SPECTROSCOPIC DETERMINATION OF RNA POLYMERASE PROMOTER SITES

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1. Introduction

That T7 DNA possesses three specific promoters for *E. coli* RNA polymerase is now well established. Dunn and Studier [1], by side-specific cleavages, Minkley and Pribnow [2] and Dausse et al. [3], by selective initiation with specific dinucleotides, demonstrated that the *E. coli* enzyme initiated mainly at three sites situated on the left DNA end. These three specific promoters were furthermore mapped by electron microscopy [4–6].

Using ultraviolet differential absorbance, we have shown [7] that binding of RNA polymerase to DNA provoked an hypochromic variation of the DNA spectrum. The percent of hypochromicity at a chosen wavelength versus concentrations of enzyme to DNA gave a two-step linear relation; the intersection of the two slopes reflected the affinity of the enzyme for the nucleic acid. Moreover, this relation allowed to deduce quite exactly the number of strong binding sites which intervene in the interaction.

Binding of RNA polymerase to DNA varies according to the incubation conditions. Mangel and Chamberlin [8] have shown that temperature affected binary complexes formed between enzyme and nucleic acid. They suggested that two kinds of complexes, open or closed ones, could exist.

Dausse et al. [9] have also recently evidenced that the utilization of T7 DNA early promoters differed according to temperature. It was therefore interesting to test if differential absorbance would reflect these variations.

Abbreviations: DNA dependent RNA polymerase (EC 2.7.7.6) core enzyme is noted PC. Enzyme with the full σ complement is noted E^{σ}

2. Materials and methods

2.1. *Enzymes*

E. coli RNA polymerases were prepared by A. Ruet according to Burgess [10]. Both enzymes (PC and E^{σ}) were pure proteins as seen by gel electrophoresis.

2.2. *Nucleic acid*

T7 DNA was extracted by phenol from T7 bacteriophages grown on *E. coli* B. The separated strands were prepared by J. P. Dausse according to Summers and Szybalski [11].

2.3. *Spectroscopic method*

Ultraviolet difference spectra were recorded on a Zeiss DMR 10 spectrophotometer with thermostated cell holders, using 0.1 sensitivity scale. Procedure was the same as previously described [7].

3. Results and discussion

At 5°C, Dausse et al. [9] have observed that one initiation site was preferentially used by *E. coli* RNA polymerase, while two sites were utilized between 10–17°C and three sites at higher temperatures. Figure 1A evidences that differential absorbance at 260 nm correlates with these results and that the number of enzyme E^{σ} molecules per T7 DNA molecule are different when the temperature is maintained at different values. The stoichiometries obtained are in good agreement with the number of early promoters selected by *E. coli* RNA polymerase in vitro [9] under the same temperature conditions.

When RNA polymerase without σ factor (enzyme PC) is added to a T7 DNA solution at 25°C, no break

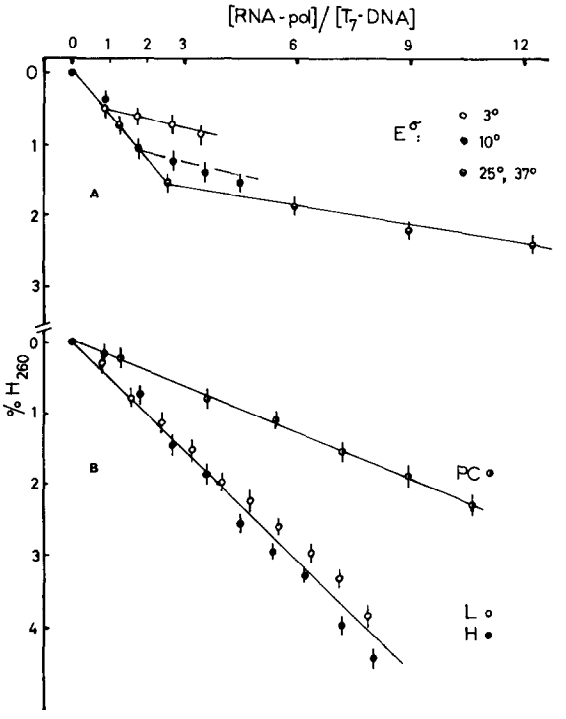

Fig.1. Absorbance variations of T7 DNA at 260 nm with increasing quantities of *E. coli* RNA polymerase. The percent of hypochromicity is plotted as a function of enzyme molecules per T7 DNA molecule. (A) Addition of E^{σ} at different temperatures as indicated. (B) Addition of PC to double-stranded DNA, or of E^{σ} to separated strands of T7 DNA, at 25°C. Binding buffer: 0.04 M Tris pH 7.9–0.01 M $MgCl_2$–0.05 M NaCl–0.0001 M dithiothreitol.

is found (fig.1B). This agrees with the fact that PC does not bind DNA at specific sites [12,13].

In addition, we studied the binding of complete enzyme E^{σ} to separated strands L and H of T7 DNA. The observed hypochromicity is directly proportional to RNA polymerase concentration and there is no change in the slope. This implies that double-stranded structure is necessary for specific site recognition. This also suggests (and confirms) that specificity does not merely come from a chosen base sequence but is determined by a special conformation.

The generally accepted hypothesis for RNA polymerase-promoter interaction implies that the DNA is locally melted by RNA polymerase [14,15]. It seems surprising to observe a hypochromic effect when the enzyme is added to DNA. In addition, since only a small part of the genome is involved in the interaction, this hypochromicity exceeds largely what could be expected even if, as suggested by Niyogi and Underwood [16], the RNA polymerase–DNA interaction concerns 50 base pairs.

Nevertheless, differential absorbance allows to differentiate a specific binding from a non-specific one ([7], and fig.1A). It permits, moreover, to observe that, according to the temperature, only one, or two, or three early promoters of T7 DNA are bound by RNA polymerase, as previously shown in vitro [9].

The origin of such an effect is yet to be explained. One might suggest that the binding of RNA polymerase to DNA implies a different distribution of charges which might involve a hypochromic variation of the DNA spectrum.

Another suggestion might be a long range effect of the enzyme which might propagate along the DNA molecule and provoke a conformational change over more than the 25 or 50 base pairs involved in binding. One might therefore envisage for *E. coli* enzyme a higher probability of being at the specific promoters rather than at other sites on the DNA molecule and which could be at the origin of the change in hypochromism at higher RNA polymerase concentrations.

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