UV Cross-linking of the Bacillus subtilis RNA Polymerase to DNA in Promoter and Nonpromoter Complexes*

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Complexes between Bacillus subtilis RNA polymerase and ^32P-labeled DNA were irradiated with UV light and digested with nuclease; electrophoresis and autoradiography were used to identify the polymerase subunits cross-linked to DNA. These experiments showed: 1) that cross-linkage of promoter complexes yielded predominantly the $\beta$ and $\sigma$ subunits; 2) that $\beta$, $\beta'$, and $\sigma$ were detected in nonpromoter complexes; 3) that addition of the $\delta$ subunit or high concentrations of NaCl decreased cross-linkage of all subunits, especially the cross-linkage of the $\sigma$ subunit in nonpromoter complexes and the binding of polymerase at DNA ends; 4) that different patterns of cross-linkage were obtained at $0^\circ$C (conditions favoring the formation of closed complexes) and $37^\circ$C (conditions favoring the formation of open complexes); and 5) predominantly $\beta$ and possibly $\beta'$ were cross-linked by irradiation of core-DNA complexes whereas similar experiments with core-$\delta$ complexed to DNA showed the efficient cross-linkage of $\beta'$ and $\beta$.

The RNA polymerases of most procaryotes consist of a core ($\beta'$-$\sigma$), which functions primarily in chain elongation and one or more peptides ($\sigma$) which determine transcriptional specificity; some polymerases also contain small peptides ($\omega$) whose function is not known. Extensive studies of Escherichia coli RNA polymerase have established that the in vitro formation of specific complexes with promoters requires the presence of the $\sigma$ subunit and is affected by a number of parameters including temperature, cation concentrations, and the polymerase/DNA ratio. The $\sigma$ subunit is responsible for discriminating between promoter and nonpromoter sites, for forming stable complexes with promoters and for initiating transcription (reviewed in Ref. 1). The holoenzyme can also form several types of nonspecific complexes. One type, "transient complexes," is formed rapidly at both $0$ and $37^\circ$C and involves primarily electrostatic interactions between polymerase and DNA. Two types of more stable nonspecific complexes can also be detected: weak complexes formed preferentially at the ends of DNA molecules (2) and "tight binding complexes" at internal DNA sites (3, 4). The transient complexes are presumed to facilitate the search for promoters both in vitro and in vivo (reviewed in Ref. 5); the functions of the more stable nonspecific complexes are not known nor is it known whether such complexes are formed in vivo.

In the RNA polymerase of Bacillus subtilis (and other species of Bacillus (6, 7)) and of Lactobacillus curvatus (8), two subunits participate in providing optimal discrimination between promoter and nonpromoter sites. In these two Gram-positive species, the $\sigma$ subunit provides recognition of promoter sequences and is required for the initiation of transcription and a separate subunit, $\delta$ in Bacillus and $\gamma$ in L. curvatus, acts in conjunction with $\sigma$ and the core to prevent stable binding at nonpromoter sites (6, 8). The mechanism of action of $\delta$ has not been fully established, but it is known that addition of $\delta$ results in a significant increase in the dissociation of preformed nonspecific complexes (9). $\delta$ also decreases the number of "close contacts" between polymerase and purines in a promoter without having a significant effect on the position of polymerase on the promoter or the proportion of molecules in open complexes (10). These observations suggest that B. subtilis core-$\sigma$ may undergo a $\delta$-mediated conformational change.

The subunits of E. coli RNA polymerase which are in close proximity to DNA in specific and nonspecific complexes have been identified by means of several cross-linking procedures (11-13). Photochemical cross-linking of polymerase to DNA by conventional UV (11, 14) or high energy laser radiation (15) demonstrated the efficient cross-linkage of $\sigma$, $\beta$, and $\beta'$ to $T^\prime$ DNA. Similar experiments with specific complexes yielded $\sigma$ and $\beta$ only, indicating a different spatial arrangement of subunits relative to the template (11, 14).

We have used photochemical cross-linking of B. subtilis polymerase preparations to determine which subunits are cross-linked in specific and nonspecific complexes under conditions which favor the formation of "closed" and "open complexes" (1). We have also tested the effect of added $\delta$ on cross-linking of subunits and determined which subunits are cross-linked in complexes formed by core and core-$\delta$.

