Comparative Analysis of the Interactions of *Escherichia Coli* σ^S and σ^{70} RNA Polymerase Holoenzyme with the Stationary-Phase-Specific *bolAp1* Promoter†

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ABSTRACT: We have investigated the interactions of *Escherichia coli* σ^{70} and σ^S holoenzyme RNA polymerases (Eσ^S and Eσ^{70}) with the stationary-phase-specific *bolAp1* promoter by various footprinting methods *in vitro*. Eσ^S and Eσ^{70} have been shown to transcribe the *bolAp1* promoter *in vitro*. We have determined the effects of salt and holoenzyme concentrations on Eσ^S and Eσ^{70} open complex formation at the *bolAp1* promoter *in vitro*. We have obtained a high-resolution hydroxyl radical (OH·) footprint of Eσ^S and Eσ^{70} on the *bolAp1* promoter. The OH· footprinting data show remarkable similarities between the footprints of the heparin-resistant transcription complexes of the two holoenzymes which have the same +1 transcription start site. However, there are distinctive differences in the protection patterns in the region between −20 and −10 of the *bolAp1* promoter. $KMnO_4$ reactivity assays reveal that, at 37 °C, both holoenzymes produced similar but not identical patterns of reactivities.

Upon entry into stationary phase, *Escherichia coli* cells undergo complex physiological changes. Stationary phase *E. coli* cells are resistant to high temperature, high concentrations of H_2O_2[1] and very high medium osmolarity (Loewen & Hengge-Aronis, 1994). The stationary phase cells are also able to survive prolonged periods of starvation (Loewen & Hengge-Aronis, 1994). The *katF/rpoS* gene has been identified as a central early regulator of a large number of starvation/stationary phase genes (Lange & Hengge-Aronis, 1991) and is induced during transition from exponential to stationary phase.

BolA is one of the *rpoS*-dependent genes (Lange & Hengge-Aronis, 1992). The *bolA* gene is involved in the process of morphogenesis of stationary phase cells (Aldea et al., 1989). The *bolA* promoter activity, as measured by its mRNA level and by reporter gene fusion assays, increases 7−10-fold during the transition from log to stationary phase of growth (Aldea et al., 1989, Lange & Hengge-Aronis, 1992). A *bolAp1* promoter mutant that contains only 55 bp upstream from the transcriptional start site still shows growth rate dependence (inverse dependence on growth rate) (Aldea et al., 1990).

The *katF/rpoS* gene product, σ^S, has been shown *in vitro* to be a sigma factor. It binds to and confers promoter specificity on core RNA polymerase (Nguyen et al., 1993, Tanaka et al., 1993). Different *E. coli* sigma factors, with the exception of Eσ^{54}, recognize different promoter motifs centered around the −10 and −35 regions upstream of the transcription start site located at +1. For one σ^S-dependent promoter, *fic*, Tanaka et al. (1995) concluded based on mutagenesis studies that Eσ^S recognition does not require the specific DNA sequences upstream of −17. Espinosa-Urgel and Tormo (1993) found that σ^S-dependent promoters, including the *bolAp1* promoter, are located in regions where DNA shows intrinsic curvature.

A number of promoters that have been tested can be recognized by both Eσ^{70} and Eσ^S (Nguyen et al., 1993, Tanaka et al., 1993), including the *bolAp1* promoter. At the *bolAp1* promoter, Eσ^S initiates transcription at +1 start site. Eσ^{70} has two transcription start sites, +1 (complex A), which is the same as that of Eσ^S, and −10 (complex B), which is 10 bp upstream of the +1 start site (Nguyen et al., 1993). The *bolAp1* promoter is transcribed about equally well *in vitro* by both Eσ^S and Eσ^{70} at one set of solution conditions (Nguyen et al., 1993), suggesting that the *rpoS*-dependence of the *bolAp1* promoter *in vivo* may be indirect. It seemed possible that the *bolAp1* promoter is transcribed by both Eσ^S and Eσ^{70}, by only Eσ^{70}, or by only Eσ^S during stationary phase of growth. We asked which holoenzyme, Eσ^S or Eσ^{70}, transcribed the *bolAp1* promoter better *in vitro* at physiologically relevant salt concentrations that mimicked the *in vivo* situation. Also, how do the two different holoenzymes interact with the *bolAp1* promoter? Do their interactions closely resemble those at other σ^{70}-dependent promoters? We present the following experiments to address these questions.

MATERIALS AND METHODS

Materials. All reagents and chemicals were purchased from Sigma unless otherwise indicated. DNase I was from Promega (Madison, WI). HPLC-purified deoxynucleoside triphosphates and ribonucleoside triphosphates were from Pharmacia (Milwaukee, WI). DNA primers were synthesized by the University of Wisconsin Biotechnology Center. The NACS52 column matrix was from GIBCO-BRL (Bethesda, MD).

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[1] Abbreviations: H_2O_2, hydrogen peroxide; PCR, polymerase chain reaction; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; DTT, dithiothreitol; OH·, hydroxyl radical; $KMnO_4$, potassium permanganate; KCl, potassium chloride; KGlu, potassium glutamate; bp, base pair.

