Conformational Changes of Escherichia coli RNA Polymerase σ70 Factor Induced by Binding to the Core Enzyme*

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Mutants of RNA polymerase σ70 subunit from Escherichia coli with unique cysteine residues engineered into conserved region 1 (autoinhibition domain of σ70), region 2.4 (−10 DNA element binding domain), region 4.2 (−35 DNA element binding domain), and a nonconserved region between regions 1 and 2 were prepared. The chemical reactivity of the cysteine at each position was determined for free σ70 and σ70 in complex with the core polymerase. Binding of the core polymerase resulted in increased solvent exposure of DNA binding domains of σ70 and in more complex changes in the autoinhibition domain (region 1). Similar conformational changes in σ70 were detected using fluorescence probes covalently attached to cysteine residues engineered into σ70. Thus, the results obtained provided physical evidence supporting a model in which core enzyme allosterically regulates DNA binding activity of σ70 by “unmasking” its DNA binding domains.

Escherichia coli RNA polymerase (RNAP)1 is a multisubunit enzyme that exists as core enzyme (α2 ββ′) or holoenzyme (α2 ββ′σ) (1). Transcription initiation begins with the specific binding of the holoenzyme to promoter DNA. Although core RNAP has the enzymatic activity necessary for transcription, it lacks the ability to specifically bind promoter DNA (1). Therefore, it was suggested that the function of σ subunit was to confer specific promoter recognition to RNAP (2). There is a variety of Es. coli σ transcription factors that are expressed in response to different growth conditions and environmental stresses and that regulate the expression of genes accordingly (3). The primary σ70 factor is responsible for expression of most genes in Es. coli (4) and is the focus of these studies.

The role of σ70 as the specificity subunit was confirmed by genetic studies that identified two conserved regions of the protein, regions 2.4 and 4.2, as likely candidates for specific protein-DNA interactions with −10 and −35 promoter DNA sequences, respectively (5–7). However, physical interaction between promoter DNA and free σ70 could not be shown (8). This paradox was resolved by the finding that the N-terminal sequences in σ70 inhibit specific σ70-promoter DNA interactions (9, 10). In these studies, it was shown that the glutathione S-transferase fusion proteins containing fragments of σ lacking the N-terminal sequences (corresponding to conserved region 1) were able to specifically bind DNA fragments containing −10 and/or −35 sequences. In contrast, the glutathione S-transferase fusion proteins containing full-length σ70 or glutathione S-transferase fusion proteins containing deletion mutants of σ in which only a part of conserved region 1 was removed did not bind promoter DNA. Further extensions of these studies showed that polypeptide fragments containing region 1 of σ70, when used in trans, could inhibit specific DNA binding of polypeptide fragments containing region 4 of σ70, whereas the activity of fragments containing region 2 was unaffected (10).

Based on these observations, a model of σ70-DNA binding modulation by core enzyme was proposed (10). In this model, in free σ70, the N-terminal region 1.1 occludes region 4, preventing its specific protein-DNA interaction with the −35 region of promoter DNA. It was further suggested that binding of σ70 to core RNAP induced an allosteric conformational change in σ70, such that the N-terminal portion of the protein shifted to expose region 4, which could now contact bases in the −35 region of promoter DNA. Additionally, it was suggested that DNA binding activity of region 2 was also regulated by N-terminal sequences, but most likely not by a simple occlusion mechanism because region 2 DNA binding activity was inhibited by region 1 only in cis, not in trans (10). However, an interaction between residues from region 1 and region 2 has been proposed (11). The self-inhibition of DNA binding activity of σ subunits may be a common mechanism for other σ factors evolutionary related to σ70 (10).

In this report, we directly test the model in which σ70 promoter DNA binding activity is regulated by the core RNAP through a core binding-induced conformational changes in σ70 affecting DNA binding domains of the protein (regions 2.4 and 4.2). In our studies we used full-length σ70 protein. Our approach was to engineer, using site-directed mutagenesis, cysteine residues at specific locations in regions 2.4, and 4.2. The chemical reactivity of these residues was then used as an indicator of conformational change in both the free σ70 and σ70 in the holoenzyme. The results obtained showed that core polymerase induced conformational changes in σ70 that affected both DNA binding domains and the auto-inhibition domain of the protein.

