Complex Formation of *E. coli* RNA Polymerase with Bacteriophage T2 DNA: Long-Range Effects in DNA

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Abstract. Conformation behavior of phase T2 DNA in the process of its interaction with *E. coli* RNA polymerase was studied using spin labeling technique. T2 DNA was modified by the spin-labeled imidazole at OH-groups of glucosylated cytidine residues. It was shown that the binding of RNA polymerase under the conditions favoring the formation of open promoter complexes induces specific conformational changes in the spin-labeled DNA. The observed conformational changes encompass not only the promoter regions of DNA which are involved in direct contacts with RNA polymerase molecules but extend over remote DNA sites (long-range effect). In relation to this effect, current theoretical models of DNA dynamics are discussed.

Key words: Conformational transition, *E. coli* RNA polymerase, long-range effects, N-(2,2',5,5'-tetramethyl-3-carboxypyrrolidine-1-oxyl)-imidazole, open promoter complex, T2 DNA

For most of the studied promoters utilized by *E. coli* RNA polymerase $E\sigma^{70}$ the formation of open enzyme-promoter complexes was found to be the rate-limiting stage in the process of initiation of RNA synthesis [1]. The efficiency of the formation of open promoter complexes appeared to be dependent on the structure of promoter areas of DNA which are involved in direct contacts with RNA polymerase molecules [2]. Recently it has been established that some remote DNA sites may also participate in this process, the distance from these sites to the basic promoter elements being from several tens to hundreds of base pairs [3–7]. Thus the question arises of how these remote sites may exert their control of the transcription initiation and what physico-chemical properties of DNA give rise to these long-range distance effects.

In the present work conformational changes of DNA during its interaction with *E. coli* RNA polymerase $E\sigma^{70}$ were studied. Phage T2 DNA was modified randomly along its entire length with a spin label which covalently binds to OH-groups of glucosylated cytosine residues. It was shown that the formation of open promoter complexes induces conformational changes in T2 DNA marked by an increased mobility of the spin label. The experimental data obtained were difficult to interpret in any other way except that the conformational changes induced in promoter sites

by RNA polymerase are transmitted to a considerable part of DNA molecule. These results support our preliminary data on the existence of long-range transmission in DNA [7] providing additional evidence to assign these changes to formation of open, transcriptionally competent promoter complexes. Different theoretical models of DNA dynamics are considered to search for mechanisms of long-range transmission in DNA which may account for the observed effect.

Materials and Methods

DNA preparation. T2 DNA was purified by phenol-extraction from phage T2 concentrates [8]. Native DNA ($90 - 110 \times 10^3$ kDa) had 1–3 single-stranded breaks determined according to [9].

Purification of E. coli RNA polymerase. *E. coli* RNA polymerase was purified according to Berg [10]. Specific activity of the purified enzyme with phage T2 DNA as a template was 7000–7500 units determined according to [10].

DNA labeling procedure. The spin label N-(2,2',5,5'-tetramethyl-3-carboxypyrroline-1-oxyl)-imidazole (I) was synthesized as recommended in [11]. Spectral properties, element composition and melting temperature of the label were the same as in literature [11].

Spin labeling of T2 DNA with the label I was carried out as described earlier [12] with minor modifications [13]. 5 mg of spin label I in 0.5 ml of 0.01 M phosphate buffer, pH=7.5, containing 30% ethanol was added to 1 ml of DNA solution (0.5 mg ml^{-1} in 0.01 M phosphate buffer, pH=7.5, 0.1 M NaCl). The reaction mixture was incubated for 10 h at 35 °C. Unbound label was removed by gel filtration on Sephadex G-50. The extent of modification was determined from comparison of EPR peak intensity of standard solution of the label I and of the solution of the spin-labeled DNA of known concentration at pH 11–12 when the label is detached from DNA.

Formation of DNA-RNA polymerase complexes and RNA synthesis. Conditions for DNA-RNA polymerase complex formation and RNA synthesis were described in [14]. The standard reaction mixture for RNA synthesis (0.2 ml) contained 0.015 mg T2-DNA (either modified or unmodified), 0.01 mg of RNA polymerase in 0.01 M Tris-HCl, pH=8.0, 0.01 M MgCl_2 , 0.05 M NaCl and ribonucleoside triphosphates, 0.3 mM each. $[^{14}\text{C}]\text{ATP}$ (40 Ci mmol^{-1}) was used as a radioactive substrate and RNA synthesis was estimated by the incorporation of $[^{14}\text{C}]\text{AMP}$ into acid-insoluble pellet. Formation of promoter complexes was carried out in the standard solution as above, for 10 min at the temperatures specified below.

