Subunit topography of RNA polymerase (E. coli) in the complex with DNA

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ABSTRACT

E. Coli RNA polymerase binding to different DNAs (from E. Coli, 5-bromodeoxyuridine (BrdUrd) substituted DNA and poly [d(BrU-A)] was induced with ultraviolet (U.V.) light to form protein-DNA crosslinked complexes. Two independent methods of analysis, polyacrylamide gel electrophoresis in SDS and chloroform extraction indicated the formation of a stable complex between the enzyme and DNA. The complexes were formed under different ionic strength conditions, at low enzyme to DNA ratios in order to approach the conditions of specific binding. In contrast there was no crosslinking of the complex in 1 M KCl solution which dissociates the enzyme from DNA. The efficiency of formation of strongly bound complex was found to be much higher with holoenzyme than with core enzyme. The following results were obtained : 1) The large subunits β and β' were found to be bound to DNA. 2) Relatively small amount of σ subunit were bound to DNA while α subunits were essentially not attached to DNA. The high binding affinity of β and β' subunits was also observed in the studies of isolated subunits. These results lead to a model of enzyme-DNA complex in which the large β and β' subunits provide the contacts between the RNA polymerase and the DNA.

INTRODUCTION

DNA dependent-RNA polymerase of prokaryotes is capable in vitro to carry out the transcription of specific segments of DNA. Since this function is performed in the absence of regulatory proteins (such as repressor, etc ...) one may consider that its multifunctional activity is related to the complex structure and subunit organization of RNA polymerase. The process of transcription has been functionally subdivided into a sequence of at least four steps including the binding of the enzyme to the template, selection of the specific promoter and formation of an active complex followed by RNA chain initiation and elongation. The structural complexity of the holoenzyme is related to the presence of at least four subunits α_2 , β , β' and σ having a total molecular weight of about 500 000 daltons (1-2). Present data indicate that the four subunits of the core enzyme (β β' α_2) direct the catalytic activity whereas σ is dissociable and probably governs the selection of promoter sites (3).

Specific function has not yet been assigned to the different subunits. RNA polymerase can be dissociated in urea or guanidine solution and yields isolated subunits which can be reassociated and reactivated to full specific activity (4-7).

The three dimensional structure of the enzyme is unknown and little is known concerning the subunit interactions, spatial organization and tertiary and quaternary structure. The relatively large size of the enzyme excludes the application of X-ray diffraction in the near future and only very limited information has been obtained from electron microscopy (8-9). Low angle X-ray scattering measurements described the enzyme as an elongated molecule with a radius of gyration of 62 Å and with an axial ratio of 1:2 (10). The binding of σ to core enzyme does not change the overall shape which the authors interpreted as showing that σ is attached to the core enzyme at a point near to its center of mass. On the basis of indirect methods such as chemical modification or chemical crosslinking or from partial digestion studies, it was proposed that the β and β' subunits constitute the backbone of both core and holoenzyme (11-13). However these methods may produce displacement or modification of the native structure of the interacting subunits. There is no direct information about the structure of the holoenzyme-DNA complex. In order to understand the molecular mechanism of the transcription process, it is necessary to know the spacial organization of various subunits in the RNA polymerase-DNA complex.

In the present investigation, stable complexes of RNA polymerase holoenzyme with DNA were obtained by irradiation with ultraviolet light at 254 nm and at 310 nm using 5-bromodeoxyuridine (BrdUrd) substituted DNA or poly [d(BrU-A)] as a template. It was shown previously that ultraviolet light can induce the formation of crosslinks between DNA and protein (14). More recently the formation of stable DNA polymerase-DNA adducts was reported (15) and also of RNA polymerase-poly [d(A-T)] (16). In the latter complex the covalent crosslinking was demonstrated by its resistance to dissociate in high salt concentration or by treatment with moderate alkali or by heating. However the nature of the subunits crosslinked to DNA was not determined; the authors hypothesized, without direct evidence, that the crosslinked protein is α or σ subunit.