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DNA Templates, Primers, Strains, and Plasmid Constructions. pGN66 contains the *bolA* promoter from −381 to +175 with +1 as the transcription start site in a pSP72 vector (Promega) (Nguyen et al., 1993). pGN64B contains the *bolA* promoter from −130 to +175 cloned into the *Eco*RV and *Pvu*II sites of pSP72. pGN65T contains the *bolA* promoter from −381 to +45 cloned into the *Bgl*II and *Pvu*II sites of pSP72. The following are primers that hybridize to the *bolA* promoter region: Primer 1 is 5′-GGATCCTGCTGTG-GCAGTG-3′ (−381 to −369). Primer 2 is 5′-CTAGC-GACTGGTCGTGTTG-3′ (complementary to +157 to +175). Primer 3 is 5′-TCGCCCCTGGTAAAAGAAACACTGAT-3′ (−153 to −128). Primer 4 is 5′-CCACTACTTCGAG-GAATACGGG-3′ (complementary to +85 to +106). A 556 bp *bolA* promoter fragment from −381 to +175 was prepared by PCR with primers 1 and 2. The concentration of the 556 bp *bolA*-containing DNA fragment was determined spectrophotometrically at A_{260nm}. The 556 bp DNA fragment containing the *bolAp1* promoter yields a 176-base run-off transcript from the *bolAp1* promoter.

Protein Purification. σ^S was overproduced and purified as described (Nguyen & Burgess, 1996). Individual fractions were dialyzed against storage buffer (Lowe et al., 1979) and stored at −20 °C. Core RNA polymerase was purified by immunoaffinity and Bio-Rex-70 chromatography as described (Thompson et al., 1992; Lowe et al., 1979). The same preparation of core RNA polymerase was used for forming Eσ^{70} and Eσ^S in functional studies. σ^{70} was purified as described (Gribskov & Burgess, 1983).

Nitrocellulose Filter Binding Assays. The assay was done essentially as described (Roe et al., 1984). The 556 bp *bolAp1* promoter fragments were labeled at both 5′ ends with γ-^{32}P-ATP and T4 polynucleotide kinase and purified by using a 1 mL G50 desalting spin column to remove free labeled ATP. Binary complexes were formed by incubating, at 37 °C, 50 fmol of the labeled *bolA* promoter fragments with varying amounts of core RNA polymerase and a 20-fold molar excess of either σ^{70} or σ^S over core RNA polymerase for 20 min. The reactions were in 50 μL containing 50 mM potassium Hepes pH 7.0, 10 mM magnesium acetate, 1 mM DTT, 100 μg/μL BSA, and varying amounts of KGlu or KCl. To distinguish open complexes, 10 μg/mL heparin (CalBiochem) was added for 10 s prior to filtration. Heparin is a polyanionic competitor used to eliminate filter-retention of complexes formed between holoenzymes and ends or random interior sites on DNA promoter fragments (Roe et al., 1984). If added prior to the addition of holoenzyme, 10 μg/mL of heparin was sufficient to prevent holoenzyme binding (data not shown). Heparin did not cause dissociation of binary complexes (protein and DNA) because the extent of open complex formation was independent of heparin concentration (data not shown). Filtration was done at 2 mL/min and the filters were then rinsed with 500 μL of transcription buffer with 100 mM KCl but without BSA. The filters were then dried and subjected to scintillation counting. The background retention of DNA on the filter in the absence of holoenzyme (3−5% of total input DNA) was subtracted from each determination. The total amount of DNA was determined by spotting 50 μL of binding mixture onto a dry filter. Filter assays were carried out in duplicate.

Transcription Activity Assay. Binary complexes (protein and DNA) were formed as described above. The samples were then incubated for an additional 3 min with 500 μM ATP, GTP, and UTP to form ternary complexes (protein, DNA, and RNA). KCl (350 mM) was then added for a 3 min incubation to disrupt binary complexes (Krummel et al., 1989), followed by 50 μM α-^{32}P CTP (5000 cpm/pmol) and a 30 min incubation to allow elongation of run-off transcripts. The reactions were processed as described (Kassavetis et al., 1990) with labeled DNA recovery markers in the stop solution. RNA was resuspended in 7 μL of 95% formamide and loaded on a 5% polyacrylamide gel containing 7 M urea. The gel was then exposed to Kodak XAR5 film overnight.

The Chamberlin assay to determine the specific activity of Eσ^{70} was done as described (Chamberlin et al., 1979). Eσ^{70} was about 23% active by this assay.

