Conformational changes in *E. coli* RNA polymerase during promoter recognition

Konstantin L. Brodolin*, Vasily M. Studitsky+ and Andrei D. Mirzabekov

W.A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow 117984, Russia

Received August 3, 1993; Revised and Accepted November 3, 1993

**ABSTRACT**

We analysed complexes formed during recognition of the *lacUV5* promoter by *E. coli* RNA polymerase using formaldehyde as a DNA–protein and protein–protein cross-linking reagent. Most of the cross-linked complexes specific for the open complex (RP₀) contain the β subunit of RNA polymerase cross-linked with promoter DNA in the regions: -50 to -49; -5 to -10; +5 to +8 and +18 to +21. The protein–protein cross-linking pattern of contacting subunits is the same for the RNA polymerase in solution and in RP₀: there are strong σ-β′ and β-β′ interactions. In contrast, only β-β′ cross-links were detected in the closed (RP_c) and intermediate (RP_I) complexes. In presence of *lac* repressor before or after formation of the RP₀ cross-linking pattern is similar with that of RP_I (RP_c) complex.

**INTRODUCTION**

*E. coli* σ⁷⁰ RNA polymerase (σ₇₀) is a multi-subunit protein consisting of the core enzyme (αββ′) and the σ subunit. The interaction of RNA polymerase with promoter DNA can be described as a four-step process: R + P → RP₉ → RP_I → RP₀ → transcription (for reviews see: 1, 2).

At 37°C, σ₇₀ first contacts the surface of the promoter DNA helix forming a 'closed' complex (RP₉). Subsequently, the 'closed' complex is transformed into an 'open' complex (RP₀) in which the DNA helix is melted around the transcription initiation point. The open complex is competent to initiate transcription upon the addition of nucleotides.

For several promoters the initiation process was studied using DNase I and hydroxyl radical footprinting methods (3–6). While the footprints of the whole RNA polymerase on DNA for different intermediate complexes are well characterised, the topography of different subunits on promoter DNA and the mechanism of transcription initiation remain unknown. DNA–protein cross-linking techniques are powerful tools for solving this problem. Using photo-activated BrU-substituted DNA (7), laser and UV irradiation (8–10), DMS crosslinking (11, 12) the contacts of β,β′ and σ subunits with DNA were identified.

Formaldehyde (FD) induces 'one atom' (2 Å) protein–protein and DNA–protein cross-links between molecules in close contact (13). The wide spectrum of reactive sites on the protein (13) and on DNA (14) make cross-linking very efficient. Studies of the structure of the core nucleosome show that the yield of the protein–protein cross-linked complexes reflects the strengths of the interactions between proteins and their spatial arrangement inside the nucleosome (15, 16). FD cross-linking does not change the sedimentation characteristics or the endonuclease protection pattern of SV40 minichromosome (17, 18). FD does not cross-link proteins which do not interact with each other and DNA *in vitro* (18, 19) and *in vivo* (20).

The stability of cross-links at room temperature and the ease with which they can be reversed by heating make possible direct analysis of the contents of the cross-linked complexes (15, 16, 20).

We used FD to study the structure of free RNA polymerase and different intermediate complexes with lacUV5 promoter DNA during RP₀ formation. The influence of *lac* repressor on the structure of transcription complexes was tested. The dual character of FD cross-links allows us to compare protein–protein and DNA–protein contacts at different stages of promoter recognition. The experimental scheme is shown on Fig. 1. It contains two parts: first determination of cross-linkable subunits in the RNA polymerase using two-dimensional gel electrophoresis. Second analysis of cross-linked patterns of initiation complexes by SDS-PAGE, determination of complexes composition by the immunoaffinity chromatography and mapping of the cross-links positions by the exonuclease digestion in gel.

**METHODS**

**DNA and proteins**

Plasmid pUC19 containing EcoRI DNA fragment bearing two tandem *lacUV5* promoters (21) was provided by Dr O. Voloshin (Moscow). DNA was 3' labeled by Klenow fragment of DNA.

*To whom correspondence should be addressed at: Laboratoire de Biochimie Cellulaire, College de France, 11 Place Marcelin Berthelot, 75231 Paris cedex 05, France
+ Present address: Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA
polymerase I, digested with HaeIII and the DNA fragments (167 bp and 123 bp in length Fig. 1) were isolated using standard methods (22).