Trypsin hydrolysis of RNA polymerase. RNA polymerase in complexes with T2-

Table I. Physico-chemical and template properties of spin-labeled T2 DNA

Parameter	unmodified T2 DNA	spin-labeled T2 DNA
molecular weight, Da	9.0×10^7	9.0×10^7
number of breaks per DNA molecule	2.5	2.5
hyperchromicity, %	38.5	38.0
melting temperature T_m , °C	81.0	81.0
melting interval ΔT_m , °C	5.0	5.0
[^{14}C] AMP incorporation into RNA, nmol	3.5 ± 0.3	3.7 ± 0.3

DNA was treated with trypsin as described earlier [15]. RNA polymerase-DNA complexes were formed for 10 min at 37 °C as above, then 0.005 mg of trypsin per each 0.01 mg of RNA polymerase was added. Solution was incubated for 10 min at 37 °C; after proteolysis the reaction mixture was cooled to 7 °C and EPR spectra were recorded.

EPR measurements. EPR spectra were recorded on ‘Varian E-104A’ spectrometer with variable temperature controller (accuracy of 0.5 °C). EPR spectra were registered at 7 °C. For analysis of EPR spectra the parameter of rotational diffusion, τ_c , i.e. the time required for the label to rotate by an angle of about 40°, was calculated as described in [16].

Results

Chemical modification of nucleic bases by spin labels in native DNA is known to be nonspecific, and, as a result, to be followed by some destabilization of the double helix [7, 17]. Therefore to obtain spin-labeled DNA without disruption of its secondary structure and alteration of its functional activity, another type of modification which does not interfere with the intramolecular interaction between complementary bases was required. One of these modifications was proposed for DNA of T-even phages and it was developed for T2 and T4 DNAs [12, 13]. This modification is based on the unique feature of T-even phage DNA, in which cytosine residues are substituted by 5-hydroxymethylcytosine, the latter being glycosylated and thus providing free hydroxyl groups for chemical reaction. The application of spin-labeled imidazole derivative I which is a specific reagent with free OH-groups of sugar components of nucleosides, nucleotides, tRNA and polynucleotides [18] made it possible to introduce this radical selectively at hydroxyl groups of glucosylated cytidine units of DNA from T-even phages [12, 13, 18]. The high specificity of this modification was proved by the observation that DNAs containing no glucose such as T7 phage DNA or calf thymus DNA could not react with this spin label [13].

Figure 1. EPR spectra of the spin-labeled T2 DNA (a) and of free spin label I (b).

The conditions of the modification of T2 DNA with the spin label I have been established previously [12, 13]. The physico-chemical characteristics and template properties of the modified DNA are shown in Table I. These data bring evidence that this modification does not disrupt the DNA secondary structure. The hyperchromicity, melting temperature and melting profile, as well as molecular weight and the number of single-stranded breaks of the spin-labeled DNA are not different from those of the unmodified DNA and are in agreement with the data from literature [12, 13].

The line-shape analysis of EPR spectra of the spin-labeled DNA (Figure 1) shows that only one type of bound labels is present which is consistent with the high specificity of the modification. Comparison of EPR spectrum of the spin-labeled DNA with the spectrum of the free label shows that the motion of the label becomes more restricted after its attachment to DNA though the spectrum of the DNA-bound label can be characterized as weakly immobilized.

It is well-known that the parameters of EPR spectra are affected not only by rotational diffusion of a spin label but also by hydrogen bonding or polarity changes in its environment. The determination of traces of tensors g and A may be used to differentiate between the effects of the environmental polarity and of the motional averaging since the traces of these tensors are dependent only on hydrogen bonding or polarity changes and are unaffected by changes of the rotational correlation time, at least in the time range from 1000 to 0.1 ns [19]. The spectrum of the DNA-bound spin label is motionally narrowed and similar to the spectrum of the free label therefore the tensor values are essentially pulled toward their isotropic values. In order to recognize if the hydrogen bonding or environmental polarity of the spin label changes upon its attachment to DNA, the g and A values were determined. The data are presented in Table II. A comparison of these values indicates that

Table II. Characteristics of EPR spectra of spin-labeled T2 DNA in complexes with *E. coli* RNA polymerase