Hydroxyl Radical Footprinting Assay. The lower and upper strand of the *bolA* promoter fragment was 3′ end labeled at the unique *Xho*I site of pGN64B and pGN65T with Klenow DNA polymerase fragment and 800 Ci/mmol α-^{32}P-dCTP (Amersham). pGN65T and pGN64B were then cleaved with *Stu*I or *Bgl*II, respectively, and the labeled promoter fragment was purified by a G50 desalting spin column, native polyacrylamide gel electrophoresis, passive elution, and finally by NACS52 ion exchange chromatography (Kassavetis et al., 1989). Binary complexes were formed by incubating, at 37 °C, 0.1 pmol of the labeled *bolA* promoter fragment with 0.4 pmol of core RNA polymerase and a 5-fold molar excess of either σ^{70} or σ^S over core RNA polymerase in 50 μL containing 50 mM potassium Hepes pH 7.0, 10 mM magnesium acetate, 1 mM DTT, 100 μg/mL BSA, and 100 mM KGlu for 20 min. Heparin (10 μg/mL) was added for 10 s, and the reactions were then footprinted by diluting to 400 μL with transcription buffer and 2% (w/v) sodium ascorbate, 0.03% H_2O_2, 40 μM Fe(EDTA) at 37 °C for 2 min (Dixon et al., 1991). Fe(EDTA) (10 μM) was used in the naked DNA control samples (Mecsas et al., 1991). The samples were diluted to reduce the glycerol concentration prior to adding the footprinting reagents because glycerol inhibits the cleavage activity of Fe(EDTA) (Dixon et al., 1991). The reactions were stopped with 5% glycerol and the bound DNA isolated by nitrocellulose filter binding assays as described above to remove unbound DNA that caused background problem. DNA was then eluted from the filter with 650 μL of elution buffer (50 mM Tris-HCl pH 7.9, 100 mM NaCl, 0.2% SDS, and 500 mM ammonium acetate) at 37 °C overnight. The eluate was then extracted with an equal volume of 25 phenol:24 $CHCl_3$:1 isoamyl alcohol and precipitated with 1 M LiCl, 10 μg of glycogen, and 650 μL of isopropanol. The samples were resuspended with 7 μL of 95% formamide, boiled for 5 min, and analyzed on a 5% polyacrylamide 7 M urea denaturing gel.

Permanganate ($KMnO_4$) Assay. Binary complexes were formed as described in the hydroxyl radical footprinting section above. Ternary complexes were formed from the binary complexes by adding 150 μM ATP, GTP, and UTP for an additional 5 min. When needed, 350 mM NaCl was added for an additional 5 min to disrupt only binary complexes and not ternary complexes (Krummel et al., 1989). The assay was done essentially as described (Sasse-Dwight & Gralla, 1991). $KMnO_4$ (25 mM) was added to footprint the samples. After 30 s, the reactions were stopped with 180 μL of 3 M sodium acetate pH 5.2, 0.2 M 2-mercaptoethanol, 3 mM EDTA, and 200 μg/μL yeast tRNA. The

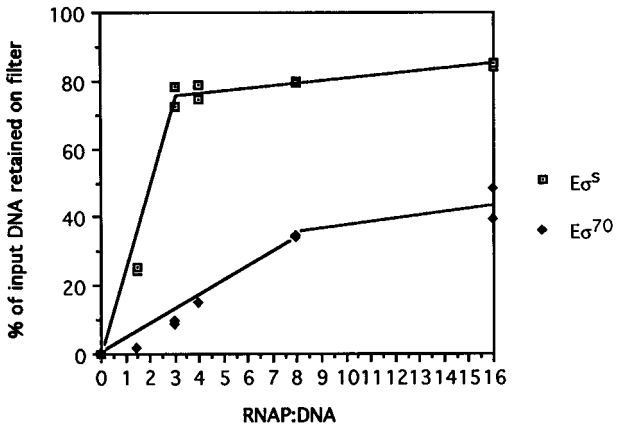

FIGURE 1: Holoenzyme titration at 50 mM KCl and 10 mM magnesium acetate. The *x*-axis indicates the holoenzyme:DNA ratio. The *y*-axis shows the percent of total *bolAp1* labeled promoter fragments retained on the filter, an indication of the amount of heparin-resistant open complexes formed in a filter binding assay. ⊡ represents Eσ^S samples. ◆ represents Eσ^{70} samples. Each point is the average of duplicate determinations.

samples were then precipitated with ethanol. Each sample was divided in half for primer extension with either primer 3 or primer 4. Primer extensions were performed in 100 μL of 20 mM Tris-HCl pH 7.9, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.05% NP-40 with 1 pmol of ^{32}P-labeled primer, 100 μM dNTPs, and 0.5 unit of *Taq* DNA polymerase. The reactions were denatured at 94 °C for 30 s, annealed at 61 °C for 30 s, and allowed to extend for 2 min at 72 °C. A total of 10 cycles were performed. The samples were then processed as described (Newlands et al., 1991). DNA sequencing was done with the labeled primers to serve as labeled markers.

DNase I Footprinting Assay. Binary complexes were formed as described in the hydroxyl radical footprinting section above with 100 mM KGlu. DNase I (15 ng or 22 ng) was added for 30 s to the no protein control and the binary complex, respectively. The reactions were stopped by the addition of 5 μL of 200 mM EDTA and the bound complex was separated from free DNA by native gel electrophoresis (Newlands et al., 1993). The samples were eluted overnight in elution buffer. The eluate was then extracted with an equal volume of 25 phenol:24 $CHCl_3$:1 isoamyl alcohol and precipitated with 1 M LiCl, 10 μg of glycogen, and 650 μL of isopropanol. The samples were resuspended with 7 μL of 90% formamide, boiled for 5 min, and analyzed on a 5% polyacrylamide 7 M urea denaturing gel. For the 3′ end labeled fragments, if a DNase I cleaved band lines up with the chemical cleavage sequencing band, then the DNase I cleavage site is 3′ of that base since both chemical cleavage and DNase I cleavage leaves a 5′ phosphate. A + G chemical cleavage of labeled promoter DNA fragments was done as described (Kassavetis et al., 1989).

