Specificity of DNA Binding and Dimerization by CspE from Escherichia coli

Danielle Johnston1, Christine Tavano2,3, Sue Wickner3, and Nancy Trun1,2

From the 1Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282 and the
2Laboratory of Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland 20895

The CspE protein from Escherichia coli K12 is a single-stranded nucleic acid-binding protein that plays a role in chromosome condensation in vivo. We report here that CspE binds to single-stranded DNA containing 6 or more contiguous dT residues with high affinity (Kd < 30 nM). The interactions are predominantly through base-specific contacts. When an oligonucleotide contains fewer than 6 contiguous dT residues, the CspE interactions with single-stranded DNA are primarily electrostatic. The minimal length of single-stranded DNA to which CspE binds in a salt-resistant manner is eight nucleotides. We also show that CspE exists as a dimer in solution. We present a possible mechanism to explain the role of CspE in chromosome condensation in vivo by CspE binding to distant DNA regions in the chromosome and dimerizing, thereby condensing the intervening DNA.

The Csp proteins are an essential group of small proteins (67–73 amino acids) (1, 2), and the genes that encode them are present in the majority of sequenced bacterial genomes, some archaea, and one lower eukaryote (3, 4). In higher eukaryotes, a sequence homologous to CspEs exists as the DNA binding domain in a family of transcription factors called Y-Box-binding proteins (5). Most bacterial species contain multiple csp genes, generally between 2 and 12 (4). At least one csp gene is required for cell viability in all organisms where it has been tested (2).

Escherichia coli K12 contains genes for nine Csp proteins (CspA to CspI), which share between 46 and 91% similarity and between 29 and 83% identity in the primary amino acid sequence (4). When expressed at high levels, CspC and -E suppress the chromosome condensation defects of a deletion in the csm6 gene for the structural maintenance of chromosomes (SMC) protein, MukB (6). CspE also prevents DNA decondensation by camphor when expressed from a plasmid (7). These properties implicate CspC and -E in chromosome condensation. In addition, CspA, -C, and -E modestly regulate specific genes (8–14), and CspA and -E increase the readthrough efficiency at transcription terminators (15).

Several activities are associated with CspEs in vitro. For CspA that have been tested, including CspA, -B, -C, -D, and -E, they bind to RNA and single-stranded DNA (ssDNA)3 (16–19). CspA alters the secondary structure of RNA, making it more susceptible to degradation (16), whereas CspC increases the stability of RNA (10). CspD inhibits DNA replication from oriC (19), and CspE binds to DNA and mRNA in transcription complexes (20). In addition, CspE melts short hairpin stems (21). It is not known which of these activities comprises the essential function(s) of the Csp proteins.

The crystal structures of several CspEs indicate that they are OB-fold proteins composed of five anti-parallel β-strands that form a β-barrel (22–24). CspB from Bacillus subtilis was crystallized as a dimer (23), and CspD from E. coli was shown to form a dimer in solution (19). For B. subtilis CspB, dimers are formed between two anti-parallel CspB molecules through interactions between the β4-β6 and β4-N terminus (23, 24). CspA from E. coli was crystallized as a monomer (22, 25). It has been speculated that 3 additional amino acids at the N terminus of CspA are responsible for its inability to dimerize (22).

We have studied both DNA binding and dimerization of CspE. CspE binds with the highest affinity to ssDNA containing 6 contiguous dT residues and has a binding site that is about 8 bases in length. In addition, we found that CspE from E. coli exists as a dimer in solution. Based on these results, we present an explanation for the role of CspE in chromosome condensation in vivo.