EXPERIMENTAL PROCEDURES

Materials—[3H]-N-Ethylmaleimide was purchased as a pentane solution from NEN Life Science Products, and 5,5′-dithiobis(2-nitrobenzoic acid) (12) was from Sigma. N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IAND amide) and...
acyrlylodon were from Molecular Probes, Inc. (Eugene, OR).

Cy5 monosuccinimidyl ester (13) was purchased from Amersham Pharmacia Biotech. The succinimidyl ester of Cy5 was converted to maleimide by reaction with the excess of ethylenediamine followed by reaction with maleimidylpropionic acid succinimidyl ester (Sigma). 5 mg of the stock Cy5 was used. The reaction mixture was then freeze-dried and dissolved in 60 μl of 1 M ethylenediamine. The mixture was incubated for 1.5 h at room temperature, and another 25 μl of 1 M ethylenediamine was added. After additional 1 h incubation at room temperature, the mixture was diluted to ~6 ml with 25 mM triethylammonium acetate buffer, pH 7.0 (Buffer A), containing 2% acetonitrile. The mixture was loaded onto a 10-ml Source 15RPC reverse phase FPLC column (Amersham Pharmacia Biotech), and 100 ml of 0–50% Buffer B gradient (Buffer B: Buffer A + 95% acetonitrile) was applied at 3 ml/min. Fractions eluting at ~26% Buffer B containing amine derivative of Cy5 were collected and lyophilized. Dried fractions were dissolved in 500 μl of N,N-dimethylformamide and 10 mg of maleimidylpropionic acid succinimidyl ester dissolved in 250 μl of N,N-dimethylformamide was added. The mixture was incubated for 1 h at room temperature, was diluted to ~8 ml with Buffer A, and was run on a FPLC reverse phase column as described above. Fractions eluting at ~27% Buffer B containing the maleimide derivative of Cy5 were collected and lyophilized. Cy5 maleimide was stored dry at ~70 °C until used.

Core RNAP was purified from E. coli containing p expression vector for Cy5 maleimide incorporation experiments described above, such that at the selected time, no more than ~50% reaction progress was achieved. Thus, both an increase and a decrease in cysteine reactivity in the presence of core RNAP could be recorded. The core RNAP contains 30 cysteine residues, 7 of which were reported to be accessible for chemical modification in the native core RNAP (21). Thus, it can be calculated that the reaction used in Cy5 labeling measurements, less than 10% of the Cy5 maleimide present in solution could be used up by its incorporation into the core RNAP. Reactions were terminated by addition of SDS-PAGE sample buffer. Reaction mixtures were analyzed on a 10% SDS-PAGE, and the amount of Cy5 incorporated into σ70 was determined by scanning wet gels using STORM and by quantifying with ImageQuant as described above.

Results

Single Cysteine Mutants of σ70

In our approach, we used the reactivity of cysteine residues engineered into specific domains of the protein as indicators of a conformational response of these domains to σ70-core RNA polymerase complex formation. The first step of this approach was to prepare single cysteine mutants of σ70 using site-directed mutagenesis. wt σ70 contains three cysteine residues at positions 192, 291, and 295 and no disulfide bonds (22, 23). Initially, a σ70 mutant (Δcys) was constructed in which all endogenous cysteine residues were replaced with structurally similar serine residues. The mutant protein was expressed, purified, and found to exhibit ~150% activity of the wt σ70 in an in vitro transcription assay (Table I). We therefore concluded that a replacement of all σ70 cysteine residues with serine residues had no negative effect on the function of the protein, as has also been observed recently by others (24, 25). All mutants used in transcriptional activity of σ70 involves numerous unique stereospecific interactions (σ70-core, σ70-duplex DNA, and σ70-single-stranded nontemplate DNA), the preservation of transcriptional activity in Δcys suggests that the conformation of the mutant protein was not also significantly affected by the Cys to Ser change. The increased activity of Δcys σ70...
compared with the wt $\sigma^{70}$ protein may be due to the resistance of the mutant to inactivation due to chemical modifications of cysteine (cross-linking, binding of heavy metals, etc.).