EXPERIMENTAL PROCEDURES

RESULTS

Subunits Cross-linked to DNA—When the B. subtilis core-$\sigma$ or core-$\sigma$ plus $\beta$ preparations were combined with pUCs-92

* Portions of this paper (including "Experimental Procedures" and Footnotes 2 and 3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-3555, cite the authors, and include a check or money order for $1.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
UV Cross-linking of B. subtilis RNA Polymerase DNA

**Fig. 1. Effect of NaCl and δ on the cross-linking of RNA polymerase subunits to DNA.** Either 20 μg of core-α (lanes 1, 3, 5, and 7) or 20 μg of core-α plus 2 μg of δ (lanes 2, 4, 6, and 8) were combined with 0.5 μCi of 32P-labeled pUC8-92 DNA per lane in the presence of increasing concentrations of NaCl: lanes 1 and 2, 15 mM; lanes 3 and 4, 100 mM; lanes 5 and 6, 200 mM; lanes 7 and 8, 300 mM. Complexes were irradiated and analyzed as described under “Experimental Procedures” by SDS-gel electrophoresis and autoradiography.

A, “internal plus end-labeled” DNA (~25 μg/sample prior to nuclease digestion); B, “end-labeled” DNA (~250–500 ng/sample prior to nuclease digestion). The position of Coomassie-stained subunits are indicated.

in the presence of 15 mM NaCl and irradiated, the α, β, and β’ subunits (M, 55,000, 140,000, and 130,000, respectively) were cross-linked (Fig. 1, A and B, lanes 1). No complexes of greater mobility were observed when identical samples were electrophoresed on gels of higher acrylamide concentration in which the Rf of the α (M, 44,000) and δ (M, 21,000) subunits were 0.5 and 0.9, respectively (data not shown) except when purified core was cross-linked to DNA (described in a later section). The presence of the α subunits in two complexes (the prominent band labeled in Fig. 1 and a fainter band immediately above in Fig. 1) and the presence of the β subunit in the complex labeled β in Fig. 1 were confirmed by precipitation of the cross-linked, DNase-treated samples with antibodies specific for β and α (16); the position of β’ cross-linked to DNA was inferred from its mobility (16).

The resolution of two bands which reacted with antibody to the α subunit could be due to the presence of two different forms of α (α-55 and a variant with a mobility corresponding to a molecular weight of ~60,000) and/or the presence of two different lengths of DNA cross-linked to α. The first explanation seems more probable since the M, 60,000 peptide can sometimes be detected in un-cross-linked preparations of RNA polymerase after electrophoresis on SDS-7.5% polyacrylamide gels and staining with Coomassie blue (data not shown and also noted by Kudo et al. (26)). The amount of the peptide varied depending on the preparation and larger amounts were detected after extensive purification of RNA polymerase or α by column chromatography. The identification of the α-55,000 peptide as a variant of α is based on its precipitation by antibody to the α subunit, its reaction with antibody to α in immunoblots and by comparison of proteolytic degradation products generated from α and its variant by treatment with *Staphylococcus* V-8 protease (16). In all experiments, both α and its variant form responded identically to each experimental condition and all subsequent discussions will refer to the complex containing the α, 55,000 peptide, the predominant α found in vegetative cells of *B. subtilis*.

**Effect of NaCl and δ on the cross-linking of Subunits to DNA**—Filter binding experiments (6, 9) showed that at low concentrations of NaCl and at moderate and high enzyme/DNA ratios, *B. subtilis* core-α forms stable noninitiated complexes with both promoter and nonpromoter sites. These stable nonspecific complexes differ from “tight binding” complexes formed by *E. coli* RNA polymerase (3, 4) with respect to several properties: they are rapidly formed at both 0 and 37 °C; they are relatively resistant to NaCl; and their formation is significantly reduced or prevented by δ. The data in Fig. 1A were obtained by irradiating core-α in the absence or presence of δ incubated with DNA at 37 °C. Although the plasmid DNA used in these experiments (pUC8-92) included several types of potential binding sites (nonspecific interior, specific interior, and end) for core-α, we assume that nonspecific interior binding predominated at the high enzyme/DNA ratios used.

Fig. 1A shows that increasing the concentration of NaCl caused a progressive decrease in the cross-linking of β, β’, and α and that δ reduced the cross-linking of all subunits at all NaCl concentrations tested. The decrease in cross-linking attributable to δ was approximately equivalent to that observed by increasing the NaCl concentration from 15 to 200 mM or from 200 to 300 mM in the absence of δ. However, δ had a greater effect on α than on β in the lower salt range and a slightly greater effect on β than α above 200 mM NaCl.

