DnaK/Hsp70 proteins are universally conserved ATP-dependent molecular chaperones that help proteins adopt and maintain their native conformations. DnaJ/Hsp40 and GrpE are co-chaperones that assist DnaK. CbpA is an Escherichia coli DnaJ homolog. It acts as a multicopy suppressor for dnaJ mutations and functions in vitro in combination with DnaK and GrpE in protein remodeling reactions. CbpA binds nonspecifically to DNA with preference for curved DNA and is a nucleoid-associated protein. The DNA binding and co-chaperone activities of CbpA are modulated by CbpM, a small protein that binds specifically to CbpA. To identify the regions of CbpA involved in the interaction of CbpA with CbpM and those involved in DNA binding, we constructed and characterized deletion and substitution mutants of CbpA. We discovered that CbpA interacted with CbpM through its N-terminal J-domain. We found that the region C-terminal to the J-domain was required for DNA binding. Moreover, we found that the CbpM interaction, DNA binding, and co-chaperone activities were separable; some mutants were proficient in some functions and defective in others.

DnaK/Hsp70 chaperones comprise a major family of ATP-dependent molecular chaperones that facilitate protein folding and reactivation. These proteins participate in many cellular functions including DNA replication, protein degradation, protein translocation across membranes, and signal transduction (1, 2). The co-chaperones DnaJ/Hsp40 and GrpE assist DnaK in protein folding to carry out these essential functions. DnaJ co-chaperones stimulate ATP hydrolysis by DnaK, thereby facilitating the binding of polypeptide substrates in the substrate-binding pocket of DnaK (1, 3, 4). GrpE facilitates nucleotide exchange by DnaK (1, 3, 4).

All members of the DnaJ family contain a characteristic J-domain of ~75 amino acids that interacts with DnaK as evidenced by its ability to stimulate the ATPase activity of Hsp70/DnaK (5). The sequences of J-domains are highly conserved and the structures of the Escherichia coli DnaJ J-domain as well as several other J-domains have been solved (6). All J-domains contain a conserved tripeptide (His, Pro, Asp) that is essential for co-chaperone activity and is located between two long α-helices. DnaJ proteins have been subdivided into classes based on their domain composition. Type I DnaJ proteins, including E. coli DnaJ, human Hdj2, and yeast Ydj1, contain four domains: An N-terminal J-domain, a glycine- and phenylalanine-rich region, a cysteine-rich zinc finger-like region, and a C-terminal region composed of two domains with similar folds (referred to as C-terminal domain I and II or CTD I and CTD II). Crystallographic studies have shown that peptides are bound by CTD I (7). Type II DnaJ proteins, including E. coli CbpA, human Hdj1, and yeast Sis1, lack the zinc-binding domain, and unlike type I DnaJ proteins, they are not able to prevent aggregation of non-native polypeptides in the absence of DnaK (8–10). Type III DnaJ proteins share with DnaJ only the J-domain and do not bind non-native proteins; this class is diverse and contains proteins with additional domains.

CbpA is one of six DnaJ homologs in E. coli. It was originally identified based on its ability to bind curved DNA (named CbpA for “curved binding protein A” (11)) and is one of the major nucleoid proteins in late stationary phase (12). Its expression is regulated by σ70 and increases during entry into stationary phase as well as during phosphate starvation and growth at low pH (13, 14). A cbpA deletion mutant exhibits no growth defects, but a dnaJ, cbpA double deletion strain resembles dnaK deletion strains in being unable to grow below 16 °C or above 37 °C, and the double deletion strain is defective in cell division at permissive temperatures (15). When CbpA is expressed from a multicopy plasmid, it suppresses the phenotypes of dnaJ deletion and dnaJ, cbpA double deletion strains by restoring growth at high temperature and allowing phase λ and plasmid mini-F growth (11, 15). These observations demonstrate that CbpA is a functional homolog of DnaJ in vivo.

Downstream from the cbpA gene and in the same operon, there is a small open reading frame, cbpM, which encodes a “CbpA modulator” (9). CbpM shares some weak homology with DafA of Thermus thermophilus, a protein found to promote the assembly of a ring-like structure containing a trimer each of DnaK, DnaJ, and DafA (16). The role of DafA in protein folding by DnaK has been explored, and the function of DafA may be to hold DnaK and DnaJ in an inactive state during non-stress conditions (17–19).