In the next step, single cysteine residues were introduced into [Cys] $\sigma^{70}$. Fig. 1 shows the schematic representation of the primary structure of $\sigma^{70}$, in which the positions of introduced cysteine residues are indicated. The logic behind selecting particular sites for introducing cysteines into region 2.4 and 4.2 was as follows. In each region, we selected one residue implicated by previous mutagenesis studies to be directly involved in the function of the particular region. Although incorporating cysteine into these positions could potentially affect DNA binding or transcriptional activity due to the replacement of a residue making a direct contact with promoter DNA, we reasoned that even if this would occur, the cysteine would still be able to report a change in conformation at the critical sites in response to binding core RNAP. Additionally, two sites in each region were chosen that were close to these residues but for which no direct interaction with DNA has been proposed so far. We reasoned that we could use these additional single cysteine mutants to monitor conformational changes in region 2 and region 4 without affecting DNA binding activity. Using this logic, mutants representing region 2.4 include [A438C] $\sigma^{70}$, [T440C] $\sigma^{70}$, and [S442C] $\sigma^{70}$. Threonine at position 440 was suggested to contact position 12 of promoter DNA (7). Residues 438 and 442 are also on the same $\alpha$-helix (26). Mutagenesis of residue 438 resulted in no change of promoter recognition activity of $\sigma^{70}$ (7), although this residue is located near residue 437, which was suggested to also be involved in recognition of position 12 of the promoter (5). Mutations at position 442 have not been described previously.

Region 4.2 single cysteine mutants include [T583C] $\sigma^{70}$, [R588C] $\sigma^{70}$, and [R596C] $\sigma^{70}$. Arginine at position 588 is located within the second helix of the proposed helix-turn-helix motif of region 4.2 and was presumed to contact the $-33$ base of promoter DNA (6). Threonine at position 583 is the last residue in the turn of the proposed helix-turn-helix of region 4.2 and is one residue away from Arg$^{584}$, which was suggested to contact position $-31$ of promoter DNA (7). Arg$^{586}$ is located outside of the second helix of the region 4.2 helix-turn-helix motif. Point mutations at this position, previously constructed by Siegele et al. (7), were found to have no observable effects on promoter recognition.

Information regarding the possible function of specific residues in region 1 is very limited. Our two mutants in this region are [1C] $\sigma^{70}$, in which cysteine was inserted between the initiation Met residue and the second residue of $\sigma^{70}$, and [A59C] $\sigma^{70}$, located within region 1.1. The residue at position 59 was selected because alanine containing no side chain was unlikely to be involved in any critical interactions, and this residue is close to residue 55, which was previously suggested to be involved in interacting with Trp$^{433}$ of region 2.4 in free $\sigma^{70}$ (11, 27). Additionally, a mutant at position 366 ([S366C] $\sigma^{70}$) was prepared. This position is in a nonconserved region of $\sigma^{70}$ (Fig. 1), within a portion of $\sigma^{70}$ determined to be necessary for efficient core binding (28). We used the reactivity of a cysteine at this position as an indicator of conformation outside conserved regions 1, 2, and 4.

Each of the single cysteine mutants of $\sigma^{70}$ was expressed and purified to near-homogeneity. One striking observation made was that the mobility in SDS-PAGE of [R588C] $\sigma^{70}$ was different from all other mutant $\sigma^{70}$ and wt $\sigma^{70}$ (not shown). The calculated molecular mass of $\sigma^{70}$ is 70,263 Da, but it is known to migrate as a band of apparent molecular mass of around 90 kDa (29). Replacement of arginine with cysteine at position 588 produced a protein that migrated in SDS-PAGE as $\sim$70-kDa band (not shown). The changes in SDS-PAGE mobility of $\sigma^{70}$ in response to a single amino acid replacement has been observed previously (11, 27). Analysis of [R588C] $\sigma^{70}$ mutant on Superdex-200 sizing column indicated that this protein was smaller than wt $\sigma^{70}$, and Western blot analysis using a C-terminal antibody as a probe suggested that the C terminus of [R588C] $\sigma^{70}$ was missing (not shown). It is thus possible that [R588C] $\sigma^{70}$ was proteolytically more unstable resulting in purification of a truncated protein. Therefore, this protein was not used for further studies.