**Effect of NaCl and δ on the Cross-linkage of Subunits to “End-labeled” DNA**—The work of Melancon et al. (2) clearly demonstrated that *E. coli* polymerase preferentially binds to the ends of DNA molecules; this binding is sensitive to heparin and NaCl. The cross-linking data of Fig. 1B indicate that *B. subtilis* core-α also binds efficiently at the ends or sufficiently near the ends of DNA molecules so that the labeled terminal bases are not released by the nuclease treatment which follows exposure of complexes to irradiation. This binding was highly sensitive to increased concentrations of NaCl. The cross-linkage of δ was reduced significantly even at 100 mM NaCl but most strikingly, the presence of δ entirely eliminated the cross-linkage of α to DNA ends at all NaCl concentrations (Fig. 1B, lanes 1 and 2). It seems likely that the cross-linkage shown in Fig. 1A in the presence of 200 and 300 mM NaCl was due to complex formation at interior sites whereas that at lower concentrations probably represents a composite of cross-linkage of subunits in complexes at interior sites and complexes formed at DNA ends.

**Interaction of Core and Core-δ with DNA**—Almost all of the α subunit can be removed from preparations of *B. subtilis* RNA polymerase to yield fractions containing predominantly the core subunits or, by an alternative method, fractions containing predominantly core-δ. It is probable that both of these forms of RNA polymerase are produced during transcription and exist in the cell, at least transiently. It may be concluded on the basis of filter binding experiments, sedimen-
tation of DNA-RNA polymerase complexes through glycerol gradients (16), and the pattern of elution from DNA cellulose (18, 27) that core binds to DNA more tightly than core-δ.

As shown in lanes 1–4 of Fig. 2, a large amount of β subunit was cross-linked when core preparations were incubated at 37 °C in the presence of two concentrations of NaCl with either end-labeled DNA or with "internal plus end-labeled" DNA. Addition of δ to the core in 200 mM NaCl reduced all cross-linking substantially (Fig. 2, lane 5). Addition of σ alone or σ plus δ restored the general patterns of cross-linking observed with core-σ and core-σ plus δ (Fig. 2, lanes 6 and 7; Fig. 1A, lanes 5 and 6). Purified σ, in the absence of core, did not cross-link to a detectable level in either 15 or 200 mM NaCl (data not shown).

Fig. 2 shows that a detectable amount of DNA cross-linked to a peptide having the mobility of the α subunit when purified core was cross-linked to DNA (Fig. 2, lanes 1–4). Cross-linking of the α subunit to DNA has not been previously reported. It is possible that this band was generated by the degradation of a larger subunit (e.g. β). It should be noted also that only 10–20% of the specific activity of core-σ was reconstituted by addition of σ to core, indicating that a large fraction of inactive core molecules were present. However, since addition of δ or σ to core (Fig. 2, lanes 5–7) yielded cross-linking patterns which were similar to those obtained in experiments with purified core-δ or core-σ, it seems unlikely that either degradation or the presence of inactive molecules accounts for the cross-linking to the presumed α subunit.

A different pattern was revealed by experiments with core-δ (Fig. 2, lanes 8–11). In the presence of 15 mM NaCl, the cross-linkage to internal plus end-labeled DNA was predominately through the β′ subunit with also a significant cross-linkage of β and a trace of radioactivity due to contaminating σ. Very little cross-linkage could be detected in the presence of 200 mM NaCl nor was there significant cross-linkage of subunits to end-labeled DNA at either NaCl concentration. These results indicate that the binding of core-δ occurred to internal sites and that it involved the β′ subunit. The results shown in lane 12 of Fig. 2 demonstrate that the addition of σ restored the usual β and σ cross-linkage pattern.

The β′ subunit was efficiently cross-linked to DNA only in experiments with core-δ. Low levels of cross-linked β′ were detected in experiments where core-α and core-α-δ were cross-linked in nonspecific complexes (Fig. 1 and see below). In those experiments, the efficiency of β′ cross-linking was not substantially altered by elevated temperature but was sensitive to increased salt concentration. It is possible that the cross-linking of β′ in experiments with core-α-δ was due to the presence of a subpopulation of core-β.
of NaCl on the binding of polymerase to DNA ends.