It is to be emphasized that the ultraviolet crosslinking can occur only if there is a close proximity, i.e. within a bond distance, of the two reactants. When thymine is replaced by BrdUrd the sensitivity to ultraviolet radiation is increased and DNA-protein crosslinks are produced

at a faster rate. Thus the mechanism in the case of BrdUrd substituted DNA or in poly [d(BrU-A)] of the formation of a crosslinked adduct is well understood. The initial event induced by the ultraviolet light is the dissociation of a bromine atom and the production of a reactive free radical at the 5 position of uracil (17). In the presence of a hydrogen donor such as amino acids containing SH group, or amino group like lysine, crosslinked product cysteine-thymine or lysine-thymine were detected in model experiments (14-18).

Direct evidence obtained in the present investigation indicates the participation of β' and β subunits in the formation of the complex with DNA whereas very little affinity for binding to DNA is indicated for the other subunits. These results were obtained under various experimental conditions using entire holoenzyme and separate subunits β β' α and σ different DNA E. Coli and BrdUrd substituted DNA and poly [d(BrU-A)] .

A spatial arrangement of the RNA polymerase-DNA complex is proposed which may have important functional implications.

MATERIALS AND METHODS

Isolation of DNA : Normal DNA and 5-bromodeoxyuridine-substituted DNA (BrdUrd-DNA) were isolated from E. Coli, MRE 600 and Q48 (thymine) respectively. For BrdUrd-DNA, E. Coli was grown for 4.5 generation times in the medium KH_2PO_4 6 g, K_2HPO_4 18.3 g, $(\text{NH}_4)_2\text{SO}_4$ 4 g, MgSO_4 0.2 g, glycerol 10 g, thiamine 20 mg, bacto-peptone 5 g, leucine 25 mg, ammonium-fer (III) citrate 22 mg and BrdUrd 20 mg per litre. The cells were concentrated by centrifugation. DNAs were isolated according to the procedure of Marmur (19) with slight modification which included deproteinization by pronase, and by chloroform-isoamyl alcohol (24:1, v/v) extraction in the presence of 1 M NaCl and 1 % SDS (20). The DNA preparation was deproteinized and virtually free of contaminating protein as determined by SDS-polyacrylamide gel electrophoresis and by U.V. absorption spectroscopy. BrdUrd-DNA was purified by CsCl gradients centrifugation. Whenever BrdUrd-DNA was used, it was handled in the dark as much as possible to minimize photochemical effects from sunlight or light from fluorescent fixtures. The analysis of substitution of thymine by BrdUrd was performed by CsCl gradients centrifugation (21).

Synthesis of poly [d(BrU-A)] : Poly [d(BrU-A)] was enzymatically synthesized using DNA polymerase I extracted from *Micrococcus luteus* by a method of Wells (22) as described previously (23). The purity of synthesized samples was

tested by I.R., U.V. absorption spectroscopy and circular dichroism.

RNA polymerase preparation : The DNA-dependent RNA polymerase was prepared from E. Coli MRE 600, according to the procedure of Burgess with slight modification (1). RNA polymerase was stored at -20° C in a storage buffer containing 10 mM Tris buffer at pH 7.9, 10 mM $MgCl_2$, 500 mM KCl, 0.1 mM EDTA, 1 mM DTT and 60 % (v/v) glycerol. The purity of each enzyme preparation was tested by U.V. absorption spectroscopy and by SDS-polyacrylamide gel electrophoresis. Gel electrophoresis exhibits the presence of α β β' and σ fractions only.

Enzyme activity test : The assay measured the incorporation of labeled nucleotide residues into acid-insoluble RNA, using calf thymus DNA (Worthington Biochemical Corp.) as a template (3). The specific activity of different enzyme preparations was similar to that found by Burgess (3). Protein was determined by the method of Lowry et al (25) using bovine serum albumin as a standard.