The activity of each single cysteine mutant was compared with the activity of wt $\sigma^{70}$ using reconstituted RNAP in an in vitro run-off transcription assay (19). All single cysteine mu-

### Table 1

Transcriptional activities of single cysteine mutants of $\sigma^{70}$

| Protein       | Activity (% of wt $\sigma^{70}$ activity) | Activity (% of [Cys] $\sigma^{70}$ activity) |
|---------------|------------------------------------------|---------------------------------------------|
| [1C]$\sigma^{70}$ | 163                                      | 109                                         |
| [A59C]$\sigma^{70}$ | 125                                      | 83                                          |
| [S366C]$\sigma^{70}$ | 96                                       | 64                                          |
| [S438C]$\sigma^{70}$ | 56                                       | 37                                          |
| [T440C]$\sigma^{70}$ | 89                                       | 59                                          |
| [S442C]$\sigma^{70}$ | 142                                      | 95                                          |
| [T583C]$\sigma^{70}$ | 94                                       | 63                                          |
| [R588C]$\sigma^{70}$ | 90                                       | 60                                          |
| [R596C]$\sigma^{70}$ | 151                                      | 100                                         |

![Fig. 1. Localization of residues replaced with cysteine in single cysteine mutants of $\sigma^{70}$.](Image)
Fig. 2. A, structure of Cy5 maleimide. B, STORM image of SDS-PAGE of maleimide modification reaction of Δcys\(\sigma^70\), free (lanes 1–3) and in the holoenzyme (lanes 4–6). C, STORM image SDS-PAGE of maleimide modification reaction of wt \(\sigma^70\)-containing holoenzyme (lanes 1–3) and core RNAP (lanes 4–6).

Reactivity (solvent exposure) of cysteine residues engineered to different positions of \(\sigma^70\) was probed by studying the kinetics of the reaction of single cysteine mutants of \(\sigma^70\) with Cy5 maleimide. Fig. 3A shows an example of SDS-PAGE analysis of a time course of Cy5 maleimide incorporation to [T\(\sigma^70\)440C]\(\sigma^70\). Fig. 3B shows results of quantitative analysis of the data from Fig. 3A. The time course of Cy5 incorporation could be described by a simple pseudo-first order kinetics (Fig. 3B).

The overall design of our experiments was to use the rate of incorporation of thiol-specific reagent Cy5 maleimide (Fig. 2A) to single cysteine mutants of \(\sigma^70\) as a measure of reactivity of unique cysteine residues of these mutants. We used Cy5 maleimide because it can be detected in STORM fluoroimager with femtomolar sensitivity, allowing the use of nanomolar protein concentrations in cysteine reactivity experiments. Because \(\sigma^70\) can be easily separated from the remaining RNAP subunits on SDS-PAGE, both reactivity of cysteines in free \(\sigma^70\) and in core-bound \(\sigma^70\) could be measured. The assumption in this experimental design is that the rate of incorporation of maleimide label into \(\sigma^70\) band in SDS-PAGE does indeed correspond to the reactivity of a single cysteine residue of a particular mutant (maleimide can also react with low efficiency with histidine and the \(\alpha\)-amino group of amino acids (30)). Thus, two control experiments were performed to validate this assumption. In the first experiment (Fig. 2B), we showed that under our experimental conditions, maleimide incorporation was entirely due to reaction with cysteines. This conclusion is based upon observation that no maleimide incorporation was observed in the case of Δcys\(\sigma^70\), free or core-bound (Fig. 2B), whereas efficient maleimide incorporation under the same conditions was observed in the case of wt protein (Fig. 2C, lanes 1–3). In the second experiment (Fig. 2C, lanes 4–6), we showed that the preparation of the core RNAP used to prepare holoenzymes with single cysteine mutants of \(\sigma^70\) was free of wt \(\sigma^70\) contamination or any other significant impurity that would incorporate maleimide, co-migrate with \(\sigma^70\) on a SDS-PAGE, and thus complicate data analysis.