EXPERIMENTAL PROCEDURES

Nucleic Acids and Purification of CspE—Synthetic oligonucleotides were purchased as high pressure liquid chromatography-purified molecules from Integrated DNA Technologies. CspE was purified from the E. coli strain BL21 (αDE3 pLysS pCH1). pCH1 contains cspE, from the AUG start codon to 11 bp downstream of the TAA stop codon, in the Ndel and BamHI sites of pET9a (Promega). Cells were grown at 37 °C in LB with 30 µg/ml kanamycin and 34 µg/ml chloramphenicol to an A600nm = 0.8. Cells were induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 2 h, centrifuged at 4000 × g for 20 min at...
4 °C and resuspended in Buffer A (20 mM Tris-HCl, pH 8.5, 1 mM EDTA, and 10% glycerol) containing 300 mM NaCl. Cells were lysed by passage twice through a French press cell at 15,000 p.s.i. followed by centrifugation at 20,000 × g for 20 min at 4 °C. The supernatant was incubated with 0.1% polyethyleneimine for 20 min on ice, centrifuged at 27,000 × g for 30 min at 4 °C, dialyzed against Buffer A, and applied to a Q-Sepharose (Amersham Biosciences) column. The pH of the flow-through containing CspE was lowered to 7.5 and subjected to a SP-Sepharose (Amersham Biosciences) column. The void volume, containing CspE, was dialyzed against Buffer B (20 mM MES, pH 5.5, 1 mM EDTA, and 10% glycerol). The dialysate was adjusted to 3 M NaCl and applied onto a phenyl-Sepharose (Amersham Biosciences) column. CspE was eluted from the column by a linear salt gradient from 3 to 0 M NaCl in Buffer B. Fractions containing CspE were pooled and concentrated using a Vivaspin concentrator (molecular weight cut-off 5000, Viva-science). The concentrated fractions were applied to a Sephacyr S-100 (Amersham Biosciences) column. CspE was eluted from the column with a constant specific activity across the peak (supplemental Fig. S1). Protein preparations were devoid of DNase activity as measured by the solubilization of 3H-labeled plasmid DNA. Edman degradation reactions (Midwest Analytical) on one preparation confirmed the identity of the protein as CspE (data not shown). Protein concentrations are expressed in molar concentrations of monomeric CspE.

Nucleic Acid Binding Measured by Gel Filtration—Separation of CspE, DNA, or CspE-DNA complexes was carried out at room temperature on a 1.5 × 18 cm Sephacyr S-100 (Amersham Biosciences) column in Buffer D (25 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, 100 mM KCl, and 5% glycerol). The column was calibrated using bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease A (Amersham Biosciences). Blue dextran (Amersham Biosciences) was used to determine the exclusion volume. Reactions containing 24 μM CspE and 24 μM ssDNA were incubated at room temperature for 15 min in Buffer D prior to application to the column. The column was run at 0.1 ml/min, and fractions of 110 μl were collected and assayed for DNA and protein using SYBR green (Molecular Probes) and Bradford dye (Bio-Rad), respectively. CspE and ssDNA were run separately as controls.

Nucleic Acid Binding Measured by Fluorescence Quenching—Fluorescence intensities of tryptophan were measured on a FluoroMax-3 spectrofluorometer with a constant temperature of 25 °C and with excitation and emission wavelengths of 292 and 349 nm, respectively. CspE (0.5 μM) and DNA template (0.01–1 μM) were mixed in 25 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, and 100 mM KCl, incubated at room temperature for 5 min and, subsequently, fluorescence intensities were measured. These conditions were used for all quenching experiments under low salt conditions; high salt conditions were carried out in 25 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, and 1 mM KCl. To determine whether quenching was due to specific interactions, 0.5 μM tryptophan (Sigma) was substituted for CspE. Fluorescence titrations were performed as individual reactions. The equilibrium dissociation constant, KD, was determined by fitting the binding curves to Equation 1 (26, 27) using the Origin 7.5 software.

\[
Q = Q_{\text{max}} \frac{A - \sqrt{A^2 - 4n_p[CspE]_{\text{total}}[ssDNA]_{\text{total}}}}{2n_p[CspE]_{\text{total}}} \tag{1}
\]

where A = [CspE]_{total} + n_p[ssDNA] + KD, n_p is the number of CspE monomers bound to one ssDNA molecule, Q is the percentage quenching of CspE Trp10 fluorescence after the addition of ssDNA, and Q_{max} is the maximum quenching obtained after complete saturation of the protein with ssDNA. The errors associated with the K_D are generated by the curve fitting. The binding model does not take into account the degeneracy of multiple binding sites within one DNA molecule, and thus, the results represent apparent equilibrium constants.