CbpA functions as a DnaJ-like co-chaperone in vitro, acting in an ATP-dependent reaction with DnaK and GrpE to remodel inactive P1 RepA dimers into monomers active in DNA binding (9, 20). CbpA, like DnaJ, forms a complex with RepA dimers, suggesting that CbpA binds native substrates and targets them...
**CbpA, a DnaJ Homolog**

for recognition and binding by DnaK (9, 21, 22). CbpM interacts with CbpA and inhibits both the co-chaperone activity and the DNA binding activity of CbpA. CbpM is specific for CbpA, having no detectable effect on the co-chaperone activity of DnaJ or on DNA binding by other DNA-binding proteins (9).

In the present study, we constructed CbpA substitution mutants and deletion mutants to identify the regions of CbpA involved in DNA binding and in CbpM interaction. Our results demonstrate that the region of CbpA essential for DNA binding is C-terminal to the J-domain and that the J-domain of CbpA interacts with CbpM.

**EXPERIMENTAL PROCEDURES**

Plasmids—CbpA domain deletions were constructed by generating cbpA PCR fragments coding for CbpA(1–78), CbpA(1–201), CbpA(79–306), and CbpA(202–306) that contained 5’ and 3’ NdeI and HindIII sites, respectively, and lacked a terminal stop codon. The PCR fragments were digested with NdeI and HindIII and ligated into similarly digested pET24B (Novagen). CbpA site-directed mutants used in these studies were constructed in the *cbpA* plasmid (pCU60) (15) using the QuickChange (Stratagene) site-directed mutagenesis kit as recommended by the manufacturer.

Proteins and DNA—CbpA (11), RepA (23), DnaK (24), GrpE (24), and CbpM (9) were isolated as described previously. CbpA site-directed mutants described above were isolated using the procedures described for isolation of the wild type protein (11). CbpA domain deletions were isolated from BL21(DE3) cells harboring plasmids pETCbpA(1–78), pETCbpA(1–201), pETCbpA(79–306), or pETCbpA(202–306) described above. The cells were grown at 37 °C to an A600 of 0.4 and then induced with 1.0 mM isopropyl-ß-D-thiogalactopyranoside. The CbpA deletion mutants were isolated using Talon metal ion affinity chromatography resin (Clontech) as described by the manufacturer. All proteins were more than 95% pure as determined by SDS-PAGE. CD spectra of the site-directed substitution mutants were essentially identical to that of wild type CbpA (data not shown). CD spectra of the domain deletions showed the presence of secondary structure indicative of protein structure (data not shown). Protein concentrations were expressed as molar amounts of RepA dimers, DnaK monomers, GrpE dimers, DnaJ dimers, CbpA monomers, and CbpM monomers.

RepA was 3H-labeled in vitro using succinimidyl N-[2,3-3H]propanetate (25). pKOSR plasmid DNA containing the origin of replication of mini-P1 was 3H-labeled in vitro as described (20).

**DNA Binding Assay**—Reactions (10 µl) containing Buffer B (20 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 0.1 mM EDTA, and 10% glycerol (v/v)), 100 mM KCl, 0.005% Triton X-100 (v/v), 50 µg/ml bovine serum albumin, 9 fmol [3H]oriP1 plasmid DNA (2700 cpm/fmol), and 270 nm CbpA were incubated for 5 min at 20 °C, filtered through nitrocellulose filters, and the retained radioactivity was measured.

**Chaperone Assay**—To measure activation of RepA, reaction mixtures (20 µl) contained Buffer B, 1 mM ATP, 100 mM KCl, 10 mM MgOAc, 50 µg/ml bovine serum albumin, 0.005% Triton X-100 (v/v), 4 nM RepA, CbpA or CbpA mutant protein as indicated, 228 nm GrpE, and 430 nm DnaK. The reaction mixtures were incubated for 10 min at 24 °C, then 13 fmol [3H]oriP1 DNA and 1 µg of calf thymus DNA were added. The mixtures were incubated for 5 min at 0 °C, filtered through nitrocellulose filters, and the retained radioactivity was measured.

