SUBSTRATE DEPENDENT HETEROGENEITY OF INITIATION BY RNA POLYMERASE FROM THERMOPHILIC *B. MEGATERIUM*

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1. Introduction

The rate of RNA synthesis, catalyzed by DNA-dependent RNA polymerase from thermophilic *B. megaterium*, drops drastically at temperatures below 30° [1, 2]. This drop is not observed when initiation takes place at 40° and RNA synthesis is followed at lower temperatures. Kinetic experiments with inhibitors of initiation such as rifampicin or high ionic strength suggest that only a fraction of RNA polymerase molecules is able to initiate RNA synthesis below 30° and that the number of such "active" molecules decreases with falling temperature [2]. Inability to initiate RNA synthesis is not due to failure of the enzyme to form a complex with DNA at low temperatures, since, as shown [3] by the nitrocellulose filter technique of Jones and Berg [4], complex formation of RNA polymerase from thermophilic *B. megaterium* with DNA is not temperature dependent.

In this communication we present data showing that such a heterogeneity of initiation, when a fraction of RNA polymerase molecules is unable to start RNA synthesis, is observed not only at low temperatures but also at low concentrations of substrates.

2. Materials and methods

The thermophilic *B. megaterium*, strain 178 [5], was kindly supplied by Dr. L.Loginova. Bacteria were grown at 60° as described previously [2]. RNA polymerase was purified by the following stages: (1) cell lysis with lysozyme; (2) DNA precipitation with streptomycin sulfate; (3) protamine sulfate fractionation by the procedure of Chamberlin and Berg [6]; (4) ammonium sulfate fractionation; (5) gel filtration through a Sephadex G-200 column. Details of the purification have been presented elsewhere [2]. Composition of the reaction mixtures and conditions of incubation are given in the legends to the figures.

3. Results and discussion

The rate of RNA synthesis catalyzed by RNA polymerase from thermophilic *B. megaterium* is very slow at 20°; incorporation of radioactivity into RNA during the first 10 min of reaction did not exceed that in the control sample without the enzyme. However, if RNA polymerase is preincubated with DNA and all four nucleoside triphosphates at 40° and then transferred to 20°, RNA synthesis continues at a considerable rate, about a quarter of that at 40° (fig. 1). Such a stimulation of RNA synthesis is observed only if initiation is achieved during preincubation: preincubation of RNA polymerase with DNA alone, nucleoside triphosphates alone or DNA and a single nucleoside triphosphate [2] does not stimulate RNA synthesis at 20°.

These results show that the rate of RNA synthesis at 20° may serve as a measure of the number of initiations during preincubation, thus providing a simple method of following the kinetics of initiation by this RNA polymerase. The kinetics of RNA initiation at 33° at different nucleoside triphosphate concentrations was measured as follows: samples containing standard concentrations of T2 DNA, enzyme, and salts were preincubated with varying concentrations of all four nucleoside triphosphates at 33°. At intervals, samples were cooled, supplied with ^{14}C-UTP and

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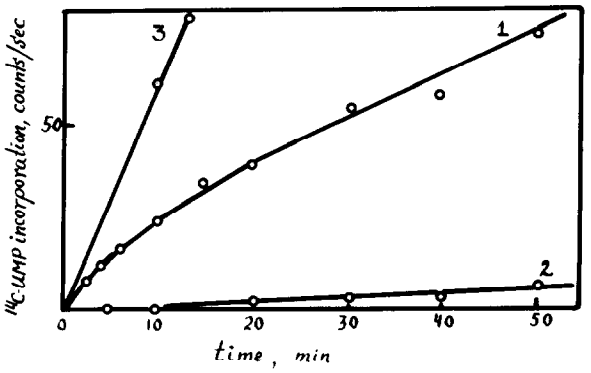

Fig. 1. Kinetics of RNA synthesis at 20° with and without preincubation at 40°. Samples (0.5 ml), containing tris-HCl, pH 8.0, 50 μmoles; $MnCl_2$, 1 μmole; T2 DNA, 50 μg; each of the four unlabeled nucleoside triphosphates, 200 nmoles, and RNA polymerase, were preincubated at 40° for 5 min. After cooling the samples were supplied with ^{14}C-UTP and transferred to 20°. At intervals samples were removed, precipitated with trichloroacetic acid (with addition of 0.5 mg per sample of albumin as a carrier), washed with cold 5% trichloroacetic acid on millipore filters, and counted in a gas-flow counter for 100 sec (curve 1). Kinetics of ^{14}C-UTP incorporation was followed in an identical manner at 20° (curve 2), and 40° (curve 3), but without preincubation.