The addition of δ resulted in decreased cross-linkage of all subunits (although the reduction in β' was less obvious). Decreased cross-linkage would be expected from the known action of δ in preventing the formation of stable nonspecific complexes. In agreement with data shown in Fig. 1, the most significant decreases were in the cross-linkage of σ at 0 and 37 °C in 15 mM NaCl (again, in part due to an effect on end binding) and in the cross-linkage of β at 37 °C. It seems likely that the δ-sensitive NaCl-resistant cross-linkage of β at 37 °C was due to the formation of nonspecific complexes at internal sites on the plasmid DNA, as shown below, σ cross-linkage was much more significant in specific complexes. Interactions of polymerase subunits with individual strands of a promoter-bearing DNA fragment—Specific complex formation was investigated using a short DNA fragment containing a SP82 promoter isolated from the plasmid pUC8-92. The 35 and -10 regions of the promoter were located by sequencing; "DNaase I footprinting" (29) showed that core-σ occupied ~60 bp of the central portion of the DNA fragment (16), thus leaving a minimal amount (~30 bp on each end) of DNA free for nonspecific interior binding. This promoter has been characterized with respect to the effect of δ on the formation of polymerase-DNA complexes, the stability of the complexes, and the rate of transcription relative to 9 other cloned promoters recognized by B. subtilis RNA polymerase.

Each strand of the double-stranded promoter DNA fragment was labeled independently and used in separate cross-linking experiments. Since the ratio of DNA ends to interior sites is relatively high with a short fragment, all experiments were done in the presence of 200 mM NaCl to minimize end binding. Experiments with the 92-bp fragment were done at both 0 and 37 °C in order to compare the subunit interactions under conditions in which the closed and open promoter complexes, respectively, would predominate. Table I shows that RNA polymerase does not interact with the two strands equally. At 0 °C, the predominant cross-linking was of the σ subunit to the nontemplate strand. Cross-linking to the template strand was similar overall to cross-linking to the nontemplate strand of the promoter but was more equally divided between the β and σ subunits. Cross-linking was generally increased at 37 °C relative to 0 °C; temperature affected cross-linking to the template strand more than to the nontemplate strand and was greater for β than for σ.

The promoter complexes (Table II) are similar to nonpromoter complexes (Table I) in that the cross-linking of the β subunit is increased substantially at 37 °C compared to the complex at 0 °C. In contrast to nonspecific complexes, the σ subunit cross-links efficiently to promoter DNA in the presence of both 200 mM NaCl and δ. With the isolated promoter fragment, there was little or no cross-linking of the β' subunit. While this may be due in part to the inclusion of 200 mM NaCl, these results are consistent with the findings of Hillel and Wu (11) that E. coli β' cross-links to T7 DNA only under conditions favoring nonspecific complex formation.

The patterns observed at 37 °C are consistent with the findings for specific complexes formed by the E. coli RNA polymerase (14) except that Park et al. did not detect any σ cross-link to the template strand. Linkage of the E. coli RNA polymerase to depurinated DNA containing the lac UV5 promoter (13) showed extensive contacts not only with β and σ in both strands, but also with β'. Possibly the cross-linking of the β' subunit in the latter work was due to differences in the methods used for cross-linking. A 77-bp fragment of SP82 DNA lacking a promoter was used in parallel with the 92-bp fragment for cross-linking analyses in 200 mM NaCl and 37 °C. Cross-linking to both strands (not shown) was similar in pattern to that observed with the entire vector DNA (Table I) indicating that the differences between the promoter fragment and the plasmid are not due to DNA length but are a property of the base sequences.

Cross-linking in the presence of initiating nucleotides—Core-σ and core-σ-δ were irradiated at 37 °C after preincubation in the presence of ATP and CTP. This would allow synthesis of a trimer from the 92-bp promoter fragment and limited initiation from some binding sites of the plasmid DNA. These conditions may permit "abortive initiation" as observed with E. coli RNA polymerase (30). No differences were found compared to parallel experiments in the absence of nucleotides (data not shown).