Chemical Cross-linking of CspE—Purified CspE was dialyzed extensively against 20 mM HEPES, pH 7.5. CspE was diluted to 5 μM and incubated with 100 μM 3,3′-dithiobis-(sulfosuccinimidyl-propionate) (DTSSP, Pierce) or 0.05% glutaraldehyde (Electron Microscopy Sciences) at room temperature for 30 min. Reactions were stopped by the addition of Tris-HCl, pH 8.0, to a final concentration of 100 mM followed by incubation for 10 min. The reaction mixtures were separated on a 10% Bis-Tris acrylamide gel (Invitrogen) in MES buffer. The gels were fixed in 10% trichloroacetic acid followed by incubation in 0.1% glutaraldehyde and silver staining (Bio-Rad).

RESULTS

CspE-ssDNA Binding as Monitored by Gel Filtration—We wanted to reinvestigate ssDNA binding by CspE since many previous reports were carried out at high protein concentrations (200 μM (17)), high ratios of CspE to nucleic acid (between 20 and 600 CspE monomers to one nucleic acid molecule (17, 20, 28)), or both (17). Additionally, the smallest ssDNA template tested in binding assays was 45 nucleotides (17, 20, 28). To lessen the possibility that the apparent binding is due to a contaminating sequence-specific DNA-binding protein, we used low μM concentrations of CspE and equal concentrations of CspE monomers and DNA in these experiments.

We first investigated the binding of CspE to ssDNA using a 30-base oligonucleotide, Ybox30 (GATAATGCATTT-GAATAAAATGGGTAA). This sequence is from the λ Pg promoter and has been shown to contain a binding site for CspE (20). CspE-Ybox30 interactions were demonstrated using gel filtration column chromatography (Fig. 1A, A–C). We found that CspE alone eluted with a peak in fraction 30 (Fig. 1A) and Ybox30 DNA alone eluted with a peak in fraction 23 (Fig. 1B). When CspE and Ybox30 DNA were mixed at 24 μM each, protein and DNA co-eluted with a peak in fraction 22 (Fig. 1C). All of the detectable CspE eluted as a single higher molecular weight species, well separated from the position of CspE alone, demonstrating that CspE binds to ssDNA.

CspE-ssDNA Binding as Monitored by Tryptophan Fluorescence Quenching—Fluorescence quenching of the single tryptophan residue, Trp10, in CspE was used as an independent
assay to measure DNA binding. Lopez et al. (29) established that the quenching of B. subtilis CspB tryptophan fluorescence correlated with CspB-ssDNA binding interactions. We observed that the intrinsic fluorescence of CspE was quenched by the addition of YBox30, indicating that CspE interacts with the ssDNA. Quenching was saturated at ~65% by 0.5 μM Ybox30 DNA (Fig. 1D). There was no detectable quenching of l-tryptophan (Fig. 1D) or 0.5 μM N-acetyl-l-tryptophanamide (data not shown) by YBox30 DNA, indicating that the quenching of the fluorescence intensity of CspE is due to direct interactions between CspE and ssDNA. Using this assay, no requirement for ATP, Mg2+, or other mono- or divalent cations was detected (data not shown).

Sequence and Length Preferences of CspE for Binding to ssDNA—We explored the sequence specificity of CspE by testing the ability of CspE to bind to 15 base homopolymers of (dA)15, (dT)15, (dC)15, and the dG-rich heteropolymer, dG5dCdG5dCdG3, using fluorescence quenching (Fig. 2A). We found that with 0.5 μM CspE and 0.5 μM oligonucleotide, there was about 10-fold higher fluorescence quenching of CspE by (dT)15 as compared with (dA)15. There was insignificant CspE binding to (dC)15 or dG5dCdG5dCdG3.

