, the ability of the modified enzyme to bind DNA and to initiate RNA chain synthesis was also investigated. The results of these assays are summarized in Table 1. The capacity of the modified enzyme to bind native DNAs was not impaired. However, a 60% decrease in γ-^{32}P-ATP chain initiations was

observed on both DNA templates. Although the native and modified polymerase showed the same net ^{3}H-CTP polymerization on both templates, they showed a net γ-^{32}P-ATP incorporation proportional to the AT-content of the template, viz calf thymus showing 1.5 times as much γ-^{32}P-ATP as micrococcal DNA. This study raises the possibility that the modification may result in the inhibition of certain class(es) of RNA. RNA product hybridization studies are in progress to probe this hypothesis, as are studies, to locate the modified cysteine and tryptophan residues.

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