RESULTS

Transcription Activity at the bolAp1 Promoter. Relatively, how well do the two holoenzymes bind to and transcribe the *bolAp1* promoter *in vitro*? The answer depends on the holoenzyme and salt concentrations. At 100 mM KCl, more Eσ^{70} than Eσ^S is required to form an equivalent amount of heparin-resistant open complexes in a nitrocellulose filter binding assay (Figure 1). The low amount of open complexes formed by Eσ^{70} is not due to the holoenzyme being inactive because it is about 23% active in a Chamberlin transcription assay, comparable to Eσ^S-specific promoter binding activity in the filter binding assay. The inability of Eσ^{70} to bind most of the promoter fragments even at highest RNAP:DNA ratio tested is possibly due to Eσ^{70}−DNA complex being unstable in the presence of heparin.

In salt titration experiments with KCl or KGlu, Eσ^S binds the *bolAp1* promoter tighter than Eσ^{70} does at high salt concentration at various holoenzyme:DNA ratios. As shown in Table 1, the ratio of *bolAp1* promoter fragment retained by Eσ^S over that retained by Eσ^{70} increases as the concentration of KCl or KGlu increases. K^+ and glutamate are the significant cytoplasmic osmolytes in cells grown in media of low osmolarity, and their levels increase with increasing external osmolarity (Cayley et al., 1991). At 400 mM KGlu, a salt concentration that mimics the *in vivo* level (Richey et al., 1987), Eσ^S retains promoter DNA 10−20-fold better than Eσ^{70} (Table 1).

We obtained the same result regarding the effect of increasing salt concentrations in single-round transcription assays: at increasing salt concentrations (KCl or KGlu), Eσ^S is more active than Eσ^{70} (Figure 2). There are more σ^S-specific transcripts than σ^{70}-specific transcripts at all salt concentrations tested. We conclude that Eσ^S forms open transcription complexes at the *bolAp1* promoter more efficiently than Eσ^{70} at higher salt concentrations.

To determine how the two different holoenzymes interact with the *bolAp1* promoter *in vitro*, we employed various footprinting assays.

$KMnO_4$ Modification. $KMnO_4$ strongly oxidizes unpaired or distorted pyrimidines in DNA, reacting much more strongly with thymine (T) than with cytosine (C). This reagent has been used to detect single-stranded regions of DNA around the transcription start site in open transcription complexes (Sasse-Dwight & Gralla, 1991).

As shown in Figure 3, $KMnO_4$ probing verifies that the 37 °C heparin-resistant complexes in the filter binding assay are open complexes and also shows similar but not identical reactivity patterns between the binary transcription complexes containing Eσ^{70} and Eσ^S. In binary complexes, both holoenzymes generate enhanced reactivities at −6, −5, and +4 Ts on the nontemplate strand and at −11, −8, −3, and −2 Ts and −10, +1 Cs on the template strand. However, only with Eσ^{70} bound (at 100 mM KGlu), −9 T and −12 T on the nontemplate strand are slightly reactive with $KMnO_4$, and −19 to −22 Ts on the template strand are strongly reactive to $KMnO_4$ (Figure, 3, panels A and B, lane 7). At higher ionic strength, such as 100 mM KCl, these upstream σ^{70}-specific signals are not present, and the $KMnO_4$ reactivity pattern is identical between Eσ^S and Eσ^{70} (data not shown).

Both holoenzymes, in the ternary complex arrested at +11 C (formed with only A, U, and GTP), give the same $KMnO_4$ reactivity pattern: −6, −5, +4 , and +8 Ts on the nontemplate strand and −9, −8, −3, −2, +3, and +10 Ts along with +1 C on the template strand are unpaired (Figure 3, panels A and B). Eσ^{70}-dependent binary complex signals at −19 to −22, −12, −9 Ts disappeared in the ternary complex (Figure 3, panels A and B, lanes 7 and 10). In the open transcription bubble, we cannot detect protein-dependent reactivities of −7, −4, +3, and +7 Ts as expected

Table 1: Extent of Retention of the *BolApl* Promoter Fragments with σ^S and σ^{70} Holoenzyme at Different Salt Concentrations[a]

	2RNAP:1DNA			4RNAP:1DNA			1ORNAP:1DNA		
salt	Eσ^S	Eσ^{70}	Eσ^S/Eσ^{70}	Eσ^S	Eσ^{70}	Eσ^S/Eσ^{70}	Eσ^S	Eσ^{70}	Eσ^S/Eσ^{70}
100 mM KGlu	53%	24%	2.2	65%	54%	1.2	67%	61%	1.1
250 mM KGlu				68%	12%	5.7	69%	15%	4.6
400 mM KGlu	29%	1.4%	20.7	58%	6%	9.7	67%	6%	11
50 mM KCl	52%	13%	4	67%	30%	2.2	76%	57%	1.3
100 mM KCl				40%	11.4%	3.5	40%	20%	2
150 mM KCl	15%	1.7%	8.8	23%	3%	7.7	22%	1.7%	12.9

[a] The percentage is percentage of total RNA retained on the filter.