Reactivity of Cysteine Residues in Single Cysteine Mutants of \(\sigma^70\)

Reactivity (solvent exposure) of cysteine residues engineered to different positions of \(\sigma^70\) was probed by studying the kinetics of the reaction of single cysteine mutants of \(\sigma^70\) with Cy5 maleimide. Fig. 3A shows an example of SDS-PAGE analysis of a time course of Cy5 maleimide incorporation to [T\(\sigma^70\)440C]\(\sigma^70\). Fig. 3B shows results of quantitative analysis of the data from Fig. 3A. The time course of Cy5 incorporation could be described by a simple pseudo-first order kinetics (Fig. 3B).
shows a summary of cysteine reactivity data for all single cysteine mutants of σ^70. In all single cysteine mutants of σ^70, cysteine residues were accessible to modification with Cy5 maleimide, albeit with rates differing by as much as 10-fold. The highest reactivity of cysteine was observed for cysteine at position 596, whereas the lowest reactivity was observed for cysteine at position 442. For a segment of σ^70 for which the crystal structure is available (26), it was possible to compare cysteine reactivity data with solvent accessibility (Fig. 4, inset) of these residues calculated from x-ray coordinates. To accomplish this, the models of single cysteine mutants of σ^70 were built using the WHAT IF program and a relative solvent accessibility (solvent accessibility of cysteine in σ^70 mutant compared with solvent accessibility of completely exposed cysteine residue) of cysteine residues was calculated using WHAT IF. Comparison of the body and the inset of Fig. 4 shows that although some correlation between solvent accessibility and cysteine reactivity at positions 366, 438, 440, and 442 exists, the correlation is not strict. The most striking discrepancy between cysteine reactivity and solvent accessibility data was observed for cysteine at position 438. The reactivity of this cysteine was much higher than expected from solvent accessibility data. One possible explanation of this discrepancy would be that replacing alanine (which has essentially no side chain) at position 438 with cysteine could produce a local perturbation of protein conformation. An alternative explanation could be that the available crystal structure is for a fragment of σ^70 corresponding to ~50% of the full-length protein. The differences between results obtained with full-length protein (cysteine reactivity data) and the fragment (crystal) may simply reflect the absence of some interactions in the fragment of σ^70 that are possible in the full-length protein.

Conformational Changes of σ^70 Induced by Core RNAP Binding

Cysteine Reactivity—Cysteine reactivity can be used as a sensitive indicator of conformational changes in proteins (31). The reactivity of a cysteine residue in a protein molecule is determined by its solvent accessibility and its pK_a value (32). Both of these properties can be changed when the local conformation of the protein in the vicinity of the cysteine changes. The effect of core RNAP on reactivity of cysteine residues in σ^70 mutants is presented in Fig. 5, A and B, and summarized as a

![Figure 4](http://www.jbc.org/)

**Fig. 4.** Pseudo-first order rate constants for Cy5 maleimide reaction with single cysteine mutants of σ^70. Inset, solvent accessibility of cysteine residues calculated from the x-ray structure of σ^70 fragment (26). Solvent accessibility was calculated using the WHAT IF program and is expressed as a percentage of solvent accessibility of Cys residue in Gly-Cys-Gly peptide (34).

![Figure 5](http://www.jbc.org/)

**Fig. 5.** The effect of core RNAP binding on the reactivity of cysteine residues in single cysteine mutants of σ^70. A, an example of core RNAP-induced decrease in cysteine reactivity. SDS-PAGE analysis of a reaction of free [S366C]σ^70 (lanes 1–3) and core RNAP-bound [S366C]σ^70 (lanes 4–6) with Cy5 maleimide. Lanes 1–3 are repeats of the same experiment, as are lanes 4–6. B, an example of core RNAP-induced increase in cysteine reactivity. SDS-PAGE analysis of a reaction of free [R596C]σ^70 (lanes 1–3) and core RNAP-bound [R596C]σ^70 (lanes 4–6) with Cy5 maleimide. Lanes 1–3 are repeats of the same experiment, as are lanes 4–6. C, summary of the effects of core RNAP on the reactivity of cysteine in single cysteine mutants of σ^70. The data are expressed as a ratio of Cy5 fluorescence incorporated to σ^70 band in holoenzyme to Cy5 fluorescence incorporated to σ^70 in free protein. The results presented are averages of 3–12 independent experiments.

