Template specific inhibitors of E. coli RNA polymerase

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ABSTRACT

Electroneutral analogs of polynucleotides, poly-9-vinyladenine and poly-1-vinyluracil inhibit E. coli RNA polymerase in all combinations where the single stranded polynucleotides are used as templates and the vinyl analogs are complementary to them. As templates both the ribo- and deoxyribopolynucleotides were tested; variation of the template concentration in the presence of vinyl analogs produced a competitive pattern of inhibition. The electroneutral analogs do not inhibit the enzyme activity when non-complementary single stranded polynucleotides or double stranded polynucleotides are used as templates; the latter fully supports transcription even when one of the strands is complementary to the analog.

INTRODUCTION

Polynucleotides have two overriding chemical features; one of them is their polyanionic backbone, another one is the bases which are capable of the specific base pairing with their complementary partners. It has been of interest to develop macromolecules which would have only one of these features and to test these compounds on enzymes of nucleic acid metabolism. Electroneutral analogs of nucleic acids have been studied with that purpose in mind and it has already been found that they interfere with the DNA synthesis templated by DNA or RNA and with the translation of RNA into protein. In these interferences the electroneutral analogs seem to act differently from synthetic polyanions. In this work we investigated effects of poly(vU) and poly(vA) on DNA dependent RNA synthesis by an enzyme from E. coli.

MATERIALS

The synthesis and properties of vinyl analogs of polynucleotides were reviewed recently. Vinyl polymers were purified by extensive dialysis. The s value of poly(vU) was 4.7; the main fraction of poly(vA) had molecular weight higher than 68,000 as estimated from electrophoresis in the presence of sodium dodecyl sulfate. Polynucleotides were obtained from Miles...
Laboratories, Elkhart, Indiana and had following s values: poly(rA)-6, poly(rU)-5, poly(dA-dT)-7, poly(dA)-6, poly(dT)-8. DNA prepared from calf thymus was used. Molarities of vinyl polymers and nucleic acids are expressed per base residue. RNA polymerase was prepared from Escherichia coli K-12 Lambda by the method of Burgess and purified by DEAE cellulose and agarose column chromatography.

METHODS

Assay mixtures were assembled by first mixing polynucleotide and vinyl polymer in water; then the remaining components were added as follows: Conditions (a) Trishydroxymethylamino methane-HCl, pH 7.9, 20 mM; MnCl₂, 2.5 mM; MgCl₂, 5 mM; mercaptoethanol, 20 mM; KCl, 200 mM; poly(rU), poly(rA), DNA, denaturated DNA when indicated, 1 mM; poly(dA-dT) and poly(dA).poly(dT) when indicated, 0.015 mM; *E. coli* RNA polymerase, 50 µg/ml; ribonucleoside triphosphates complementary to template, 0.5 mM, with one of them radioactive (³⁰ATP, 0.05 µCi/ml). Inhibitors were present at a concentration of 1 mM. Conditions (b) Trishydroxymethylaminomethane-HCl, pH 7.9, 50 mM; MgCl₂, 12.5 mM; ethylenediaminetetraacetatic acid 0.125 mM; dithiothreitol, 0.125 mM; KCl, 20 mM; concentrations of templates, substrates and enzyme were as sub (a). Inhibitors were present at a concentration of 1 mM. Conditions (c) From the conditions sub (a) KCL was omitted. Inhibitors were present at a concentration of 0.3 mM. The mixtures (total volume 250 µl) were incubated for 10 min at 37°C and then chilled on ice. One ml of a 2% solution of bovine serum albumin, which served as a carrier, was added and nucleic acid was precipitated by the addition of 3 ml of a solution containing trichloroacetic acid (5%) and sodium pyrophosphate (0.01 M). The precipitate was collected on glassfiber filters (Whatman GFC), washed with the precipitating solution, dried, and the radioactivity measured by liquid scintillation counting.
RESULTS

The effects of vinyl analogs on transcription of double stranded DNA, poly(dA-dT), poly(dA).poly(dT) and denaturated DNA were tested using three different assays: (a) under conditions which include both magnesium and manganese ions as activators and high salt concentration (200 mM KCl); (b) with magnesium ions as activator and at lower salt concentration (20 mM KCl); (c) in the presence of both magnesium and manganese ions and with no other salt except the buffer, i.e. under conditions which increase the importance of electrostatic forces between enzymes and polyanionic polymers and where poly(rA) is inhibitory (70-85%). Under all these conditions both poly(vU) and poly(vA) did not show inhibitory activity.