Reaction mixture	Incubation temperature, °C	g	$\underline{A}$, G	τ_c , ns
T2 DNA	7.0	2.0061	15.9	0.11
T2 DNA + holoenzyme	7.0	2.0060	16.0	0.11
T2 DNA + holoenzyme	37.0	2.0061	16.0	0.057
T2 DNA + core enzyme	37.0	2.0061	15.9	0.14
T2 DNA + holoenzyme (10 min, 37 °C)	37.0	2.0061	16.0	0.12
+ trypsin free spin label I	37.0	2.0061	16.0	0.025

they are almost identical for the free and DNA-bound labels and thus the change of hydrogen bonding or environmental polarity of the radical upon its interaction with DNA may be ruled out. Since the g and $\underline{A}$ values are not changed during interaction of the spin-labeled DNA with RNA polymerase (Table II), the alteration of EPR spectrum observed in this case (as it will be discussed below) may be ascribed to change of the rotational diffusion of the DNA-bound spin label.

The weak immobilization of the DNA-bound label makes it possible to use the isotropic time of rotational correlation of the spin label (τ_c) for characterization of its rotational mobility [reviewed in 7]. This parameter is 0.026 ns for the free label and it is much smaller than τ_c of the DNA-bound label (0.11 ns). In addition, the line shape analysis of EPR spectra of the DNA-bound label reveals no dipole-dipole interaction which could be observed between the neighboring labels in the case of the existence of spin-labeled cytidine tracks in the modified macromolecule. This is a further evidence for random labeling of cytidine units of T2 DNA [13] and is in agreement with the literature data on the structure of DNA of T-even phages [20, 21].

It can be seen that the modification does not disrupt T2 DNA secondary structure and has little if any effect on its template properties in RNA synthesis (Table I). Therefore the spin-labeled T2 DNA can be used to study conformational changes of DNA template during its interaction with *E. coli* RNA polymerase ($E\sigma^{70}$). For this purpose, EPR spectra of the spin-labeled DNA at different stages of DNA-RNA polymerase complex formation were registered. The values of the time of rotational correlation τ_c of the label as a characteristic of its rotational mobility were calculated and the data are presented in Table II.

The formation of open, transcriptionally competent promoter complexes between the spin-labeled T2 DNA and *E. coli* RNA polymerase (37 °C, presence of σ -factor) affects the rotational mobility of the label (Table II). The formation of open promoter complexes is known to be accompanied by a rather serious distortion of DNA structure involving local melting of 10–12 base pairs just upstream of the

transcription start site. These complexes being transcriptionally competent are able to initiate RNA synthesis in the presence of ribonucleoside triphosphates. In contrast the formation of RNA polymerase-T2 DNA nonspecific complexes resulted in no change of EPR spectrum of the label (at 7 °C or in the absence of σ -factor).

The data indicate that the formation of open promoter complexes is followed by a transition of DNA into a state with a greater mobility of the spin label (τ_c of the label decreases to 0.06 ns in this complex). In order to rule out the possibility that this increase of the rotational mobility of the DNA-bound label is due to its detachment from DNA during the formation of open promoter complexes, experiments on trypsin proteolysis of RNA polymerase in the complex with T2 DNA were carried out.

The aim of these experiments was to follow changes of the rotational mobility of the label during the release of the spin-labeled DNA from its complexes with RNA polymerase due to proteolysis of the enzyme with trypsin. The conditions of proteolysis resulting in the complete deactivation of RNA polymerase were used in the experiments. When the parameters of the spectrum of the spin-labeled DNA released from the complex with RNA polymerase are compared to that of the original spin-labeled DNA it is apparent that they are almost identical (Table II). The time of rotational correlation of the DNA-bound spin label which decreased in the open promoter complex turns back to its starting value of 0.11 ns after RNA polymerase proteolysis. These data rule out the possibility that the decrease of τ_c of the spin label during the formation of enzyme-DNA open promoter complexes is due to the detachment of the label from DNA. Therefore the increase of the mobility of the DNA-bound spin labels in the open promoter complex may be explained by a conformational transition of DNA into a state which is characterized by a faster motion of the label.

Discussion

One of the possible explanations for the observed decrease of τ_c of the DNA-bound label in the open promoter complex may be RNA polymerase-induced local melting of DNA leading to increased rotational mobility of nucleotides in the single-stranded regions. However, as it was mentioned above, the size of single-stranded regions in open promoter complexes is not greater than 10–12 bp. The spin-labeled T2 DNA used in these experiments contained 1 spin label per each 50–100 bp, spin-labeled cytosines being distributed randomly along its entire length. Taking into account such character of DNA labeling and the dimensions of the melted sites in open promoter complexes, the amount of radicals falling to the single-stranded regions should be negligibly small to contribute significantly to the observed EPR spectra.