*E. coli* RNA polymerase (Ec70) was isolated by method (23) modified as described (24) and was kindly provided by Dr L. Savochkina (Moscow). SDS-PAGE indicated that the polymerase was at least 80% saturated with the $\sigma$ subunit and contained at least 90% pure RNA polymerase. Enzymatic activity of the preparation was measured by promoter titration using a run-off transcription assay. RNA polymerase (60 nM) and different amounts of 123 bp fragment were preincubated in 30 $\mu$l of cross-linking buffer (50 mM HEPES (pH 7.5), 50 mM NaCl, 5 mM MgCl$_2$, 5% glycerol) for 10 min at 37°C, then NTPs in 5 $\mu$l of the buffer were added to start reaction (final concentrations: 0.25 mM each of ATP, CTP, and GTP, 50 $\mu$M of UTP and 5 $\mu$M of [\(\alpha\text{-}^{32}P\text{]UTP}]). After 10 min the reaction was stopped adding an equal volume of the loading buffer (95% formamide, 45 mM Tris-borate, 2 mM EDTA and dyes) and transcripts were resolved on 10% denaturing gel. The bands corresponding to 60 nucleotides run-off transcript were quantitated on scintillation counter. The holoenzyme was about 70% active. Polyclonal antibodies to RNA polymerase subunits were kindly provided by Dr S. Nikiforov (Moscow). *lac* repressor was kindly provided by Dr C. Muller-Hill.

**RESULTS**

Contacts between subunits of RNA polymerase in solution

The formaldehyde (FD) cross-linking pattern of free RNA polymerase (not bound to DNA) at different temperatures and salt concentrations was analysed by SDS-PAGE electrophoresis (Fig. 1A; Fig. 2). The presence of 0.1% FD induces the formation of protein–protein cross-linked complexes, designated C1, C2, C3 and a minor complex Cx. The accumulation of these complexes was accompanied by a decrease in the amounts of free $\beta, \beta', \alpha$, and $\sigma$ subunits, but the amount of the $\sigma$ subunit remains unchanged, suggesting that only $\beta, \beta'$ and $\sigma$ subunits are present in the cross-linked complexes. The same three major complexes occur at temperature range from 0°C to 37°C and at salt concentrations from 50 mM to 400 mM NaCl; the rate of C3 complex formation greatly increases with temperature (Fig. 2). At a given temperature, the relative yields of each complex depend only on the time of incubation in the presence of FD. After a long incubation with FD, C3 complex becomes predominant in parallel with disappearance of the C1/C2 complexes. This results suggest that C1/C2 are the intermediate complexes in C3 complex formation and C3 is the final FD cross-linking product.

**EXONUCLEASE FOOTPRINTING IN GEL SLICE**

The procedure is similar to that described in (28). All steps were done at 22°C. After separation of cross-linked complexes by SDS-PAGE, gel slices (approximate volume 50 $\mu$l) containing radioactive bands corresponding to different complexes were cut out of the gel and soaked in 10 gel volumes of 20 mM HEPES (pH 7.4), 0.1 mM EDTA buffer for 20 min. at 22°C. Then 5 $\mu$l of exonuclease buffer containing 67 mM Glycine/KOH pH 9.4; 50 $\mu$g/ml BSA and 0.1 U of phage $\lambda$ exonuclease were added and incubated for 40 min. Then 50 mM MgCl$_2$ was added to concentration of 2.5 mM and incubated for 15 min. The reaction was stopped by addition of 1 $\mu$l of 0.5 M EDTA and 2 $\mu$l of 10% SDS. Gel slices were crushed in Eppendorf tubes and DNA eluted for 4 hr at 65°C in 0.3 M Tris/HCl pH 8.0; 150 mM NaCl; 5 mM EDTA, precipitated with 3 vol. of ethanol, dissolved in formamide and loaded on 8% sequencing gel (29).

**Gel-retardation of initiation complexes**

Initiation complexes were pre-formed and separated by 4% PAGE 0.5×TBE (45 mM Tris borate pH 8.3, 1 mM EDTA). Electrophoresis was performed at 14°C or 37°C, as indicated. Gels were dried and autoradiographed.