To determine the minimum size of ssDNA to which CspE binds, we tested oligonucleotides of (dT)5, (dT)7, (dT)9, (dT)11, (dT)13, and (dT)15 (Fig. 2B). Because binding is influenced by the number of protein molecules bound as well as the affinity of the protein for the DNA, we initially compared binding as the relative binding competency (defined as the concentration of ssDNA that gives 50% quenching; [ssDNA]0.5 (30)). The titration curves showed that with respect to binding competencies, the different oligonucleotides segregate into three groups. CspE binds with similar competencies of 0.11, 0.14, and 0.15 μM to (dT)11, (dT)13, and (dT)15, respectively. CspE binds to (dT)7 and (dT)9 with binding competencies of 0.33 and 0.20 μM, respectively. Under these conditions, CspE did not appreciably bind to (dT)5 (binding competency of ~2 μM; Fig. 2B). These results suggest that the minimum binding site is greater than 5 bases. It has been previously reported that CspA from E. coli has a binding site requirement for 6–7 dT residues within a 23-nucleotide dC oligonucleotide (18, 29), and CspB from B. subtilis has a minimal binding site size of 6 dT residues (29, 31). Thus, our observations that CspE binds with the lowest competencies to (dT)11, (dT)13, and (dT)15 could reflect that these oligonucleotides contain more than one CspE binding site. By this logic, (dT)7 and (dT)9 should contain a single CspE binding site and, therefore, the apparent K_D could be determined by fitting the fluorescence data to Equation 1 under “Experimental Procedures.” The K_D for binding to (dT)7 was 52.7 ± 21.2 nM and for (dT)9, the K_D was <28 nM.

To examine the quantity and spacing of dT residues required in the binding site, we tested 8-base oligonucleotides containing 2, 4, or 6 dT residues flanked by dC residues. CspE bound to

![FIGURE 1. Interaction of CspE with ssDNA as measured by gel filtration (A–C) and tryptophan quenching (D). Gel filtration elution profiles of CspE (A, filled squares), Ybox30 (B, open circles), or a mixture of CspE and Ybox30 were performed (C) as described under “Experimental Procedures.” Experiments were carried out three or more times, and representative results are shown. D, tryptophan quenching assay to measure DNA binding by CspE (filled squares) or l-tryptophan (open triangles) as described under “Experimental Procedures.” Experiments were carried out three times, and the results shown are the averages of three experiments ± S.E. The lines through the points have no meaning but to guide the eye. BSA, bovine serum albumin.](image-url)
an oligonucleotide with 6 contiguous dT residues with an apparent $K_D$ of 123 nM, similar to that determined for (dT)$_9$ (Fig. 2C). CspE bound to an oligonucleotide with 4 dT residues with a much greater $K_D$ of 1349 ± 319 nM and had no significant binding to an oligonucleotide with 2 dT residues (Fig. 2C). We next tested the ability of CspE to bind to sequences with variable spacing between the dT residues. CspE bound poorly to an oligonucleotide with 6 contiguous dT residues with an apparent $K_D < 23$ nM, a $K_D$ similar to that determined for (dT)$_9$ (Fig. 2C). CspE bound to an oligonucleotide with 4 dT residues with a much greater $K_D$ of 1349 ± 319 nM and had no significant binding to an oligonucleotide with 2 dT residues (Fig. 2C). We next tested the ability of CspE to bind to sequences with variable spacing between the dT residues. CspE bound poorly to

**FIGURE 2. Quenching effect of ssDNA on wild-type CspE fluorescence.** A, nucleic acid binding by CspE (0.5 μM) to four different ssDNA templates (0.5 μM) was assayed using tryptophan quenching as described under “Experimental Procedures.” B–D, nucleic acid binding by CspE (0.5 μM) to different ssDNA templates of varying lengths (A) or with 8-nucleotide ssDNA templates with different dT compositions (B). All experiments were measured using tryptophan quenching assays as described under “Experimental Procedures” in low salt (gray bars) or high salt (filled bars) buffer conditions. The results are shown as the averages of three experiments ± S.E.