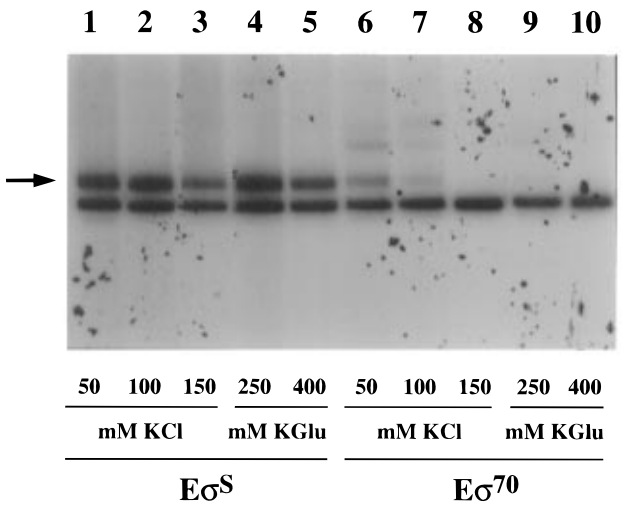

FIGURE 2: Transcription activity of Eσ^S and Eσ^{70} at different salt concentrations. The assay is done with 50 fmol of *bolAp1* promoter fragments and 200 fmol of Eσ^S or Eσ^{70} formed as described in the Material and Methods section. Eσ^{70} is about 23% active based on the Chamberlin transcription assay. Eσ^S is about 25% active in binding to the *bolAp1* promoter (Figure 1). Lanes 1−5 are Eσ^S samples. Lanes 6−10 are Eσ^{70} samples. Lanes 1 and 6 are 50 mM KCl samples. Lanes 2 and 7 are 100 mM KCl. Lanes 3 and 8 are 150 mM KCl. Lanes 4 and 9 are 250 mM KGlu. Lanes 5 and 10 are 400 mM KGlu. The arrow indicates *bolAp1*-specific transcripts. The band below the *bolAp1* transcripts is labeled DNA used as a recovery marker.

because these residues were already highly reactive in naked DNA. The $KMnO_4$ data are summarized in Figure 3C.

DNase I Footprinting. The protection profiles of binary complexes for both holoenzymes are qualitatively similar (Figure 4). On the nontemplate strand, both holoenzymes protect from −57 to +22 in four areas: −57 to −47, −41 to −35, −34 to −26, and −22 to +22 with DNase I hypersensitive sites at −35, −34, and −26, −25. On the template strand, both holoenzymes protect three areas similarly: −56 to −48, −45 to −37, and −34 to −28. There is a major difference downstream of +1. Eσ^S protect −25 to +20 while Eσ^{70} gives a longer footprint from −25 to about +30. With both holoenzymes, there is a DNase I hypersensitive site only at −37. To obtain a higher resolution of backbone protection patterns of both holoenzymes, we used the OH· cleavage reagent.

OH· Footprinting. Due to their small size, OH· are useful probes for studying backbone contacts at high resolution. In the presence of Eσ^S, small regions of strong protection upstream of −20 are visible, centered at approximately −49, −39, and −29 on the nontemplate strand and at −53, −42, −32, and −21 on the template strand (Figure 5). The protected regions on each strand are offset from those of the other strand by 2−3 bp in the 3′ direction, indicating protein interactions are across the minor groove of the DNA, since the backbone positions closest to each other across the minor groove are 3 bp apart in sequence (Dixon et al., 1991). Since on each strand, the protected regions are 10−12 bp apart upstream of −20, Eσ^S appears to contact primarily one face of the helix (Dixon et al., 1991).

Downstream of −10, Eσ^S protects from −9 to +17 on the nontemplate strand and, on the template strand, two regions from −14 to −3 and +4 to +17. The region around the transcription start site from −2 to +3 on the template strand is accessible to OH· cleavage (Figure 5), analogous to the Eσ^{70} footprint on the T7A1 (Metzger et al., 1989) and the λP_r promoters (Craig et al., 1995). Overall, the sugar−phosphate backbone structure of the Eσ^S−*bolAp1* complex is very similar to that observed for Eσ^{70} interactions with *rrnB* P1, λP_r and T7A1 promoters (Newlands et al., 1991; Craig et al., 1995; Metzger et al., 1989), and for Eσ^{32} interactions with three heat shock promoters (groE, rpoD, and dnaK) (Mecsas et al., 1991).

The Eσ^{70} footprint shows similarities to Eσ^S footprint but also some differences in the region around −10. Downstream of −10, on the nontemplate strand, the Eσ^{70} footprint extends from −9 to +17, similar to that of Eσ^S (Figure 5A). However, on the template strand, the Eσ^{70} footprint is shorter than that of Eσ^S, extending only from −11 to −3 and from +4 to +17 (Figure 5B). The transcription start site on the template strand is still accessible to OH· cleavage. Immediately upstream of −10, in direct contrast to the Eσ^S footprint, Eσ^{70} does not protect over the 5′ half of the −10 box on the template strand. Eσ^{70} protects strongly the entire region from −20 to −12 on the nontemplate strand, while Eσ^S shows weak protection from −17 to −13 on the nontemplate strand (Figure 5A).