Core polymerase and subunits preparation : Core polymerase was isolated on phosphocellulose column following the method of Chamberlin (24).

Each subunit was separated from the holoenzyme by dissociation in 7 M urea according to the procedure of Lill and Hartmann (5) with slight modification. The subunits were isolated by phosphocellulose chromatography in the presence of 7 M urea, the σ subunit being eluted between α and β . The α subunit fraction was chromatographed again on a phosphocellulose column. This method yielded after reconstitution biologically active material. The enzymatic activity of the reconstituted enzyme from isolated subunits represented 30 % of the native enzyme.

Formation of binary complexes : The formation of the complex was performed in the following solutions : low salt buffer A : 1 mM Tris buffer (pH 7.8); 4 mM KCl ; 4 mM dithiothreitol (DTT) ; 0.1 mM EDTA.- high salt buffers B : 1 mM Tris buffer (pH 7.8) ; 1 M KCl ; 10 mM DTT ; 0.1 mM EDTA. - buffer C : 1 mM Tris buffer (pH 7.8) : 40 mM or 100 mM KCl ; 4 mM DTT ; 0.1 mM EDTA. - buffer D : 1 mM Tris buffer (pH 7.8) ; 1 mM KCl ; 1 mM DTT ; 0.1 mM EDTA ; 6 M urea. The pH was checked during and after the dialysis and in some experiments 10 mM Tris buffer was used. E. Coli DNA, BrdUrd-DNA or poly [d(BrU-A)], and the enzyme (weight ratio Enzyme/DNA = 0.08) were mixed and dialyzed against a low salt buffer A, high salt buffer B, C or urea containing buffer D, for 5 hours at 4° C to make a binary complex under well determined salt conditions.

Irradiation : Solution of the binary complex RNA polymerase-DNA was adjusted to the optimum optical density ($O.D._{260} = 0.8$) and was deoxygenated by passing helium gas. Glass stoppered cuvettes containing the deoxygenated solution were kept at constant temperature during irradiation either on ice or at $25^{\circ}C$ in a thermostated cell holder. Irradiation at 254 nm was carried out using a germicidal 15 W, low pressure mercury lamp (Hanovia). In order to avoid high energy irradiation at wavelengths below 200 nm, a liquid filter of 1 M NaCl solution in a 0.2 cm quartz cell was applied. The flux at 254 nm measured with a Lajarjet dosimeter, corresponded to 8.5×10^3 ergs/ mm^2/min . Ultraviolet irradiation at about 305 nm was obtained from 85 W RUL 3000 Å low pressure mercury and phosphor conversion lamp filtered by pyridine solution in a 0.2 cm quartz cell. Pyridine was renewed every hour during irradiation. The determination of the light intensity at about 305 nm was carried by chemical actinometer using potassium ferrioxalate system (26). The flux at about 305nm corresponded to 2.2×10^3 ergs/ mm^2/min . As control, a non irradiated sample and samples which were placed under the U.V. lamp but protected from irradiation, were used.

Chloroform extraction assay : This assay was performed according to the procedure of Smets and Cornelis (20). Irradiated solutions containing RNA polymerase and DNA were dissociated with 1 % SDS, 1 M NaCl and subsequently extracted with 2 volumes of chloroform-isoamyl alcohol (24:1) after vortexing for 15 sec. The solutions were separated into two phases by centrifugation at 6.000 rpm for 20 min., and the absorbance at 260 nm of the DNA in the aqueous phase was measured. The aqueous solution contained free DNA while protein and covalently bound protein-DNA complex was concentrated at the interphase.