**FIGURE 3. Comparison of wild-type CspE to ssDNA templates under different salt conditions.** CspE (0.5 μM) was mixed with oligo(dT) ssDNA templates of varying lengths (A) or with 8-nucleotide ssDNA templates with different dT compositions (B). All experiments were measured using tryptophan quenching assays as described under “Experimental Procedures” in low salt (gray bars) or high salt (filled bars) buffer conditions. The results are shown as the averages of three experiments ± S.E.
CspE DNA Binding and Dimerization

**TABLE 1**

| Binding of CspE to ssDNA | Length of ssDNA (No. of nucleotides) | Quenching of CspE<sup>a</sup> % |
|-------------------------|--------------------------------------|-------------------------------|
| dT oligonucleotides     |                                      |                               |
| TTTTTT                  | 5                                    | 43 ± 1.3                       |
| TTATTTT                 | 7                                    | 71.8 ± 3.2                     |
| TTATTTT                 | 9                                    | 85.8 ± 1.6                     |
| TTATTTTT                | 11                                   | 89.4 ± 7.8                     |
| TTATTTTTT               | 13                                   | 89.5 ± 3.0                     |
| TTATTTTTTT              | 15                                   | 92.3 ± 2.9                     |
| dT/dC oligonucleotides  |                                      |                               |
| CCTTTCCC                | 8                                    | 12.9 ± 2.6                     |
| CTTTTTCC                | 8                                    | 27.8 ± 10.5                    |
| CTTTTTTC                | 8                                    | 84.8 ± 1.4                     |

**Predicted CspE binding sequences<sup>b</sup> (17)**

- AATTT
- GCATTTTTTT
- ACAAAATTC
- GCATTTTTTCTG
- CCTTTTTTCCC
- TTTTTTTTTTTTTTTTTTT

**Y-Box or related sequences<sup>c</sup> (20)**

- TATTTTTTTTTTTTTT
- AATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

<sup>a</sup> The average tryptophan quenching ± the standard error of three trials for CspE (0.5 μM) and ssDNA (0.5 μM) as described in "Experimental Procedures."

<sup>b</sup> The underlined sequences are the AT rich regions reported to be binding sites for CspE (17).

<sup>c</sup> The bold, underlined sequences are the Y-Box consensus sequences and the underlined sequences indicated the mutated residues.

We tested whether CspE has a higher affinity for contiguous dT residues over the previously reported AT-rich sequences AATT, AATTT, AATTTT, AAATTT, AAAATT identified using SELEX (17). We examined the binding of CspE to 16 different oligonucleotides containing the individual AT-rich sequences listed above or combinations thereof (Table 1). The binding to the AT-rich sequences was compared with the binding of oligo(dT) of various lengths and to the 8-base oligonucleotides containing 2, 4, or 6 dT residues (Table 1). With the 16 oligonucleotides tested, we observed between 3 and 55% quenching of CspE fluorescence using 0.5 μM CspE and 0.5 μM DNA. CspE bound all of the oligonucleotides with much lower affinity than the dT-rich sequences, where we observed about 80% quenching with (dT)<sub>9</sub>, or CTTTTTTC and ~90% quenching of CspE fluorescence with (dT)<sub>11</sub>, (dT)<sub>13</sub>, and (dT)<sub>15</sub> using the same conditions. Moreover, the affinity of CspE for a given oligonucleotide did not correlate with the number of proposed CspE binding sites present in the oligonucleotide. For example, there was 15% quenching of CspE fluorescence with two oligonucleotides containing three proposed CspE binding sites, whereas there was 55% quenching of CspE fluorescence by an oligonucleotide containing a single proposed site. In summary, CspE has a higher affinity for (dT)<sub>9</sub> than for any of the various oligonucleotides containing the proposed CspE binding sequences identified by SELEX.
We quantified the binding of CspE to the proposed consensus CspE binding sequence by measuring the apparent $K_d$. We chose the 10-base oligonucleotide, GCAATTTC and, because it should contain one CspE binding site. It exhibited 40% quenching of the CspE fluorescence at 0.5 μM of CspE and oligonucleotide, significantly more than the 3.7% quenching seen with the other 10-base single CspE consensus binding site tested, ACAATAAGC (Table 1). Our results indicate that CspE binds with an apparent $K_d$ of 887.0 ± 267.1 nM to GCAATTTC (Fig. 2C), comparable with the binding to CCTTTTC, which also contains 4 contiguous dT residues. CspE bound with a much greater affinity to either CTTTTTTC or (dT)$_9$ with apparent $K_d$ values in the 30–50 nM range. Thus, 6 consecutive dT residues constitute a much better binding site than the SELEX selected sequences.