DISCUSSION

In view of the complexity of the structure of RNA polymerase and the multistep process of transcription, the existence of several conformations of RNA polymerase is probable. One change in conformation has been documented—i.e. when σ

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**Table I**

Cross-linking of RNA polymerase to plasmid pUC8

<table>
<thead>
<tr>
<th>RNA polymerase</th>
<th>NaCl [μM]</th>
<th>T °C</th>
<th>β</th>
<th>β'</th>
<th>σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core-σ</td>
<td>15</td>
<td>0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>15</td>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>15 + 37</td>
<td>1.0</td>
<td>0.1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Core-σ</td>
<td>200</td>
<td>0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>200</td>
<td>0 t*</td>
<td>t t</td>
<td>t t</td>
<td></td>
</tr>
<tr>
<td>Core-σ</td>
<td>200</td>
<td>37</td>
<td>0.9</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>200</td>
<td>37</td>
<td>0.2</td>
<td>t t</td>
<td>t t</td>
</tr>
</tbody>
</table>

a, t, trace.

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**Table II**

Cross-linking of RNA polymerase to the 92-bp promoter fragment

<table>
<thead>
<tr>
<th>RNA polymerase</th>
<th>Strand</th>
<th>T °C</th>
<th>β</th>
<th>σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core-σ</td>
<td>Nontemplate</td>
<td>0</td>
<td>t'</td>
<td>0.4</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>Nontemplate</td>
<td>0</td>
<td>t</td>
<td>0.3</td>
</tr>
<tr>
<td>Core-σ</td>
<td>Nontemplate</td>
<td>37</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>Nontemplate</td>
<td>37</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Core-σ</td>
<td>Template</td>
<td>0</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>Template</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Core-σ</td>
<td>Template</td>
<td>37</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Core-σ + δ</td>
<td>Template</td>
<td>37</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

a, t, trace.

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*E. C. Achberger and H. R. Whiteley, manuscript in preparation.*
associates with core (31)—and several conformational changes have been proposed on the basis of kinetic studies (32–34). In considering how the subunits might interact with DNA, an association of α, β, and β' would be expected from the known functions and properties of these subunits. Hillel and Wu (11) used photochemical cross-linking to demonstrate that α and β were in close contact with DNA in specific complexes formed by E. coli RNA polymerase. The finding that α, β, and β' of E. coli polymerase were cross-linked in nonspecific complexes indicates that the interactions of polymerase with promoter and nonpromoter sites is different. In the present investigation we found that B. subtilis RNA polymerase also interacts differently in promoter and nonpromoter complexes. Furthermore, we found altered interactions depending on the temperature and on the concentration of NaCl. The cross-linking reported in the present investigation supports a model in which each subunit that interacts with DNA may have a different primary role (Table III).

It has been suggested (35) that transient nonpromoter binding to DNA may be entirely electrostatic. Core-δ may represent a form of RNA polymerase capable only of transient nonspecific binding since it lacks a specificity (α) subunit. Consistent with this possibility are the findings that core-δ forms no filter-retainable complexes with fragments of phage SP82 DNA (6) and is cross-linked to DNA only in the presence of low salt. It is interesting that this is the only form of B. subtilis RNA polymerase in which significant cross-linking of β' to DNA was found; low levels of cross-linkage of β' were found with core-α-δ and core-α in nonpromoter complexes.

Binding of RNA polymerase to both promoter and promoter sites is sensitive to elevated salt concentrations, indicating that electrostatic interactions contribute significantly to the free energy of binding to both types of sites (35). Since the base sequence is a significant distinguishing feature of promoter sites, it has been proposed that promoter recognition may occur by specific nonionic (hydrogen bond) interactions between donor and acceptor groups on the RNA polymerase and the DNA bases (5). The greater sensitivity to salt of the cross-linking of the δ subunit of RNA polymerase when bound to nonpromoter sites compared to when bound to promoter sites provides direct evidence in support of this proposal. Although it is probable that other definable intermediates exist on the pathway to initiation of RNA synthesis (32, 36, 37), such intermediates would not be distinguished by the methods used in the present investigation. For this reason, only the two general complexes (prior to and after strand separation) will be discussed. Most of the cross-linking at 0 °C (presumed to be closed promoter complexes) was of the δ subunit to the nontemplate strand. α interactions would be expected since this subunit is the determinant of promoter sequence recognition (1, 6). The possibility that early recognition of the promoter site involved contact with the nontemplate strand was previously suggested by experiments to determine which purines or phosphates of the promoter sequence would, if previously modified, interfere with the initial recognition and binding of RNA polymerase to the lac UV5 and T7 A3 promoters (38).