**Figure 1. Schematic representation of methodological approaches used for investigation of RNA polymerase subunits contacts.** A: Cross-linking of RNA polymerase in solution. B: Cross-linking of RNA polymerase/ lacUV5-DNA complexes. C: The 290 bp DNA fragment bearing two tandem lacUV5 promoters used in experiments. Positions of transcription start sites (arrows) and −10; −35 consensus regions of promoter (black boxes) indicated.
The protein content of cross-linked complexes were analyzed in a second dimension gel after reversal of FD-induced cross-links by incubation of gel slices in Tris-containing buffer at high temperature (Fig. 3). As expected, there was no detectable amounts of α subunit in cross-linked complexes. C1 complex consists of the β and σ subunits, C2 complex contains the β and β' subunits and C3 includes β, β' and σ; consistent with the proposal that C1/C2 are are intermediates in C3 complex formation. Only weak bands corresponding to β, β' subunits are present in Cx.

Using the free subunits as molecular weight markers and assuming that the mass of C2 is 305 kDa (the sum of the masses of the β and β' subunits), a calibration curve was obtained. Calculated molecular weight masses for Cx, C1 complexes were 192 kDa and 220 kDa, respectively. Comparison with expected molecular weight for the cross-linked complexes suggests that Cx may be the complex of β (186 kDa) or β' (192 kDa) subunits with the α subunit. The expected molecular weight for C1 (β'/σ complex, 225 kDa) also correlates with the observed mobility of the complex.

The inter-subunit cross-linking patterns of initiation complexes formed at 14°C and 37°C are different. At 37°C ‘closed’ and ‘intermediate’ initiation complexes are short-lived and therefore are not easily accessible to biochemical analysis. Kinetic and structural studies have provided good evidence that different complexes predominate at different temperature intervals; the structure of the ‘frozen’ intermediates could be analysed by biochemical methods. For the lacUV5 promoter, the predominant complex is RP_C at 4°C, RP_P between 10°C and 20°C, and RP_O above 20°C (3, 30, 31).

We used a DNA fragment that contains two cloned tandem lacUV5 promoters (Fig. 1). Such a construction permits analysis of the cross-linking patterns on both the transcribed and non-transcribed DNA strands after 3'-end-labeling and restriction (21).

In order to obtain detectable cross-linked complexes, initiation complexes were incubated in presence of FD for 10 to 20 min. Under these conditions some complexes could contain several cross-links per complex and modification of aminoacids exposed on the surface of the protein as well as DNA bases could occur (13,14). As a result, structures of the complexes could be distorted.

To determine if FD cross-linking perturbs the structure of initiation complexes, RNA polymerase/promoter complexes were formed at 4°C, 14°C or 37°C (RP_C, RP_P, or RP_O respectively). cross-linked with FD and analysed by gel mobility shift assay (Fig. 4). Structure of the complexes were analysed previously using hydroxyl radical footprinting and DNA–protein cross-linking (12). It was shown that lower complex corresponds to closed complex (RP_C) and upper complexes correspond to intermediate (RP_P, 0°C and 14°C) or open (RP_O 37°C) complexes, respectively. Neither the mobility nor the yield of RP_P and RP_O were changed on incubation with 0.1% FD; similar data were obtained for RP_O (not shown). Moreover, fixation with FD partly prevents RP_C → RP_P and probably RP_P → RP_O transitions because complexes cross-linked at 14°C give the electrophoretic pattern characteristic of RP_P even if electrophoresis is run at 37°C (Fig. 4; lane 4); while without

---

Figure 2. Protein–protein cross-linking pattern of RNA polymerase in solution. 3.5–15% gradient SDS-PAGE of the RNA polymerase cross-linked by 0.1% FD in presence of 0.4 M (lane 1) or 150 mM NaCl (lanes 2, 3 and 4). Lanes 2, 3 and 4 contain 1 µg and lanes 1 and 5 contain 2 µg of protein. The RNA polymerase was incubated with FD for 20 min. at 0°C (lane 2) and 22°C (lane 1, 3) or 10 min. at 37°C (lane 4). Lane 5 contains the RNA polymerase not treated with FD. Cross-linked complexes having different mobilities in the gel (Cx, C1, C2 and C3) and RNA polymerase subunits are marked.

Figure 3. Analysis of protein content of the cross-linked complexes in two-dimensional SDS-PAGE. Electrophoresis of cross-linked complexes (0.1% FD, 400 mM NaCl for 20 min at 22°C) in the first dimension was performed as described in Fig. 2. The gel slice from the first dimension gel was polymerised on the second dimension 8% SDS-PAGE after reversal of the FD cross-links by heating. Mobilities of the cross-linked complexes (C1, C2 and C3) in first dimension marked. Free RNA polymerase was used as MW marker (M).