Polyacrylamide Gel electrophoresis in a dissociating medium : Analysis of RNA polymerase subunits was carried out on 5 % SDS-polyacrylamide gel electrophoresis as described by Schapiro (27) and Burgess (1), Weber and Osborn (28). Samples containing RNA polymerase were concentrated either by lyophilization or in some cases (e.g. samples in 7 M urea or in high salt) proteins were precipitated by 5 % trichloroacetic acid and collected by centrifugation and washed with acetone. RNA polymerase fraction crosslinked to DNA was calculated by estimating the disappearance of the bands of each subunit and indicated in percentages. Under the conditions of present experiments neither E. Coli DNA nor protein-DNA complex will enter the polyacrylamide gels.

FIGURE 1 - Analysis by chloroform extraction assay of the formation of U.V. crosslinked complex as a function of time. Samples of E. Coli DNA with RNA polymerase (holoenzyme) in buffer A (O-O-O), in buffer A minus DTT (Δ - Δ - Δ), or in Buffer B containing 1 M KCl ($\bullet$ - $\bullet$ - $\bullet$) and samples of E.Coli DNA with core enzyme in buffer A minus DTT ($\square$ - $\square$ - $\square$). DNA fraction crosslinked to RNA polymerase was calculated by the reduction of free DNA and expressed in percentages (see "Methods").

RESULTS

Formation of crosslinked complex measured by chloroform extraction method.

Figure 1 shows the results of the formation of crosslinked DNA-RNA polymerase holoenzyme and core enzyme complexes as measured by the chloroform extraction method. The ultraviolet induced crosslinking at increasing time of irradiation (at 254 nm) is more pronounced with the holoenzyme than with the core enzyme since it reaches about 65 % of DNA fraction bound to protein. Crosslinking of the core enzyme is reduced by more than 50 % with respect to holoenzyme.

It is considered that the core enzyme binds with a low affinity to all segments of DNA. In contrast, the holoenzyme can recognize promoter sites and form stable complexes (29). Our results are in agreement with these notions and suggest that a stable complex of holoenzyme on tight binding sites of DNA can form covalent bonds by ultraviolet irradiation whereas the complexes of core enzyme on loose binding sites of DNA are less well suited for crosslinking reaction.

Comparing the results on the binding of holoenzyme and core enzyme under identical conditions, i.e. in the absence of dithio-treitol (DTT), one can clearly see that the holoenzyme is characterized

by a much higher initial rate (Figure 1 - triangles and squares respectively).

Crosslinking of the holoenzyme and DNA in buffer A and in buffer A minus dithiotreitol (Fig. 1) shows no difference in the final amount of the covalently bound DNA-enzyme complexes which corresponds in each case to about 65 % of the total amount of DNA. It should be noted that the absorption of DTT was taken into consideration and the total absorbancy was identical in both experiments. The only changes are in kinetics. Thus the maximum crosslinking occurs after about 50 minutes and 15 minutes of irradiation in buffer A and in buffer A minus dithiotreitol, respectively. Presumably the thiol groups of DTT interfere with the crosslinking of DNA to RNA polymerase.

A longer time of irradiation leads to the reduction of the fraction of DNA crosslinked to RNA polymerase in Figure 1. A similar effect was observed in crosslinking of lac repressor to BrdUrd-DNA detected by filter binding assay (30). A longer time of irradiation may introduce breaks in the DNA and may liberate the enzyme.

In all these experiments, a non-irradiated sample was used as a control. Another control is represented by the irradiated sample (at 254 nm) with holoenzyme in the dissociating buffer B containing 1 M KCl (see Fig. 1 full points). As expected under these conditions of high ionic strength, the dissociation of the complex DNA-protein occurs and there is no detectable crosslinking.

Electrophoretic analysis of crosslinked subunits.

Gel electrophoresis allows one to determine which particular subunit of RNA polymerase participates in the crosslinked complex with DNA. In fact, after denaturation and dissociation in SDS solution only subunits not crosslinked to DNA migrate following their molecular weights. Figure 2 shows the electrophoretic pattern of holoenzyme for various time periods of crosslinking whereas Figure 3 shows the quantitative estimate of each fraction crosslinked to DNA. The ratio of the holoenzyme to DNA was kept as low as possible in order to avoid protein aggregation and to favour specific interactions. At ratios of 0.08 and of 0.04 RNA polymerase to DNA yielded essentially the same crosslinking curve as that shown in Fig. 3.