Finally, we tested binding of CspE to four 30-base oligonucleotides, two containing a Y-Box and two containing an identical sequence except that the Y-Box had been mutated, since we and others have seen binding by CspE to Y-Box-containing ssDNA (Fig. 1) (20). In eukaryotes, the DNA binding domain of Y-Box transcription factors is highly homologous to the Csp proteins in its primary amino acid sequence and secondary structure and binds with specificity to CCAAT (the Y-Box (5)). At 0.5 μM each CspE and ssDNA, we found that CspE fluorescence was quenched about 50% by all four oligonucleotides (Table 1). Thus, CspE does not show preferential binding to Y-Box containing sequences.

**DISCUSSION**

We have investigated how CspE interacts with DNA and with itself. We and others have shown that CspE binds to ssDNA (17, 21, 28) (Fig. 1). Importantly, we have shown that CspE binds with the highest affinity to oligonucleotides containing 6 or more consecutive dT residues. This high affinity binding site is different from the CspE consensus sequences reported by Phadtare et al. (17), yet strikingly similar to the binding site for...
B. subtilis CspB (29). The binding site is greater than 5 bases in length and is more likely to be 8 or 9 bases, as judged by the salt-resistant binding to CTTTTTTC and (dT)₉. We have calculated that the $K_D$ for CTTTTTTC or (dT)₉ is $\sim 30$ nM, and the $K_D$ for a 10-base oligonucleotide containing a previously reported CspE binding site is $\sim 900$ nM, assuming that only one CspE binds per oligonucleotide. Thus, CspE has $\sim 30$-fold greater affinity for the sequences identified here than for the SELEX selected sequences.

It is known that protein-nucleic acid interactions can occur through contacts between the amino acid side chains and the bases, the sugar moiety, or the phosphodiester backbone of the DNA. For the bases or the sugar moiety, the associations would include mainly non-covalent interactions that would be not be disrupted by high ionic strength. For the backbone, the associations would have a significant ionic component that could be destabilized by weakening the charge-charge interactions through increasing the ionic strength (33). To investigate the mode of binding for CspE-ssDNA interactions, we tested binding under different salt conditions. We found that binding of CspE to ssDNA containing 6 or more contiguous dT residues was largely resistant to 1 M KCl, indicating that a significant component of the binding is from CspE interacting directly with the dT bases (Fig. 3).

Because Csp proteins are highly homologous, we constructed molecular models of CspE as a monomer and a dimer, both with and without ssDNA (Fig. 5). A model of the monomeric form was generated using the known crystal and NMR structures of E. coli CspA, the crystal structure of Bacillus caldolyticus wild type Csp, and four mutant B. caldolyticus Csp proteins as chosen by SwissModel (34) (Fig. 5A). We have also fashioned a model of CspE bound to (dT)₆ based on the recent co-crystal structure of monomeric CspB from B. subtilis with (dT)₆ (Fig. 5B). The model indicates that the ssDNA could fit into a cleft on one side of CspE, much like it does in CspB from B. subtilis. A dimeric model of CspE, more likely representing the structure we have determined for CspE in solution (Fig. 4), was generated based on the crystal structure of dimeric B. subtilis CspB (Fig. 5C). Additionally, we have modeled (dT)₆ bound by each protomer of the dimer (Fig. 5D). Because the CspE dimers are predicted to be antiparallel, the DNA binding sites are likely on opposite sides of the dimer.