**Chemical Cross-linking**—CbpA and CbpA deletion mutants were dialyzed against 40 mM Hepes, pH 7.5, 100 mM NaCl, and 10% glycerol (v/v). Reaction mixtures (20 µl) containing 40 mM Hepes, pH 7.5, 100 mM NaCl, and 8.7 µM CbpA or CbpA mutant protein were incubated with or without 5 mM bis(sulfosuccinimidyl) suberate (Pierce) as indicated in Fig. 7 for 15 min at 24 °C. Reactions were stopped with 2 µl of 1 M Tris-HCl, pH 7.5, and the samples were subjected to SDS-PAGE. Protein bands were detected by staining with Coomassie Blue.

**CbpA-RepA Binding Assay**—Reactions (100 µl) contained 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM dithiothreitol, 0.1 mM EDTA, 10% glycerol (v/v), 0.005% Triton X-100 (v/v), 100 µg/ml bovine serum albumin, 125 nm [3H]RepA (960 cpm/pmol), and CbpA or CbpA mutant protein as indicated. The reactions were incubated for 10 min at 23 °C and then centrifuged in 100,000 MWC0 filters (Millipore, Microcon YM-100) at 2,700 × g for 12 min. Retentates containing CbpA-RepA complexes were recovered in 100 µl of 10% SDS, and the radioactivity measured.

**RESULTS**

**Comparison of CbpA to Other DnaJ Homologs and the Construction of Mutants**—We wanted to identify the regions of CbpA that make CbpA a unique DnaJ homolog, namely the regions essential for CbpM interaction and those necessary for DNA binding activity. To gain information about the potential domain structure of CbpA, we aligned CbpA with DnaJ homologs from several organisms (Fig. 1A). The J-domain residues of CbpA (amino acids 1–78) are 55% identical and 78% similar to *E. coli* DnaJ J-domain and are also highly homologous to many other J-domains, including yeast Sis1 and Ydj1, *Drosophila* DroJ1 and human Hdj1, which are shown in Fig. 1A. A molecular model of the CbpA J-domain from residues 1–75 was generated with reference to the NMR structures of the J-domains of *E. coli* DnaJ and human Hdj1 (Fig. 1B). The C-terminal portion of CbpA from residue 118 to 306 was aligned with several type II DnaJ proteins: yeast Sis1, *Drosophila* DroJ1, and human Hdj1 (Fig. 1A). The crystal structure of Sis1 has been solved (26), and a molecular model could be generated, although the homology between the C-terminal domains of CbpA and Sis1 is not as high as between the J-domains (24% identity and 38% similarity) (Fig. 1B). Thus, this is only a model used to guide the construction of mutants and may not reflect accurately the structure of CbpA. The spatial relationship between the J-domain and the CTD I and CTD II domains is unknown because the structure of a full-length DnaJ family member remains to be solved.

The 42 amino acid linker region of CbpA from amino acids 76 to 117 that connects the J-domain to the predicted CTD I did not align with any proteins of known structure and could not be modeled. This region is significantly smaller than the linker regions of Sis1, DroJ1, and Hdj1, which are 104, 81, and 86 amino acids, respectively (Fig. 1A). In other DnaJ proteins, the
FIGURE 1. Alignment of CbpA and DnaJ homologs. A, the J-domain of CbpA (from residue 1 to 78) was aligned with J-domains of *E. coli* DnaJ, *Saccharomyces cerevisiae* Sis1, *S. cerevisiae* Ydj1, *Drosophila* DroJ1, and human Hdj1. The C-terminal domain of CbpA (from residue 118 to 306) was aligned with those of Sis1, DroJ1, and Hdj1. The nonhomologous linker regions of CbpA, Sis1, DroJ1, and Hdj1 are also shown. Identical residues are yellow, and conserved residues are green. B, molecular models of CbpA. Models of the separate J-domain and the C-terminal portion of CbpA were generated using Swiss model (29). The structures of several J-domains (Protein Data Bank codes: N. Kobayashi, N. Tochio, S. Koshiba, M. Inoue, T. Kigawa, and S. Yokoyama, unpublished data, 2ctr; 1xbl (30); 1bq0 (31); and 1hdj (32)) were used as the template for the J-domain and the structure of Sis1 CTD I and II (26), Protein Data Bank code 1c3g, for the template for the C-terminal domain. The J-domain is shown in red, the CTD I is shown in blue, the CTD II is shown in gold, and the region of CbpA that lacks homology with any proteins of known structure is indicated by a dotted line connecting the N- and C-terminal domains.
linker region is typically rich in glycine and phenylalanine. In CbpA the linker contains only six phenylalanine residues as compared with 11 in Sis1 and DroJ1 and 12 in Hdj1. The CbpA linker contains only four glycine residues in contrast to 38 in Sis1 and 15 in DroJ1 and Hdj1. Thus, in some aspects CbpA is similar to other DnaJ homologs but in others it differs.