DISCUSSION

The filter binding data of Figure 1 show that Eσ^S forms more heparin resistant complexes than Eσ^{70} does at all RNAP:DNA ratios tested at 100 mM KCl. Even at the highest RNAP:DNA ratio, Eσ^{70} does not bind a majority of *bolAp1* promoter fragments or reach the plateau level of Eσ^S as expected since, in the absence of heparin, Eσ^{70} binds as much promoter DNA as Eσ^S does at saturating holoenzyme concentration (data not shown). One possible explanation is that since Eσ^{70} forms two transcription competent complexes, complex A (+1 transcription start site) and complex B (−10 start site) [see Figure 2, Nguyen et al. (1993)], one complex is unstable in the presence of heparin and it dissociates during the filtration step of the filter binding assay. Our interpretation is that the filter-bound heparin resistant complex is Eσ^{70} complex A since its OH· footprint shows a similar footprint over the +1 start site (Figure 5) as

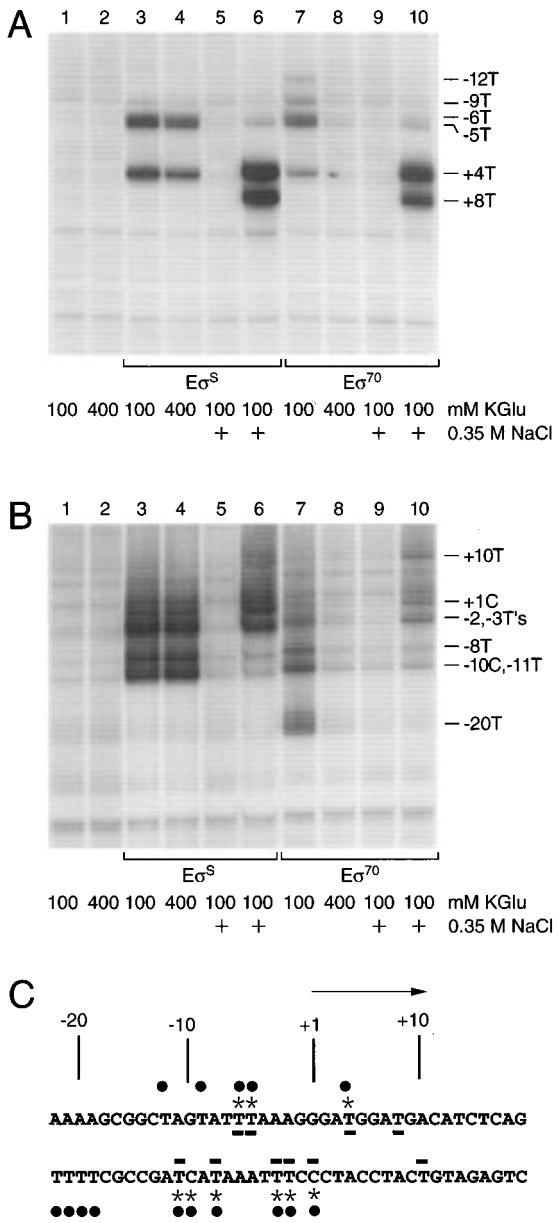

FIGURE 3: $KMnO_4$ reactivity patterns of Eσ^S and Eσ^{70} at the *bolAp1* promoter at 37 °C. A, nontemplate strand. B, template strand. Lanes 1 and 2 show naked DNA that has been treated with $KMnO_4$. Lanes 3−6 are Eσ^S samples. Lanes 7−10 are Eσ^{70} samples. All reactions are in 100 mM KGlu except lanes 2, 4, and 8, which contained 400 mM KGlu. All reactions contained 10 mM magnesium acetate. Lanes 6 and 10 represent ternary complexes formed from binary complexes with 500 μM A, G, and UTP followed by 350 mM NaCl addition. Lanes 5 and 9 are identical to lanes 6 and 10, respectively, except that 350 mM NaCl was added to the binary complexes before nucleoside triphosphates addition. C, summary of $KMnO_4$ reactivity data. The asterisk represents Eσ^S binary complex pattern. ● represents Eσ^{70} binary complex pattern. The solid rectangle represents the identical ternary complex pattern of both holoenzymes. The transcription start site is +1.