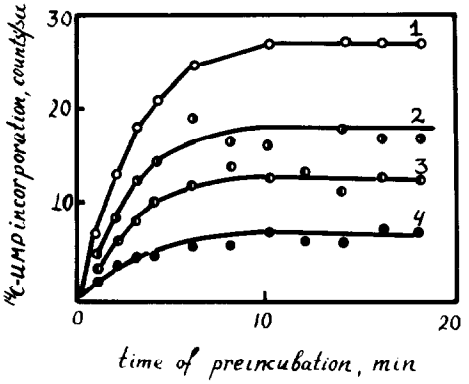

Fig. 2. Kinetics of initiation at 33° at different concentrations of substrates. Samples identical to those in fig. 1, except for nucleoside triphosphates concentrations, were preincubated at 33°. At intervals samples were quickly cooled, supplied with all four nucleoside triphosphates up to a concentration 0.4 μmoles/ml of each and ^{14}C-UTP and immediately transferred to 20° for 10 min. Incorporation at 20° is a measure of initiation during preincubation (see text). Nucleoside triphosphates concentrations during preincubation were (in μmole/ml): 0.4 (curve 1), 0.08 (curve 2), 0.04 (curve 3), and 0.02 (curve 4).

unlabeled nucleoside triphosphates to a standard concentration and transferred to 20° for 10 min. After 8–10 min of preincubation at 33°, the rate of RNA synthesis at 20° reaches a plateau which decreases with decreasing substrate concentration (fig. 2). Similar curves were obtained with preincubation at 37° and 40°. The time for initiation to occur was independent of nucleoside triphosphate concentration at all temperatures tested.

These results indicate that it is not the *rate* of initiation but the *number* of RNA polymerase molecules able to initiate which depends on the concentrations of substrates. In fact, if the concentrations of substrates had determined the rate of the initiation, all the curves would have reached the same plateau but at different times.

Inability of a fraction of RNA polymerase molecules to initiate is not caused by inactivation of either the enzyme or template since addition of standard concentrations of substrates to a reaction mixture raises RNA synthesis at 40° to a normal value even after a prolonged preincubation at low substrate concentrations. Nor is failure to initiate RNA synthesis a result of exhaustion of nucleoside triphosphates, since with the amounts of RNA polymerase used only a small percentage of the added substrates were incorporated into RNA during preincubation.

As was shown by Anthony et al. [7], initiation performed at high nucleoside triphosphate concentrations results in a pronounced stimulation of RNA synthesis after a transfer of RNA polymerase from *E. coli* to a low concentration of substrates. Although these authors explain their results in terms of initiation rates, it may be suggested that the main effect here is actually caused by inability of a fraction of RNA polymerase molecules to initiate at low nucleoside triphosphate concentrations.

Just as with low temperatures, failure of a fraction of the enzyme molecules to initiate at low substrate concentrations may be attributed to heterogeneity of either the RNA polymerase molecules themselves or of the promotor sites in DNA to which the enzyme attaches. The existence of threshold levels of both substrate concentrations and temperatures must be

postulated below which initiation is impossible, and which differ for different RNA polymerase molecules. Existence of such critical substrate concentrations and temperatures may indicate that cooperative change in conformation of the RNA polymerase molecule is required for initiation.

If heterogeneity of initiation is indeed based on heterogeneity of promotor sites in DNA, changes in substrate concentration might be expected to influence the proportion of different species of mRNA transcribed from the same DNA template and the balance of activities of different genes may be regulated by substrates. This hypothesis is now under investigation.

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