How does binding to ssDNA translate into chromosome condensation? Previous studies have indicated that the folded chromosome contains ssDNA. The most direct demonstration of this point is from the in vivo induction of the T7 gene 3 protein. When this ssDNA-specific endonuclease is induced at low levels in E. coli, the cells die, and the chromosome is completely degraded (35). Further indication of ssDNA regions in the condensed chromosome comes from studies on replication bubbles (36), transcription bubbles (37), DNA repair (38), and recombination (for review, see Ref. 39). Previous studies have shown that CspE binds to active transcription complexes (20), in the vicinity of known ssDNA. Thus, there are many opportunities for CspE to bind to single-stranded regions of chromosomal DNA.

Binding to ssDNA alone is not sufficient for chromosome condensation. However, ssDNA binding in combination with dimerization would provide a simple explanation for how CspE helps condense the chromosome. If CspE monomers were bound to separated regions of the chromosome, CspE dimerization would bring distant regions of DNA closer together (Fig.

**FIGURE 5. Model of CspE dimerization and ssDNA binding.** A, a model of the predicted CspE structure was generated using Swiss-Model (34) with reference to three crystal structures (Protein Data Bank Codes 1MJC (CspA), 1C90 (Bc-Csp), 1I5F (mutants of Bc-Csp)), and 16 solution NMR structures (Protein Data Bank Code 3MEF (CspA)) of sequence homologues. B, a model of CspE and (dT)₆ DNA from the co-crystal of B. subtilis CspB-DNA (Protein Data Bank Code 2ES2) (31). C, two monomers of CspE were manually modeled into a dimer based on the crystallized CspB dimer (23). D, two models of CspE monomers with DNA were manually configured into a dimer. All models were generated using Deep View Swiss-PDB Viewer and rendered using PyMol.
5D). Binding to DNA either prior to or after dimerization would suffice for condensation. A survey of the *E. coli* chromosome indicates that there are 4662 (dT)₃, or potential CspE binding sites in the genome. Calculations based on the size of the *E. coli* chromosome, which contains independently supercoiled loops, and the persistent length of DNA indicate that proteins that condense the chromosome are only responsible for a 2–3-fold reduction in the volume of the nucleoid (4). CspE, in combination with several other previously identified proteins in *E. coli*, would contribute to this 2–3-fold reduction. We would argue that in *E. coli*, which replicates its DNA every 40 min, can induce genes up to 1000-fold, and is very proficient at recombination, this 2–3-fold condensation could be accomplished by a transient mechanism. In fact, it has been shown in eukaryotic cells that condensing proteins bind differentially to the chromosomes (40, 41).

There are some reasons to suspect that chromosome condensation by Csp proteins may be linked to their ability to dimerize. Using the N-terminal sequence differences between CspA from *E. coli* and CspB from *B. subtilis* as the criteria for dimerization, *E. coli* CspB, -G, -I, -F, and -H should not be able to form dimers, whereas CspC, -D, and -E should (Fig. 4A). We have shown that CspE exists as a dimer (Fig. 4, B and C). As expected, CspD, which has perfect alignment of the N terminus with CspB, has also been shown to form dimers (19). The genetic screen that implicated CspE in chromosome condensation also implicated CspC but no other CspS (32). CspD may not have been identified in this screen because overexpression of CspD is lethal (Ref. 19 and data not shown). However, electron micrographs of CspD bound to ssDNA show that CspD appears expected, CspD, which has perfect alignment of the N terminus with CspB, and CspC, -D, and -E should (Fig. 4B). We have shown that CspE DNA Binding and Dimerization

In summary, we have demonstrated two novel properties of CspE, preferred DNA binding to contiguous dT residues in ssDNA and homodimerization. Further experimentation will be aimed at testing whether these biochemical properties are responsible for the role of CspE in chromosome condensation.

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