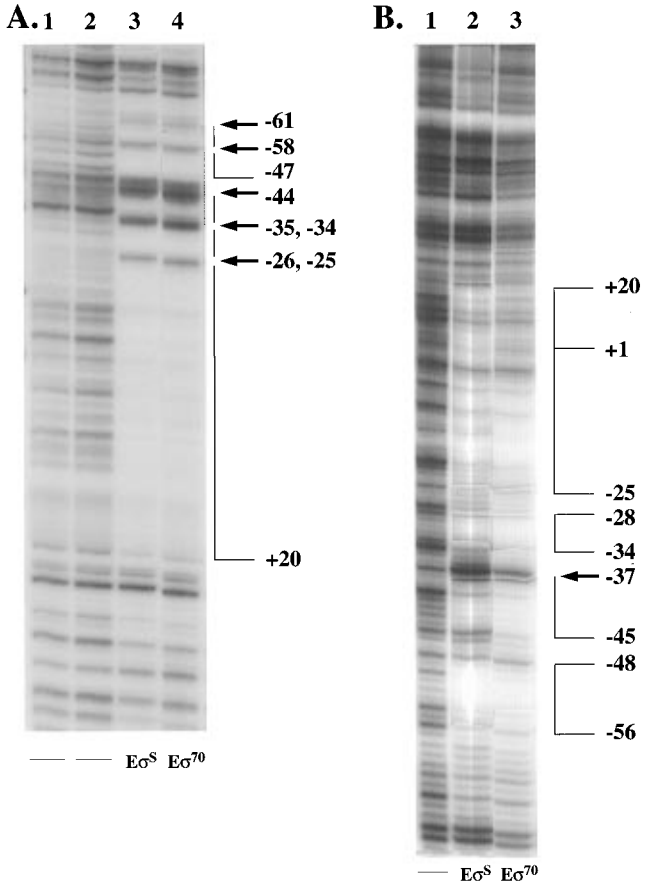

FIGURE 4: DNase I footprint of binary complex containing Eσ^S or Eσ^{70} at the *bolAp1* promoter. A, nontemplate strand data: lanes 1 and 2 are the no holoenzyme controls, lane 3 is the Eσ^S sample, and lane 4 is the Eσ^{70} samples. B, template strand data: lane 1 is the no holoenzyme control, lane 2 is the Eσ^S sample, and lane 3 is the Eσ^{70} sample. Arrows denote DNase I hypersensitive sites. Vertical bars on the side of the figure represent areas protected from DNase I cleavage. The protection patterns of Eσ^S and Eσ^{70} are summarized on the right.

that of Eσ^{70} complexed with T7A1 or λP_r promoter (Craig et al., 1995; Metzger et al., 1989).

The filter binding result in Table 1 suggests that Eσ^S forms more open transcription complexes than Eσ^{70} does at increasing concentration of salt. It is also possible that the low amount of labeled DNA retained on the filter with Eσ^{70} at high salt concentration is an indication of dissociation of DNA from Eσ^{70}−DNA complexes during the filtering (2 s) and washing (15 s) even though 60−70% of the DNA was retained in the presence of Eσ^{70}, comparable with that of Eσ^S at high RNAP:DNA and low salt concentrations. However, the transcription data of Figure 1 shows that indeed Eσ^{70} forms fewer open transcription complexes at high salt concentration than Eσ^S does. *In vitro*, Eσ^S binds specifically to the *bolAp1* promoter and, at physiologically relevant salt concentrations, transcribes the promoter much better than Eσ^{70} (Figure 1). These data support the hypothesis that, *in vivo*, the *bolAp1* promoter is directly transcribed by Eσ^S during the transition from log to stationary phase. Increasing salt concentration also enhanced Eσ^S transcription over Eσ^{70} transcription of the osmoregulated promoters, *osmY* and *osmB* (Ding et al., 1995). Our data do not exclude the possibility of the involvement of a stationary-phase-specific activator in *bolAp1* transcription. At the present, no known *bolAp1*-specific activator protein has been found. Such an activator would have to bind to the same promoter region as holoenzyme footprints since the *bolAp1* promoter activity does not require sequences upstream of −55 (Aldea et al., 1990).

The *bolAp1* promoter is located in a DNA region that has intrinsic curvature (Espinosa-Urgel & Tormo, 1993). Whether or not the *bolAp1* promoter needs an intrinsic DNA bending for Eσ^S to bind, the Eσ^S footprint is not distinctively different from the Eσ^{70} interaction patterns at the T7A1, λP_r, or *rrnB*