Figure 4. Gel mobility shift assay of initiation complexes with or without cross-linking by FD. The '167 bp' DNA fragment was used. Initiation complexes were preformed at 4°C (lane 1) 14°C (lanes 2, 3, 4, 5) or 37°C (lane 6) for 15 min and cross-linked by 0.1% FD (lanes 1, 2, 3, 4) for 20 min or directly loaded on the gel (lanes 5, 6). The gel was run at 14°C (lanes 1, 2, 3) and 37°C (lanes 4, 5, 6).
fixation pre-formed RPc and RPi complexes transform to RPo on electrophoresis at 37°C. We conclude that 0.1% FD does not induce significant perturbation of the structure of the initiation complexes; however we cannot exclude a possibility of minor rearrangements of the complexes which are not detectable by gel shift assay.

To find out if DNA-protein or protein-protein interactions are different in the different initiation complexes, RPc, RPi and RPo, were cross-linked with FD and analysed by gradient SDS-PAGE (Fig. 1B; Fig. 5). The pattern of bands of slow mobility (D1, D2, D3) complexes obtained at 37°C (Fig. 5A, lanes 2, 3) is similar to that obtained for free RNA polymerase (Fig. 2).

We propose that the low mobility complexes D1, D2 and D3 correspond to C1 (β′/σ), C2 (β/β′) and C3 (β′/β′/σ) complexes cross-linked to DNA. This proposal is consistent with the observation that the relative yields of D1, D2, D3 at given concentration of FD correspond to the yields of C1, C2 and C3, respectively. The complexes having highest mobility in the gel were identified as single subunits of the RNA polymerase cross-linked to DNA by comparing these complexes with those formed after DNA-protein cross-linking at 14°C (Fig. 5B; Fig. 5). The pattern of bands of slow mobility (D1, D2, D3) complexes obtained at 37°C (Fig. 5A, lanes 2, 3) is similar to that obtained for free RNA polymerase (Fig. 2).

We propose that the low mobility complexes D1, D2 and D3 correspond to C1 (β′/σ), C2 (β/β′) and C3 (β′/β′/σ) complexes cross-linked to DNA. This proposal is consistent with the observation that the relative yields of D1, D2, D3 at given concentration of FD correspond to the yields of C1, C2 and C3, respectively. The complexes having highest mobility in the gel were identified as single subunits of the RNA polymerase cross-linked to DNA by comparing these complexes with those formed after DNA-protein cross-linking using DMS (12). Electrophoretic analysis of the fraction bound to Protein A-Sepharose containing polyclonal antibodies to β, β′ subunits shows that all the cross-linked RPo-specific complexes are specifically retained except the σ/DNA complex (not shown). Therefore all protein-protein cross-linked complexes contain β or β′ subunits.

The cross-linking patterns at 4°C (RPc) and 14°C (RPo, RPc, Fig. 5) are different from patterns obtained for free RNA polymerase (Fig. 2) and RPo. Both at 4°C and 14°C only one low-mobility complex is observed (D2; β/β′-DNA); the two complexes containing the σ subunit (D1 and D3) are not visible. The pattern of high mobility cross-linked complexes (monomeric subunits cross-linked to the DNA fragment) is also changed but the σ, β and β′-specific complexes are present; the changes in mobility of the complexes relative to RPo reflect different sites of cross-linking to DNA (10, 12). Therefore the absence of the D1 and D3 after cross-linking of RPc cannot be explained by absence of σ- or β′-DNA contacts. Because C1 and C3 complexes were observed after cross-linking of free RNA polymerase even at low temperature, the difference in the pattern of cross-linked complexes cannot be explained by a difference in specificity of cross-linking at 14°C vs. 37°C.

These data suggest that cross-linking between β′ and σ subunits is much less efficient in RPc and RPi probably due to disruption of contacts between the β′ and σ subunits on binding of the RNA polymerase to the promoter DNA; the protein-protein contact pattern similar with that of RNA polymerase in solution is restored in RPo.

The presence of lac repressor prevents formation of RPo.

It has been shown that lac repressor inhibits the transformation from 'closed' to 'open' complex (32). To test whether the presence of lac repressor changes the structure of the intermediate complexes, it was added either before or after formation of the RPo; cross-linked complexes were analysed after incubation.
with FD (Fig. 5). The cross-linking pattern of the RNA polymerase/lac repressor/DNA complex was identical to that of the RP0 and RP1 complexes. This shows that the difference in the pattern between RP0 and RP1 is not due to different chemistry of FD cross-linking at different temperatures and also suggests that protein—protein interaction are different in the two complexes. The pattern does not depend on the order of addition of the components: lac repressor induces a change of the pattern of the completely formed stable RP0. The same pattern was observed in the presence of lac repressor at 14°C (not shown). Repressor alone is not cross-linked to DNA and does not induce new contacts of RNA polymerase with DNA. We also were unable to detect any protein—protein cross-linked complexes containing lac repressor subunits.