The results of Figures 2 and 3 clearly show that essentially β' and β fractions are participating directly in the stable complex with DNA. After 30 minutes of U.V. crosslinking 60 % of β' and β subunits are covalently bound to DNA, while only a small amount (about 15 %) of σ

FIGURE 2 - SDS polyacrylamide gel electrophoretic analysis of crosslinked complexes of RNA polymerase-DNA (weight ratio 0.08) as a function of time of irradiation, visualized with Coomassie Blue staining : (1) 0 min. - (2) 15 min. - (3) 30 min. - (4) 45 min. - Sample 5 represents a control test : complex of RNA polymerase and DNA protected from irradiation (45 min.). Samples of holoenzyme and E.Coli DNA in buffer A (4 mM DTT, 4 mM KCl, 0.1 mM EDTA, 1 mM Tris pH 7.8) at 0°C, were irradiated at 254 nm.

subunit and none of the α subunits are bound. The results of Figure 3 indicate that one molecule of holoenzyme is covalently linked per about 17,000 base pairs after 30 minutes of U.V. crosslinking. Since similar results were obtained for a lower of ratio of enzyme to DNA (0.04 w/w) which corresponds to about one molecule of enzyme per 34,000 base pairs (date not shown). Thus under these conditions a molecule of enzyme is bound by its β' and β subunits to DNA every 17,000 or 34,000 base pairs. At much higher ratio of enzyme to DNA a nonspecific binding started to occur which was expressed in a absence of specificity of subunits binding and in a much faster rate of crosslinking, thus one could not observe any subunits dependency.

The results of Fig. 2 and Fig. 3 are in good agreement with the results of chloroform extraction shown in Fig. 1 ; similar amounts of crosslinking detected by these two completely different methods. After

FIGURE 3 - Electrophoretic analysis of crosslinked complexes of RNA polymerase-DNA induced by U.V. light, a quantitative estimate of the data is shown in Fig.2 after densitometer tracing, β' (-O-O-), β (-●-●-), σ (Δ - Δ - Δ -), α (-□-□-). Subunit crosslinked to DNA is calculated from the disappearance of the bands of each subunit and is expressed in percentage. Non irradiated sample and samples which were protected from irradiation were used as controls.

37-45 minutes of U.V. crosslinking, about 50 % of enzyme is covalently bound to DNA as measured by chloroform extraction and about 50 % as determined by gel electrophoresis. Chloroform assay shows the crosslinking of DNA whereas the electrophoresis assay shows crosslinking of RNA polymerase. The good agreement between these two different methods clearly indicates that the results reflect protein-DNA crosslinking and no other secondary effect like a possible protein aggregation.

Interactions of BrdUrd containing DNAs with RNA polymerase.

Particularly interesting for the ultraviolet crosslinking investigations is to study DNA in which thymidine is replaced by bromodeoxyuridine. It was previously shown that the lac repressor binds more tightly to the operator substituted by BrdUrd, and also can be crosslinked to the DNA after ultraviolet irradiation (30-31).

In the present experiments, we used DNA in which 80 % of thymidine residues were replaced by BrdUrd as estimated from buoyant density determination in CsCl. Figure 4 shows the results of crosslinking of BrdUrd-DNA to RNA polymerase in buffer A, as a function of irradiation time at 254 nm and analyzed by gel electrophoresis in an SDS dissociating

FIGURE 4 - SDS polyacrylamide gel electrophoretic analysis of crosslinked complexes of RNA polymerase and BrdUrd-DNA (0.08 weight ratio). Complexes in buffer A, at 0°C, were irradiated at 254 nm as a function of time. Subunits β' (O-O-O), β (●-●-●), σ (Δ - Δ - Δ), α ($\square$ - $\square$ - $\square$), were estimated from densitometer tracing. For explanation see the legends of Fig. 2 and Fig. 3 and the "Methods".

medium. It can be clearly seen that β and β' subunits are crosslinked almost immediately to DNA, about 20 to 30 % being crosslinked after 7 minutes of irradiation, whereas no α or σ subunits are crosslinked even after 30 minutes of irradiation.