It can be also suggested that direct contact of the DNA-bound spin labels with RNA polymerase could result in the faster motion of the label. It should be mentioned that promoters in DNA of T-even phages are spaced at intervals of 1000–

1500 bp [21], and therefore the concentrations of T2 DNA and RNA polymerase which provide the binding of the enzyme exclusively to promoter sites were chosen in the experiments. Taking into account the size of promoter regions interacting with RNA polymerase (40–60 bp) and the random labeling of DNA, the amount of spin-labeled cytosines contacting the enzyme in open promoter complexes should be negligible, not exceeding 5–10 percent of all spin labels attached to DNA. Again, the increase of the mobility of this small fraction of spin labels could not explain the observed changes of EPR spectra of the spin-labeled T2 DNA during the formation of open promoter complexes.

Therefore the data obtained could be attributed to structural changes of DNA itself indicating that the local conformational changes induced in rare promoter sites by RNA polymerase binding could be transmitted over a long distance thus initiating a cooperative change of most DNA duplex. The technique employed did not permit the distances for which these long-range effects propagate to be determined exactly. However it is obvious that they should be large enough compared to the length of promoter sites.

In order to search for a mechanism which could be responsible for the long-distance transmission in DNA, the main known types of internal motions of the double helix [22] were analyzed in relation to the results obtained and were considered in their correspondence to the current models which offer different explanations of long-range transmission in DNA [3, 22–24]. It should be emphasized that a distinguishing feature of the label used is its weak immobilization on T2 DNA. The value of τ_c of the DNA-bound label is about 0.1 ns and this parameter is pulled towards smaller values in complexes of the spin-labeled T2 DNA with RNA polymerase. This time scale of motions in nucleic acids was suggested to be related to motions of individual bases and base pairs or oscillations of small groups of atoms (sugars, phosphates and nitrous bases) [22, 25]. Thus, analyzing the mechanisms of the long-range effect observed, these dynamical modes of DNA rather than such slow motions as collective large-amplitude bending, twisting and global tumbling should be taken into consideration.

One of the mechanisms which may account partially for the observed effects is DNA wrapping around RNA polymerase molecule in open promoter complexes [3]. It is based on the ability of some DNA sequences to form bends and kinks [3, 24, 26]. Due to DNA wrapping around RNA polymerase molecule, some remote DNA regions could approach the enzyme closely enough thus enlarging the fraction of the DNA-bound spin labels in direct contact with the enzyme molecule. There is some evidence that DNA sequences surrounding promoter regions of T-even phages are bent [20], and therefore this mechanism may be one of the factors contributing to the observed experimental results.

Another explanation is based on the suggestion that a local change of DNA structure may induce some alteration of physico-chemical parameters of the surrounding DNA regions extending to sufficiently large distances. Examples of such effects were reported in literature. Namely, it was shown that the transition of

a 16-mer GC sequence in the center of a restriction fragment of 1097 bp to Z-conformation results in the change of physico-chemical characteristics of the surrounding DNA such as torsional constant, susceptibility to S1 nuclease cleavage *et al.* [27]. This alteration of the physico-chemical characteristics covers the DNA region of 330–550 bp. Analogously in our experiments the local melting of 10–12 bp (or some other structural change) induced in promoter sites by RNA polymerase binding may affect the physico-chemical parameters of DNA over a distance of hundreds bp which in turn may lead to altered mobility of spin-labeled cytosines in this region.

Quite a different group of models assumes the propagation of a local conformational excitation as solitary waves along DNA chain [28–30]. Mathematical description of these models is based on nonlinear differential equations, their soliton solution being mathematical analogs of conformational changes moving along DNA chain. The models were developed for the description of the contribution of the rotational and/or transverse internal motions to the formation and dynamics of base-pair opening. Comparative analysis of the main characteristics of DNA internal motions involved in these models and in the rotational mobility of the DNA-bound spin labels is consistent with the view that solitary waves may be related to long-distance transmission. The view is reinforced by the existence of base-pair open state in the open promoter complexes induced by RNA polymerase binding.

It should be emphasized that all the above mechanisms are only hypothetical schemes and their applicability for the interpretation of our experimental results presents a problem due to the absence of many details. Thus further investigation, both theoretical and experimental, is required to clarify the mechanisms of long-range effects in DNA. The above models representing different approaches to the problem can be useful for designing this future experimental work.

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