Mapping of the linear arrangement of cross-links to promoter DNA in RP0

To localise the positions of cross-links on DNA, cross-linked complexes were resolved by SDS-PAGE (Fig. 5), bands corresponding to cross-linked complexes were cut out and digested in situ with phage λ exonuclease in the gel slices. DNA was isolated and analysed in a sequencing gel (Fig. 6). The most intensive cross-links are specific for the non-transcribed DNA strand and are localised in the —10 to —5 promoter region; less strong interactions were detected at —50 to —48; +5 to +8 and +18 to +21 regions. This pattern of contacts is characteristic of all DNA—protein cross-linked complexes with the exception of α/DNA complex. The cross-linked pattern specific for the transcribed DNA strand (Fig. 6B) is much less pronounced (detected near the positions +5 and —14); however, it is also clearly different from the digestion pattern of free DNA and similar for all the cross-linked complexes with the exception of the α-specific one. Only weak α-specific contacts were detected at —10 to —8 region of non-transcribed DNA strand.

The pattern of contacts on both strands of promoter DNA is almost identical for all the complexes. Because only the β′ subunit is present in all the cross-linked complexes (β′/α/DNA, β′/β′/DNA and β′/β′/α/DNA, Fig. 3), this pattern probably reflects the cross-linking of the β′ subunit to DNA. If this interpretation is correct, β-specific cross-links do not contribute significantly to the pattern. We do not exclude the contribution of α-specific cross-links in DNA—protein contacts of D1 and D3 complexes. But it seems highly unlikely that it will be responsible for major observed pattern of contacts.

DISCUSSION

The results of our work show that FD-induced protein—protein cross-linking patterns of subunits of E.coli RNA polymerase are different for RP0 and RP1 initiation complexes formed on lacUV5 promoter; the RP0-specific cross-linking pattern is similar with that of RNA polymerase in solution. All the major cross-linked complexes specific for RP0 contain the β′ subunit cross-linked at and downstream of the melted promoter region. Addition of lac repressor leads to a cross-linking pattern characteristic of RP0.

Protein—protein cross-linking of the RNA polymerase in solution

The predominant form of E.coli RNA polymerase in vivo is the multisubunit holoenzyme complex (α2ββ′α; (1)). The holoenzyme monomer is stable in vitro at ionic strengths higher than 350 mM; at lower concentration of NaCl, the predominant form of RNA polymerase is a dimer of holoenzymes (33). The cross-linking products obtained after incubation with FD at low (150 mM) vs. high (400 mM) ionic strength are very similar (Fig. 2); indicating that cross-links are formed mainly between subunits inside the holoenzyme.

Incubation of the holoenzyme in presence of FD leads to formation of two major protein—protein cross-linking products: β/β′ and β′/α (Fig. 3). This indicates that the structure of RNA polymerase could be asymmetric, with the α subunit interacting mainly with the β′ subunit. Data on RNA polymerase assembly in solution and data from scanning immuno-electron microscopy also shows that the β′ and α subunits are located in close proximity in the holoenzyme (34, 35). In previous studies of the subunit arrangement of RNA polymerase in solution using bifunctional di-imido esters of different length (from 11.5 to 15 Å) as cross-linking reagents contacts of all subunits with each other were detected (36, 37). Di-imido esters induce protein—protein cross-links only between Lys residues (13, 36). Because FD can induce cross-linking between different amino acids including Lys, the difference in specificity of cross-linking can not explain the selective formation of only β/β′ and β′/α cross-links by FD. Probably, the cross-linking reagents used in previous studies were too long to identify only proteins in close contact. It is also possible that α and β′ subunit might actually be in contact but no cross-linked complex is formed because of absence of cross-linkable amino acid residues on contacting surfaces of the proteins. Thus, according to scanning immuno electron microscopy data, the α and σ subunits are localised close to each other (35), but we did not detect the α subunit in any of the major cross-linked complexes. The amount of a subunit cross-linked by di-imido esters is also very low (36).

Protein—protein cross-linking of RNA polymerase in initiation complexes

Earlier, the change of conformation of the RNA polymerase during RC—RP0 transition was suggested on analysis of temperature dependence of αP promoter recognition (38). But it was not considered in terms of RNA polymerase structure.