It was shown that the lac repressor binds to poly [d(BrU-A)] about 40 times more efficiently than to poly [d(A-T)], suggesting the existence of some specificity in the protein-DNA interaction (32). The complexes of poly [d(BrU-A)] and RNA polymerase were irradiated under the specific binding condition - in high ionic strength buffer C containing 100 mM KCl at 25°C. As shown in Fig. 5, 65 % to 75 % of β and β' subunits are crosslinked after 30 minutes of irradiation. In contrast, under 10 % of σ and α subunits are crosslinked.

In order to demonstrate the binding and the crosslinking of RNA polymerase to DNA in the absence of any possible protein-protein crosslinking, we tried the irradiation at about 305 nm. At this wavelength, only BrdUrd-DNA but not the protein absorbs U.V. light. Poly [d(BrU-A)] is particularly suitable DNA for these experiments, since protein-DNA crosslinking occurs at BrdUrd and in the absence of protein-protein interaction. Figures 6.a and 6.b show the results of the irradiation at about 305 nm of poly [d(BrU-A)] complex with RNA polymerase at lower and

FIGURE 5 - SDS polyacrylamide gel electrophoretic analysis of U.V. cross-linked complexes of RNA polymerase and poly [d(BrU-A)] in buffer C containing 100 mM KCl, at 25°C, as a function of time of irradiation at 254 nm, β' (○-○-○-), β (●-●-●-), σ (-Δ-Δ-Δ-), α (-□-□-□-). The subunits were estimated after densitometer tracing. For explanation see the legends of Fig. 2 and Fig. 3 and the "Methods".

higher ionic strength in buffers A and C containing 4 mM and 40 mM KCl respectively at 0°C at low enzyme to DNA ratio (0.08 w/w). Higher ionic strength buffer C containing 40 mM KCl was used in order to favour the specific binding of RNA polymerase to DNA (2). The results clearly show that under both experimental conditions only β and β' subunits are cross-linked to poly [d(BrU-A)] whereas there is almost no indication of the crosslinking of α or σ . It is to be noted that the efficiency of crosslinking at 305 nm is much lower and the kinetics is much slower than at 254 nm. The smaller efficiency of crosslinking and the relatively slow kinetic must be related to the differences in energy, since as the wavelength increases the radiation energy is decreased. Under the experimental conditions of the present work (see "Methods"), the quantum yield at 305 nm measured corresponds to 2.2×10^3 ergs/mm²/min., which represents about one fourth of that at 254 nm, i.e. 8.5×10^3 ergs/mm²/min. This may also mean that the dose rate seems to be more important than the total dose. The use of the irradiation at 305 nm allows one to avoid protein-protein binding and the results are entirely due to crosslinking of RNA polymerase to DNA, most probably at 5-uridine position.

Experiments at different ionic strength indicate that the

FIGURE 6 - SDS polyacrylamide gel electrophoretic analysis of crosslinked complexes between RNA polymerase and poly[d(BrU-A)] (0.08 weight ratio) in low salt buffer A (Fig. 6,a) and high salt buffer C containing 40 mM KCl (Fig. 6.b). Samples were irradiated at about 305 nm, at 0°C, through a solution of pyrimidine used as a filter, as a function of time. Each subunit was estimated after densitometer tracing, β' (O-O-O-), β (●-●-●), σ (Δ - Δ - Δ), α ($\square$ - $\square$ - $\square$). Buffer A : 4 mM KCl, 4 mM DTT, 1 mM Tris (pH 7.8), 0.1 mM EDTA ; Buffer C : 40 mM KCl, 4 mM DTT, 1 mM Tris (pH 7.8), 0.1 mM EDTA.

kinetics of crosslinking are more rapid at higher ionic strength (buffer C) than at lower (buffer A), but the final amounts of crosslinked complexes are similar. One may thus conclude that $\beta\beta'$ subunits are directly involved in the complex, which makes contact with the DNA at the 5-position of at least some of the uridine residues.