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C. The examples of data shown in Fig. 5A and B, were chosen to illustrate the case when core binding induced a decrease in cysteine reactivity ([S366C]σ70, Fig. 5A) and the case when core binding induced an increase in cysteine reactivity ([R596C]σ70, Fig. 5B). Generally, three classes of cysteine residues can be identified from inspection of Fig. 5C. The first class consists of residues 438 and 442, the reactivity of which remained unchanged in the presence of the core RNAP. Both of these residues are located in region 2.4 of σ70. The second class consists of residues 1, 440, 583, and 596, the reactivity of which was increased in the presence of core RNAP. These residues are located in regions 1, 2.4, and 4.2, respectively. The third class consists of residues 59 and 366, the reactivity of which was decreased in the presence of core RNAP. These residues are in region 1 and in the nonconserved region of the protein, respectively. Decrease of the reactivity of a given cysteine residue in the presence of core RNAP could be due to a direct steric protection by the core enzyme or could be due to a core-induced conformational change in σ70. However, an increase in cysteine reactivity could be only due to a core-induced conformational change in σ70. Thus, results presented in Fig. 5C, provide a direct evidence that a large scale conformational change in σ70 is triggered by an interaction with the core RNAP.

Fluorescence Spectroscopy—Conformational changes in σ70 induced by binding to the core enzyme were also studied using fluorescence signal of IANBD covalently attached to several selected single cysteine mutants of σ70 ([A59C]σ70, [T440C]σ70, [S442C]σ70, [R596C]σ70, and [S366C]σ70). All spectra were recorded at 25 °C, in 50 mM MOPS (pH 7.5), 0.3 M NaCl, 1 mM EDTA, 5% glycerol buffer with the excitation at 495 nm. Each panel shows the spectrum of free IANBD-labeled σ70 (curve f) and IANBD-labeled σ70 bound to the core RNAP (curve h). The concentration of labeled σ70 was 250 nM and the spectra shown were corrected for instrument response. F, bar plot representation of a difference between a position of fluorescence emission maximum observed in the holoenzyme and in the free IANBD-labeled σ70.

Overall, fluorescence data support cysteine reactivity data and provide additional evidence that large scale conformational

Fig. 6. Fluorescence emission spectra of single cysteine mutants of σ70 labeled with IANBD: [A59C]σ70 (A), [T440C]σ70 (B), [S442C]σ70 (C), [R596C]σ70 (D), and [S366C]σ70 (E). All spectra were recorded at 25 °C, in 50 mM MOPS (pH 7.5), 0.3 M NaCl, 1 mM EDTA, 5% glycerol buffer with the excitation at 495 nm. Each panel shows the spectrum of free IANBD-labeled σ70 (curve f) and IANBD-labeled σ70 bound to the core RNAP (curve h). The concentration of labeled σ70 was 250 nM and the spectra shown were corrected for instrument response. F, bar plot representation of a difference between a position of fluorescence emission maximum observed in the holoenzyme and in the free IANBD-labeled σ70.
changes in $\sigma^{70}$ occur upon binding to the core enzyme and that these changes affect at least regions 1, 2.4, and 4.2.

**DISCUSSION**

Cysteine residues at several positions in regions 2.4 and 4.2, which contain promoter recognition elements, become more accessible to Cy5 maleimide when $\sigma^{70}$ binds to the core RNAP. The increased accessibility could be caused by an increased solvent exposure of these cysteines or by a change in the $pK_a$ values for cysteine. Irrespective of the exact nature of cysteine reactivity increase, these results clearly indicate that regions 2.4 and 4.2 undergo a major conformational change induced by $\sigma^{70}$-core interaction. Because the increase of cysteine reactivity is observed in several locations within regions 2.4 and 4.2, it is unlikely that a change in $pK_a$ of these cysteines would be responsible for the observed increased cysteine reactivity in the holoenzyme. Such interpretation would require that core-induced conformational changes in $\sigma^{70}$ produced very similar changes in $pK_a$ of cysteine in different locations of $\sigma^{70}$. Therefore, it is more likely that the increased reactivity of cysteines simply reflects an increase in solvent exposure of regions 2.4 and 4.2 induced by core RNAP. This conclusion is further supported by fluorescence experiments, which were consistent with increased solvent exposure of fluorescence probes at several locations in region 2.4 and 4.2 in the presence of core RNAP. Therefore, taken together, these data provide direct experimental evidence that binding of $\sigma^{70}$ to the core polymerase induces “unmasking” of DNA binding domains of $\sigma^{70}$. Such unmasking of DNA binding domains is likely to be important for the ability of $\sigma^{70}$ to bind promoter DNA and is consistent with the model for regulation of $\sigma^{70}$-DNA binding by the core RNAP activity proposed by Dombroski et al. (9, 10).