The transcription of single stranded polynucleotides was inhibited in all cases where the template was single stranded and the vinyl analog was complementary to the template; i.e. in the pairs poly(rA)-poly(vU), poly(dA)-poly(vU), poly(rU)-poly(vA) and poly(dT)-poly(vA) (Fig. 1). Poly(vA) was a more potent inhibitor than poly(vU) and the polyribonucleotide templates were more easily inhibited than the polydeoxyribonucleotide ones (Fig. 1). The nature of the inhibition was investigated by variation of the template concentration. The results (Fig. 2) show that in both the studied combinations [poly(vA)-poly(rU) and poly(vU)-poly(rA)] the inhibition

![Graph](image.png)

Fig. 1: Effect of vinyl analogs on RNA synthesis by E. coli RNA polymerase using single stranded polyribonucleotide or polydeoxyribonucleotide templates as given. When poly(rA) and poly(dA) were used as templates MnCl$_2$, 2.5 mM was used as the activator ion and $^{14}$C UTP was the substrate. When poly(rU) and poly(dT) were used as templates, MnCl$_2$, 2.5 mM, and MgCl$_2$, 5 mM were included as activators and $^{14}$C ATP was the substrate.
by analog was prevented by a high concentration of template and that maximum velocities are about the same for both the inhibited and uninhibited reactions. Thus the inhibitions are of the competitive type and are compatible with a mechanism in which the enzyme, template, and analog react to form a complex which excludes free template from the enzyme binding site.

No inhibition was observed in the non-complementary pairs: poly(rA)-poly(vA), poly(dA)-poly(vA), poly(rU)-poly(vU) and poly(dT)-poly(vU).
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Analogs in these experiments were used in 0.3 mM concentrations. Conditions of assay are given in Methods under b.

DISCUSSION

RNA polymerase is an enzyme which can utilize a variety of single or double stranded polynucleotides as templates. The present studies show that inhibition occurs only under conditions which favor the formation of a stable complex between the template and poly(vA) or poly(vU). Vinyl polymers are ineffective as inhibitors in cases where the template which is complementary to vinyl polymer is already complexed with the corresponding polydeoxyribonucleotide. Apparently double stranded complexes are more stable than any polynucleotide-vinyl polymer complex and thus no interference with template activity occurs. Also transcription of denatured DNA, a template which presumably does not contain any extensive stretches complementary to the vinyl polymers, is not affected by the latter.

These results are in agreement with studies of effects of vinyl polymers on other enzymatic systems. Vinyl polymers in a similar way act on systems containing α, β and γ DNA polymerases (isolated from mouse myeloma) and on the reverse transcriptase (from murine leukemia virus), the only difference being that the present results are less complex since the requirements of RNA polymerase for templates are less limiting than for the other studied enzymes. A similar situation was also found in studies of effects of vinyl polymers on protein synthesis; translation is blocked, or potentially blocked, only when vinyl analog and message are complementary.

This situation contrasts with the pattern of inhibition which is observed when polyanionic vinyl analogs of polynucleotides are used. These compounds can be prepared by the copolymerization of N-vinyl bases with acrylic acid or maleic anhydride. Some of these copolymers affect the protein synthesis in a way which is not sensitive to the complementarity of bases of the copolymer and of the message, but which occurs through the formation of a complex between the copolymer and protein component of ribosomes. A similar copolymer was also tested for its effect on RNA polymerase (β type isolated from calf thymus) and was found to be inhibitory even when double stranded DNA was used as a template. RNA polymerase from E. coli can be inhibited in a similar way by other polyanions which have only the polyanionic character and not any other feature in common with nucleic acids, like heparin or copolymer of glutamic acid.
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and tyrosine^13. All these inhibitions occur probably by competition for polynucleotide binding site(s) on the enzyme.

Thus the introduction of electronegative charge into an analog of the polynucleotide apparently changes its fundamental mode of action. Electro-neutral analogs of polynucleotides mimic exclusively the base pairing ability of nucleic acids and are not effective in any interaction where the electronegative charges are important, as they apparently are in interactions of polymers with the enzymes of nucleic acid metabolism. Introduction of electronegative charge can bring into play these interactions and thus the inhibitory potency of polymers is increased, but apparently in a rather non-specific manner, since the potential of a polymer to act in any specific base pairing fashion with the natural templates is lost. This follows from the observation that the electronegative analogs of the above type do not form complementary complexes with polynucleotides^15-16.

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