Interactions of separated subunits with DNA.

In order to test directly the crosslinking in the absence of protein-protein interactions RNA polymerase was dissociated in 6 M urea containing buffer D, into subunits and in the presence of DNA crosslinked under identical conditions as in previous experiments*. Under these conditions of complete dissociation and at relatively low ratio of RNA polymerase to DNA (i.e. 0.08), protein aggregation is completely avoided and each subunit would be attached independently to DNA. The results indicate that up to 15 minutes of U.V. crosslinking at 254 nm, essentially only β and β' are bound to DNA which confirms the previous results shown in Fig. 3. It is not surprising to find that the binding of these subunits in 6 M urea is following a different kinetics.

The binding affinities of different subunits for DNA were investigated by testing the crosslinking of individual subunits. Figure 7

(*) Data not shown.

FIGURE 7 - SDS polyacrylamide gel electrophoretic analysis of the complex of β' subunit and DNA (0.08 w/w) as a function of time of irradiation at 254 nm. The samples in buffer A, at 0°C, were irradiated at different time : 1) 0 min., 2) 15 min., 3) 30 min., 4) 45 min. Sample 5 represents a control test : the solution containing β' subunit and DNA was placed under the U.V. lamp for 45 min. but was protected from the radiation.

shows a typical example of electrophoretic analysis after irradiation of solution containing isolated β' subunit and DNA, while in Figure 8 the quantitative results from all four subunits are summarized. It can be seen clearly that β' and β subunits exhibit the highest affinity for DNA. The σ subunit exhibits much less binding affinity while there is very little if any detectable binding of α subunit. Comparing these results on isolated subunits with the results on the complexes of holoenzyme with DNA (Fig. 3), some differences in kinetics and in quantities of crosslinked complex are observed. This may be explained as a result of differences in conformation, molecular weight and environment of individual subunits and of holoenzyme. In conclusion, this direct evidence confirms the participation of β and β' subunits in the binding of the enzyme to DNA.

FIGURE 8 - Formation of crosslinked complexes of isolated subunits RNA-polymerase with DNA induced by U.V. light. Samples containing individual subunit (β' , β , σ or α) and DNA in buffer A were irradiated at 254 nm at different times as described in the legend of Fig. 7. The crosslinked protein fraction (in %) was estimated from densitometer tracing. The result on crosslinking of β' with DNA represents a quantitative summary of the data shown in Fig. 7.

DISCUSSION

There is no information in the literature about the structure E.Coli RNA polymerase-DNA complex. In agreement with previous data, the results of the present studies indicate that U.V. induced crosslinking leads to the formation of a stable covalently bound RNA polymerase-DNA complex (for a review see Ref. 14 and 16). Since the binding and interactions between two macromolecules are occurring within a bond length distance (14), this allows us to establish the nature of the subunits which are in direct contact with DNA and to propose the topography of the subunit arrangement in the complex. The crosslinked complexes were resistant to the dissociation in 1 M NaCl and in 4 % SDS solutions. The course of formation of the stable DNA-enzyme complex was followed during irradiation at 254 nm and at about 305 nm outside of the protein absorption band using BrdUrd substituted DNA and poly [d(BrU-A)]. The latter provided strong evidence that protein-DNA and not protein-protein adducts were being observed. Furthermore, the formation of the U.V. crosslinked complex was prevented under dissociating conditions in 1 M KCl (see Fig. 1). Using different DNA's E.Coli DNA, BrdUrd substituted DNA and poly [d(BrU-A)] RNA polymerase holoenzyme, core enzyme and individual subunits separately, all the results obtained demonstrated clearly the β' and β subunits of RNA