The N-terminal region of $\sigma^{70}$ (region 1) was previously suggested to be involved in masking the DNA binding domains in the free $\sigma^{70}$ (9, 10). This region was proposed to interact with region 2.4 and/or region 4.2 in the free protein and was proposed not to interact with any domains of the protein in $\sigma^{70}$ bound to the core RNAP. Thus, this model predicts only an increase in solvent accessibility in region 1 upon binding to the core RNAP. We observed an increase in solvent accessibility of cysteine at position 1, but a decrease of accessibility was observed at position 59. Thus, the changes within region 1 as a result of core RNAP binding must be more complex than described by the model. In particular, decrease of reactivity of cysteine at position 59 suggests that in holoenzyme, a new interdomain interaction involving region 1 (in the vicinity of residue 59) is formed or that upon interaction with core, region 1 undergoes a conformational transition itself.

It is interesting to compare solvent accessibility deduced from our cysteine reactivity data with the presumed function of some of these residues. Previous studies determined that mutations of threonine 440 could compensate for promoter downstream mutations (7). It was suggested that this residue directly contacts promoter at the –12 position. The expectation was thus that Thr$^{440}$ would be solvent-exposed in the holoenzyme in order to make direct DNA contact. We found this residue to be relatively exposed in free $\sigma^{70}$, and its exposure was increased upon interaction with the core RNAP, consistent with a direct DNA contact role proposed for this residue. Position 366 is located within the domain of $\sigma^{70}$ that is indicated by deletion mutagenesis experiments to be involved in $\sigma^{70}$-core interactions (28). The crystal structure of $\sigma^{70}$ fragment shows this residue to be on the periphery of the hydrophobic face proposed to interact with core RNAP (26). Cysteine at this position is reactive in free $\sigma^{70}$, and its accessibility is decreased in the holoenzyme. Fluorescence probe at this position upon core RNAP binding exhibited spectral changes (red shift in fluorescence emission maximum) characteristic for transfer of the probe to a more polar environment. The simplest interpretation of these observations is that residue 366 is near (but not at) the core binding site of $\sigma^{70}$. Formation of $\sigma^{70}$-core RNAP complex would reduce (but not eliminate) accessibility of cysteine to modification with Cy5 maleimide. The fluorescence changes suggest that some polar residues of bound core RNAP could be located in the vicinity of residue 366 of $\sigma^{70}$. Arginine at position 588 is located in the second helix of the putative helix-turn-helix of region 4.2 and was predicted to contact –31 and –33 positions of promoter DNA, respectively (7). We found that replacement of Arg$^{588}$ with cysteine resulted in a protein that in comparison to wt $\sigma^{70}$ had altered electrophoretic mobility and was likely to be more susceptible to degradation but was active in a transcription activity assay. This was a rather unexpected observation because a charged arginine residue should be located on the surface of the protein exposed to a solvent. Replacing such a residue should not affect protein structure significantly. Thus, it is likely that arginine at position 588 in free $\sigma^{70}$ is involved in some interactions important for maintaining the structure of the protein. One obvious candidate could be an interaction between regions 1 and 4.2.

In summary, we present here a direct evidence for core RNAP-induced large scale change in $\sigma^{70}$ resulting in unmasking of DNA binding domains (region 4.2 in particular). The next step now should be an elucidation of the molecular mechanism by which these conformational changes are accomplished.

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