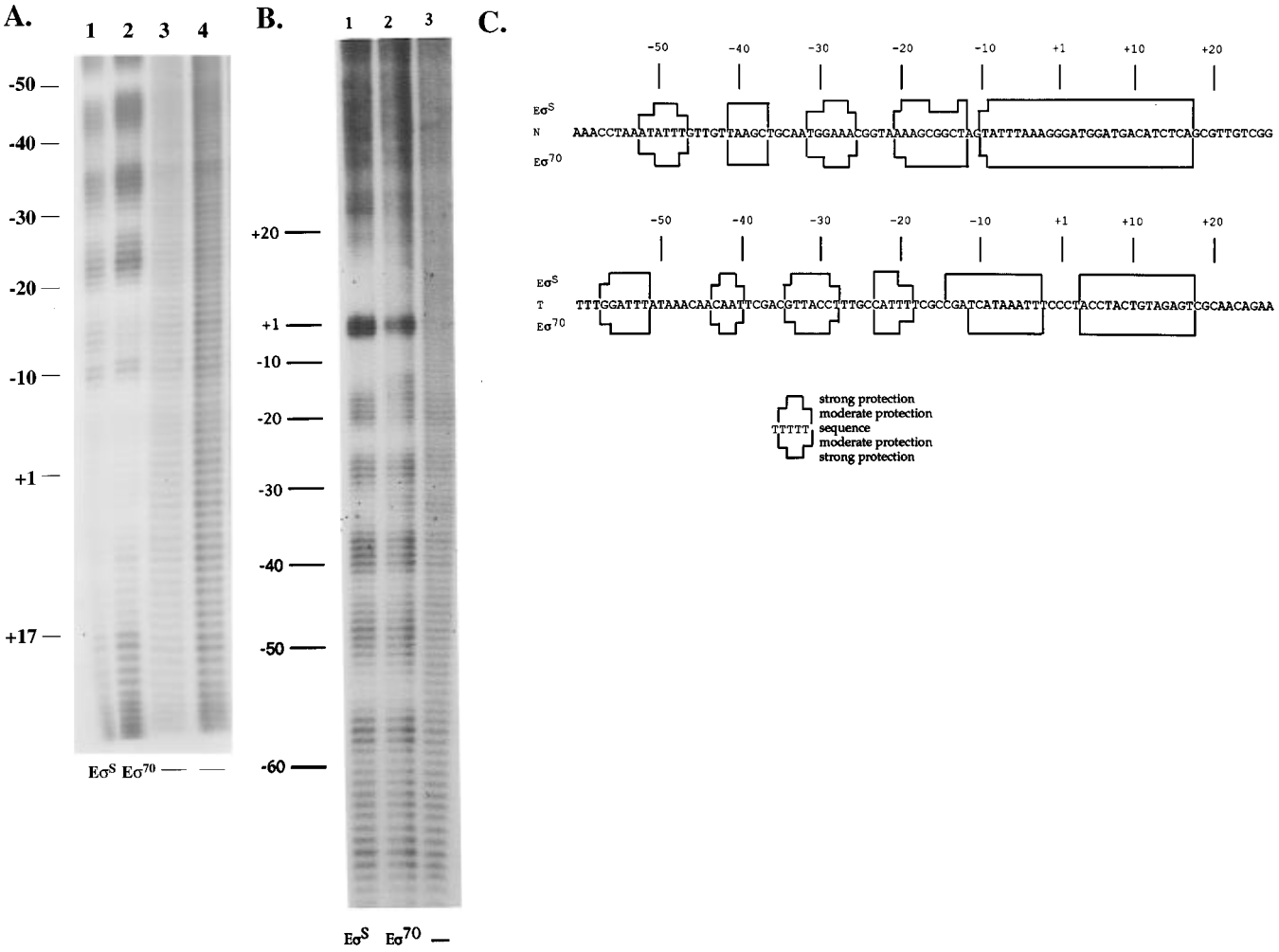

FIGURE 5: OH· footprint of transcription complexes containing Eσ^S or Eσ^{70} at the *bolAp1* promoter. A, nontemplate strand. Lane 1 shows the Eσ^S sample, lane 2 is the Eσ^{70} sample, and lanes 3 and 4 are the no holoenzyme control samples (naked DNA). B, template strand. Lane 1 is the Eσ^S sample. Lane 2 is the Eσ^{70} sample. Lane 3 is the no holoenzyme sample. C, summary of binary complex OH· and DNase I footprinting data: +1 is the transcription start site, T is the template strand, and N is the nontemplate strand. Eσ^S protection is above each DNA strand. Eσ^{70} protection is below each DNA strand.

P1 promoters or the Eσ^{32} interaction patterns at heat shock promoters. Compared with the analogous OH· footprints of Eσ^{70} at the above-mentioned promoters (Newlands et al., 1991; Schickor et al., 1990; Craig et al., 1995), and Eσ^{32} at three heat shock promoters (Mecsas et al., 1991), the Eσ^S footprint at the *bolAp1* promoter has nearly identical polymerase-DNA backbone interactions. The accessibility of the transcription start site of the *bolAp1* promoter to the OH· reagent in the presence of either Eσ^S or Eσ^{70} has also been observed for the T7A1 and λP_r promoters with Eσ^{70} (Schickor et al., 1990; Craig et al., 1995).

The OH· footprint of Eσ^{70} on the *bolAp1* promoter is similar but not identical to that of Eσ^S. In contrast to Eσ^S, Eσ^{70} footprints strongly the −17 to −13 region on the nontemplate strand but does not footprint the −14 to −9 region on the template strand, where part of the −10 hexamer is located (Figure 5). This latter difference in interactions may cause the weak transcription activity of the Eσ^{70} complex A. Although the OH· footprint pattern of Eσ^{70} might be a composite footprint of both complex A and B, we believe it to be the footprint of complex A (+1 start site). If there is also a complex B, then we would expect the region downstream of −20 (the location of the −10 box of the promoter of complex B) to be protected and that there would be protected regions centered at −60 of the nontemplate strand and at −65 of the template strand. Both expectations are based on the usual footprint pattern of Eσ^{70}. We do not see these patterns in Figure 5 and other OH· footprinting experiments (data not shown).

On the other hand, the $KMnO_4$ reactivities at −9, −12, and −19 to −22 Ts in the presence of Eσ^{70} indicated that the $KMnO_4$ footprint may be a composite footprint of complexes A and B. These specific signals probably belong to the B complex because they are too far upstream of the expected transcription bubble size for a complex that would start at +1 (complex A).

The difference between the OH· and $KMnO_4$ footprint data can be rationalized by the way the transcription complexes are manipulated in the assays. Exposure of transcription complexes to heparin is longer in the OH· assay (3 min) than that of in $KMnO_4$ (40 s). If the complex B is less stable than the complex A, then longer exposure to heparin and a 100 mM KCl salt wash can lead to the loss of the complex B in the OH· assays. Consistent with this hypothesis, $KMnO_4$ assays done at 100 mM KCl did not show the presumed complex B specific signals (data not shown). The Eσ^{70} complex A has the same $KMnO_4$ pattern as that of Eσ^S at the *bolAp1* promoter.

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