We used the potential structural similarity between CbpA and other DnaJ homologs to design deletion mutants and site-directed substitution mutants for our studies. The mutants are shown in Fig. 2.

CbpM Interacts with CbpA through the J-domain—We previously showed that CbpM, an 11-kDa protein, specifically interacts with CbpA, and as a result of the interaction, both DNA binding and co-chaperone activities of CbpA are inhibited (9). To determine which domain of CbpA participates in the protein-protein interaction, we incubated CbpM with the domain deletions and then separated the protein species by native PAGE. When CbpA(J) and CbpM were incubated together and analyzed, a protein species appeared that migrated to a new position in the gel, distinct from the position of CbpM and CbpA(J) separately (Fig. 3A, lanes 1–3). Similarly the mixture of CbpM and wild type CbpA produced a protein complex that migrated on native PAGE distinct from either CbpM or CbpA (Fig. 3A, lanes 4 and 5). To confirm that the new species seen following native PAGE contained both CbpA(J) and CbpM, the protein band was excised, eluted, and analyzed by SDS-PAGE. The results showed that the new species contained both proteins (Fig. 3B, lane 1). Quantification of CbpA(J) and CbpM in the complex is most consistent with a 1:1 ratio of CbpA to CbpM (data not shown). No complexes were detected between CbpA(L,CTDI,CTDII) and CbpM (data not shown). These results show that the J-domain is sufficient for CbpM interaction.

To identify the region of the CbpA J-domain involved in CbpM interaction, we tested site-directed mutants. Using the rationale that CbpA, but not DnaJ, interacts with CbpM (Ref. 9 and data not shown), several CbpA mutants were made in which DnaJ amino acids were substituted for the CbpA counterparts, including CbpA(D16A, D17E, L18E, K19R), CbpA(S57T, E59S, R61K), and CbpA(M68Y, W69G, Q70H, H71A) (shown in Fig. 2). These mutants were tested for CbpM interaction by native PAGE as described above. All of the mutants formed complexes with CbpM indistinguishable from complexes formed between wild type CbpA and CbpM (data not shown). Two other mutants in the J-domain, CbpA(H33Q) and CbpA(V36G, S37G, K38G), also formed complexes with CbpM. DNA binding activity of all of the J-domain mutants was inhibited by CbpM (data not shown), further showing that the CbpA-CbpM interaction site had not been inactivated. Taken together, the results show that the J-domain of CbpA interacts with CbpM, but the specific interacting residues remain to be identified.

The Region C-terminal to the J-domain Is Required for DNA Binding—To identify the DNA-binding domain of CbpA, we tested the domain deletion mutants (Fig. 2, A and C) for their ability to bind DNA. CbpA(L,CTDI,CTDII) bound DNA with similar apparent affinity to wild type (Fig. 4A). CbpA(J,L,CTDI) retained some DNA binding ability; however, it exhibited 5–10-fold lower affinity for DNA than wild type (Fig. 4A). In contrast, CbpA(J) and CbpA(CTDII) had no detectable DNA binding activity (Fig. 4A). These results indicate that residues C-terminal to the J-domain are important for DNA binding. The linker region and CTD I are essential for DNA binding and CTD II contributes. None of the deletion mutants possessed detectable co-chaperone activity using an assay in which inactive RepA
dimers are remodeled by DnaK, GrpE, and CbpA to active monomers that bind to labeled oriP1 DNA with high affinity (9, 20) (Fig. 4B).