At 37 °C the majority of complexes formed with promoter DNA should be in the open (strand-separated) state. While the absorbance and (and hence the efficiency of cross-linking) of double-stranded DNA is not substantially affected by temperature under the experimental conditions used in this investigation (39), the melted region would have a higher absorbance than the surrounding double-stranded DNA. This may account for the increased cross-linking of the α and β subunits at 37 °C, although the increases may also be due to the greater stability of the open complexes as compared to closed complexes. The greatest increase was in the cross-linkage of the β subunit to the template strand. An increased interaction of the β subunit with the template strand in the open complex would agree with the previously proposed role of this subunit in the catalysis of polymerization.

In comparing the rapidly formed, temperature-independent nonspecific complexes formed by E. coli core and core-δ, Melancon et al. (2) found that core-α partially bound to DNA ends relative to random interior sites whereas core bound more equally to ends and interior sites. Furthermore, the constants for preferential end binding by core-α were comparable to constants calculated for the reaction of polymerase with promoters. Since it seemed unlikely that end binding was due to preferential reaction with single-stranded DNA produced by unraveling of the ends of the molecules, Melancon et al. (2) proposed a steric model involving two conformational states of RNA polymerase, R and R*. According to this model, the more stable R conformation can bind to DNA ends or in open promoter complexes. The R* conformation can bind to interior sites either in random complexes or in closed promoter complexes. They further proposed that allosteric effectors could alter the conformation of the enzyme and that the transition of the R* to R conformation could accompany the formation of stable open promoter complexes. Salt-sensitive binding to DNA ends was also observed in the present work. Since this binding is also sensitive to the presence of δ, it is tempting to speculate that the δ subunit may be an allosteric effector favoring the putative R* conformation. However, δ reduced cross-linking under conditions (0 °C, 200 mM NaCl) in which few open complexes are expected as well as under conditions (37 °C, 15 mM NaCl) in which most complexes would be open. Thus, δ affects the conformation of the polymerase-DNA complex, but we find no evidence that δ favors a particular step on the pathway to transcription.

The α subunit was cross-linked efficiently to nonpromoter DNA only if the salt concentration was low and δ was absent but was cross-linked efficiently to the promoter fragment in the presence of 200 mM NaCl and δ. This indicates that the α subunit of RNA polymerase is able to interact with DNA in either of two separate (different) modes: the first is base sequence-specific and is not substantially dependent on temperature, salt concentration, or the presence of δ and the second is nonspecific for base sequence and involves salt-sensitive, i.e. electrostatic, bonds. This second mode is reduced or excluded by the presence of δ. This may indicate that there are separate domains on the α subunit which can be modulated independently or may indicate that α and/or the entire RNA polymerase assembly may assume two conformations. Earlier experiments (6) using the filter binding assay

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**Table III**

<table>
<thead>
<tr>
<th>Subunits cross-linking to DNA</th>
<th>PUTATIVE COMPLEX</th>
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<tbody>
<tr>
<td>++, ++, +</td>
<td>Transient nonspecific</td>
</tr>
<tr>
<td>+, +</td>
<td>Stable nonpromoter (closed and open)</td>
</tr>
<tr>
<td>+ ++, +, +</td>
<td>Closed promoter</td>
</tr>
<tr>
<td>++ +, +, +</td>
<td>Open promoter</td>
</tr>
</tbody>
</table>

+++, Strongest cross-linking; ++, moderate cross-linking; +, weak cross-linking.
showed that binding to nonpromoter DNA fragments by core-
σ is not severely inhibited by 200 mM NaCl and remains
significant at 300 mM NaCl. This suggests that although the
salt-sensitive σ interactions detected in the present investiga-
tion may reflect the conformation of the RNA polymerase,
they are not solely responsible for the aberrant binding and
transcription observed in the absence of δ. From the present
cross-linking data it may be concluded that the β subunit
is most probably responsible for the stable binding of RNA
polymerase to nonpromoter DNA at 200 mM NaCl and above.
Hence, δ acts to reduce both β and sigma (and possibly β')
interactions with nonpromoter DNA. The most plausible
explanation for this observation and the finding that δ affects
complexes at both 0 and 37 °C is that δ acts as an allosteric
effector of RNA polymerase conformation, reducing the num-
ber of bonds formed with DNA so that only when specific H
bonds are formed, primarily by σ, can the polymerase remain
stably bound at a site.