FIGURE 9 - Schematic representation of the spatial arrangement of subunits in RNA polymerase-DNA complex based on our crosslinking results. All the subunits are assumed to be of spherical shape reflecting their relative volume corresponding to their respective molecular weights, whereas the DNA segment is about 40 base pairs long (for explanation see the text).

polymerase participate directly in the stable binary complex with DNA. Thus the DNA binding sites on RNA polymerase are located mainly on β' and β subunits. Our results do not support the interaction of α subunits with DNA under the conditions used in present experiments, i.e. at low enzyme to DNA ratios. Since several results indicate that a small fraction of σ subunit is crosslinked, this probably reflects its direct or indirect interaction with DNA.

Figure 9 illustrates a model which we propose for the spatial arrangement of subunits in RNA polymerase-DNA complex based on our crosslinking results. The model indicates that the β' and β subunits are the template binding site of the enzyme whereas the σ subunits is in close proximity to the DNA and in direct contact with β and β' subunits, which can explain its functional role of correct selection of specific binding site. In this model the backbone of the holoenzyme in the complex is the $\beta'\beta$ subunits. The core enzyme must form the complex with the template following a similar arrangement as shown in Fig. 9, except that the σ subunit will be eliminated.

The following additional results are supporting this model :

- 1) limited proteolysis with various proteases is occurring on the β' and β subunits of RNA polymerase but is restricted by the addition of the template (33). From the studies of digestion of RNA polymerase with proteases, Lill and Hartmann (12) have concluded that the σ subunit interacts with both β and β' subunits which is in agreement with our model.
- 2) Poly deoxy-4-thiothymidylic acid is preferentially linked to the β' subunit of RNA polymerase by photooxidation (34).

- 3) the tsX mutation (in E. Coli strains T16 and Ts19) which affects the binding of the enzyme to the template has been located in β' subunit (35-36).
- 4) the binding of poly [d(A-T)] protects about 10 lysyl ϵ -amino groups of the holoenzyme as determined by fluorescamine (37) which are preferentially located on β' and β subunits.
- 5) it is also known from charge density electrophoresis that β' and β are the most basic subunits suggesting a relation to DNA binding function (38-41).

The question now arises of whether the proposed model is related to the specific binding of RNA polymerase to DNA. Although there is no direct evidence that the crosslinking involves only enzyme-promoter complexes, the conditions were chosen to approach the conditions of the specific interactions :

- 1) The formation of the complex was at very low enzyme to DNA ratios, i.e. 0.08 and 0.04 (w/w) which corresponds to an efficient binding of one molecule of RNA polymerase holoenzyme per about 17,000 to 34,000 base pairs respectively. With templates such as T7 DNA and fdRF DNA, it was found that the complexes are quantitatively at the promoter sites when enzyme concentration were chosen below the saturation of the available sites (42, 43).
- 2) The comparison of the efficiency of crosslinking of the holoenzyme and of the core enzyme indicates a reduction of crosslinking to about 50 % in the latter case (see Fig. 1). Since the presence of the σ subunit is related to the function of the recognition of sequence (1), this result supports the specificity of interaction for the holoenzyme.

We may suggest that also some elements of specificity are probably present in our experiment of the binding of RNA polymerase to poly [d(BrU-A)] or BrdUrd substituted DNA from E.Coli (see paragraph "Interactions of BrdUrd containing DNAs with RNA polymerase").

In conclusion, by means of U.V. crosslinking we have obtained information on the topography of subunits in RNA polymerase-DNA complex. The spatial organization of the enzyme-DNA complex may be a primary importance for the understanding of the mechanism of the recognition process and the mechanism of the transcription process.

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