In present work, clear changes in protein—protein contacts upon binding of the polymerase to promoter DNA and during RP1—RP0 transition were detected (Fig. 5). β′/β′ and β′/α major protein—protein cross-linked complexes were detected on cross-linking of the polymerase in solution and in RP0. In contrast, in RP0 and RP1 only β′/β′ complex was observed (Fig. 5). Therefore in terms of protein—protein cross-linking pattern the structure of RNA polymerase changes on binding to promoter DNA and RP0 formation and changes back again during RP1—RP0 transition. Detailed analysis of the cross-linking sites of the subunits in RP0 and RNA polymerase in solution is necessary to prove the identity of contacting surfaces in both complexes.

Arrangement of cross-linking sites along lacUV5 promoter DNA

The changes in contacts between the subunits of RNA polymerase during the RP1—RP0 transition occur in parallel with the changes in DNA—protein contacts. Using dimethylsulphate as a DNA—protein cross-linking reagent, we have previously shown that the arrangement of cross-linking sites along the promoter DNA is different in RP1 vs. RP0 (12). The results of mapping FD-induced cross-linking sites along DNA in RP0 consistent with these data. The strongest contacts on the non-transcribed DNA strand (—50 to —48, —10 to —5, +5 to +8 and +18
to +21) correspond to the main DMS-induced cross-links in this regions (Fig. 7). The identity of the patterns specific for cross-linked complexes having different composition of proteins (Fig. 6) suggests that the β′ subunit is the major protein cross-linked to DNA in this region. Only weak contacts of σ subunit with −10 promoter region (in α/DNA complex) were detected (Fig. 6). Probably this reflects the limits of exonuclease mapping (the cross-linked protein of smaller size give the weaker protection from exonuclease) and the specificity of FD cross-linking because strong σ-specific contacts along promoter DNA were detected by other cross-linking methods (7, 10, 11).

The primary cross-linked site (−10 to −5) is located in the melted promoter region (−10 to +2) (39), probably because partially melted AT-rich DNA regions are the primary sites of FD attack (40). However, this does not explain the highly preferential cross-linking of β′ subunit only to the non-transcribed DNA strand. Our data are consistent with the proposal that active centre of RNA polymerase contacts tightly only non-transcribed DNA strand in RPO while transcribed DNA strand is free. Protection of the same region of the non-transcribed DNA strand in RPO from KMnO4 has been detected in previous experiments (41). Also transcribed DNA strand in melted region of promoter is more accessible to hydroxyl radicals while non-transcribed DNA strand is protected (5).

Interaction of lac repressor with the intermediate complexes

Straney and Crothers (32) have reported that the lac repressor can inhibit transcription without occluding the binding of the RNA polymerase to lacUV5 promoter; the authors suggested that the repressor blocks the closed-to-open complex transition. However, as was noted by Krummel and Chamberlin (42), this data are consistent with a proposal that the repressor blocks the transition from open to stable elongation complex, based on data showing that short abortive transcripts accumulate even in the presence of the repressor (32, 43).

Our data show directly that lac repressor induces formation of RPO or RPS on lacUV5 promoter at 37°C even when the repressor is added after formation of a stable RPO (Fig. 5). The data showing that abortive transcription occurs in the presence of the repressor (32, 43) in combination with above data indicate that it is more likely RPS complex, which initiates transcription. This conclusion is consistent with our previous data showing that contacts of the β′ subunit of the polymerase with the promoter do not change during the RPS → RPO transition; the structure of the catalytic centre is established during the RPO → RPS transition (12).

Our data and results of previous work (12) show that rearrangement of the σ-DNA and σ-β′ contacts is the only detectable change during the RPO → RPS transition leading to effective initiation. We speculate that the driving force for this transition could be the tendency of RNA polymerase to adopt a stable conformation, similar to the conformation in solution. The presence of lac repressor blocks this transition as well as promoter clearance by RNA polymerase.

ACKNOWLEDGEMENTS

We thank L. Savochkina for the preparation of the RNA polymerase, O. Voloshin for pUC19 plasmid containing the tandem lacUV5 promoter, S. Nikiforov for polyclonal antibodies to RNA polymerase subunits, C. Muller-Hill for lac repressor, M. Lazar and D. Clark for critical reading of the manuscript. We are grateful to Olga Mamaeva for technical assistance in preparation of manuscript.

REFERENCES