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UV Cross-linking of B. subtilis RNA Polymerase DNA

EXPERIMENTAL PROCEDURES

Purification of RNA Polymerase - Methods for the isolation of B. subtilis DNA polymerase, core and core-delta were described previously. Core was isolated from core-promoter on chromatography on phosphocellulose (17, 18). Nativiomy of photographs of electrophoretic gels stained with Coomassie blue and the gels were vertically separated (119 substrate) with signs of radioactivity in core and core-delta preparations, respectively. More than 90% of cells formed by core-promoter preparations were resistant to challenges by rifampicin (6) in assays with S92 DNA at the template. Immunoprecipitation experiments (described in a later section) showed that purified preparations of core-delta contained a trace of signal activity although none was detectable on a stained SDS gel. Core-delta activity could be stimulated by more than 7-fold when an excess of purified signal-T subunits (data not shown) was added using S92 DNA as a template (11).

RNA polymerase subunits were isolated from purified core-promoter by digestion with RNase and HindIII and treated with exonuclease III and ethidium bromide (19). The resulting insert-fragment was isolated from the vector with HindIII and used to prepare subunits by digestion with RNase and HindIII. The resulting insert-fragment was dialyzed against subunit stock solutions and materials were re-equilibrated on internal materials. The isolated subunits were used as a standard.

Cross-linking - The efficiency of cross-linking of DNA to RNA polymerase was measured by filtration through nitrocellulose after addition of 5 volumes of 1 M NaCl to displace noncovalent complexes. The efficiency of cross-linking (the fraction of radioactivity retained by the filter compared to the total input radioactivity) was a linear function of time through 16 minutes. Cross-linking of core-delta to RNA polymerase was measured by a similar procedure except that the DNA was cleaved from the vector by digestion with EcoRI and HindIII (as described (19)). The core-delta preparation was assayed by determination of core-sigma activity in the presence and absence of each of 2 Moles of added delta per core-delta using two templates, S92 DNA (delta stimulates 7 to 8) and 77 DNA (delta retains transcription by more than 50%). For some experiments, the signal subunit was purified on phosphocellulose chromatography under non-denaturing conditions (17, 18). Four to 6 Moles of signal purified by either technique were required per Moles of core-delta for maximal stimulation of activity.

Protein concentrations were determined according to Bradford (20) with bovine serum albumin (Biorad) as a standard.

Plasmid DNA - A 92 base pair fragment, originally isolated from plasmid S92 and containing a promoter recognized by the B. subtilis RNA polymerase, was inserted into the insertion site of each plasmid DNA was designated pJcbs-12-23. Plasmid pJcbs-1277 has an insert of 77 of S92 DNA lacking a promoter sequence. The promoter fragment was cleaved from the vector by digestion with EcoRI and HindIII, the resulting insert fragment contained 97 Mo of S92 DNA and 24 bp of vector DNA from the (multiple cloning site) but was not digested by HindIII. The resulting insert fragment was obtained from the same method and included 11 Mo of vector DNA. Both inserts were originally cloned into phage M13 DNA, sequenced and characterized by E. C. Hahnberger and were a generous gift. Plasmid DNA was isolated from E. coli JM83 by a 25 M-MnCl2 lysis procedure and SDS-phosphocellulose chromatography (20).

Dilution of DNA to 1 ng DNA at internal sites and near the ends ("internal" also exo-labeled DNA) transcribed plated DNA (200,000 cpm) was isolated with 200 ng/ml exo-labeled DNA. Isolated samples were ethanol precipitated and washed with ethanol at 4°C. Ethanol-washed, ethanol-precipitated samples were resuspended at 15°C, 20 ml ethanol and extracted with phenol (utta). DNA was precipitated with ethanol and resuspended in 20% glycerol, 20% SDS, and 0.1 U/ml of RNase A. DNA was linearized by digestion with HindIII and treated with EcoRI to 1 ng DNA at internal sites and near the ends ("internal" also exo-labeled DNA) was obtained with 1 ng of DNA in 20 ml containing 40 M-Tris-HCl pH 6.8, 100 M-EDTA, 200 M-Mel and 0.5 M-glycerol and 100 M-Mel. DNA was separated from the vector with HindIII and 77 DNA (delta stimulates 7 to 8) and 77 DNA (delta retains transcription by more than 50%). For some experiments, the signal subunit was purified on phosphocellulose chromatography under non-denaturing conditions (17, 18). Four to 6 Moles of signal purified by either technique were required per Moles of core-delta for maximal stimulation of activity.

To label the non-template strand, the same procedure was followed except that DNA was labeled with [3H]-UTP. The labeled DNA was purified by DEAE-Sephadex chromatography and ethanol precipitation.

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