Knowing that CbpM inhibits DNA binding by CbpA, a prediction from the observation that CbpA(L,CTD,CTDII) binds DNA like wild type is that its DNA binding activity should not be inhibited by CbpM. Indeed, CbpM did not significantly inhibit DNA binding by CbpA(L,CTD,CTDII) (Fig. 4C). Under the same conditions, CbpM inhibited DNA binding by full-length CbpA and by CbpA(J,L,CTDII) more than 90% (Fig. 4C). This observation confirms that CbpM interacts through the J-domain and that DNA binding activity is C-terminal to the J-domain.

To further explore the DNA-binding domain, alanine substitution mutants were constructed in the region between 79 and 202 (Fig. 2, B and C). Interestingly, mutants with alanine substitutions in 87–90, 104–107, or 133–136 had two to five-fold lower DNA binding activity than wild type CbpA (Fig. 5A). Other mutants in this region, including CbpA(72–74), CbpA(77–80), CbpA(82–85), CbpA(156, 162), and CbpA(192, 194, 196) retained wild type DNA binding activity (Fig. 5B and

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**FIGURE 3.** Involvement of the J-domain in CbpA-CbpM interaction. A, reaction mixtures (25 µl) containing 240 pmol CbpM (lane 1), 80 pmol CbpA(J) (lane 2), a mixture of 80 pmol CbpA(J) and 240 pmol CbpM (lane 3), 80 pmol wild type CbpA (lane 4) or a mixture of 80 pmol wild type CbpA, and 240 pmol CbpM (lane 5) in Buffer B were incubated for 10 min at 24 °C. Two sets of identical reaction mixtures were prepared and applied to the same Tris-glycine gel and subjected to native PAGE. Following electrophoresis, one half of the gel was stained with Coomassie Blue to detect the proteins. The new protein species, referred to as “CbpA(J)–CbpM complex,” that appeared following the mixing of CbpA(J) and CbpM is indicated. B, the protein bands were excised from the unstained half of the gel, eluted in Buffer B at 42 °C for 1 h, subjected to SDS-PAGE, and stained with Coomassie Blue as follows: lane 1, the CbpA(J)–CbpM complex species; lane 2, molecular weight markers; lane 3, CbpM; and lane 4, CbpA(J).

**FIGURE 4.** The region C-terminal to the J-domain is involved in DNA binding. A, DNA binding by domain deletions of CbpA. DNA binding assays were carried out as described under “Experimental Procedures” with wild type CbpA (blue circles), CbpA(J) (red triangles), CbpA(J,L,CTDII) (green diamonds), CbpA(J,L,CTDI,CTDII) (pink squares), or CbpA(CTDII) (orange circles). B, co-chaperone activity of deletion mutants. RepA activation reactions were carried out as described under “Experimental Procedures” with the indicated amounts of wild type CbpA (blue circles), CbpA(J) (red triangles), CbpA(J,L,CTDI) (green diamonds), CbpA(J,L,CTDI,CTDII) (pink squares), or CbpA(CTDII) (orange circles). C, CbpM does not inhibit DNA binding by CbpA(L,CTD,CTDII). DNA binding by wild type CbpA (blue circles), CbpA(J,L,CTDI) (pink squares), or CbpA(J,L,CTDII) (green diamonds) was measured as described under “Experimental Procedures” using the indicated amounts of CbpM. Results shown are means (± S.E.) of three or more experiments.
Together with the data from the deletion mutants, these results show that the linker region and CTD I are essential for DNA binding; CTD II plays a part in DNA binding; and specific residues between 87 and 136 are important.

FIGURE 5. DNA binding and co-chaperone functions of CbpA are separable. A, DNA binding by substitution mutants. DNA binding assays were performed as described under “Experimental Procedures” with wild type CbpA (blue circles), CbpA(87–90) (black squares), CbpA(104–107) (pink diamonds), or CbpA(133–136) (green triangles). B, DNA binding was measured as in A with wild type CbpA (blue circles), CbpA(77–80) (black triangles), CbpA(33) (pink squares), CbpA(82–85) (green circles), or CbpA(72–74) (red diamonds). C, co-chaperone activity of substitution mutants. Co-chaperones assays were carried out as described under “Experimental Procedures” with wild type CbpA (blue circles), CbpA(104–107) (pink diamonds), or CbpA(133–136) (green triangles). Results shown in A–C are means (± S.E.) of three or more experiments.

FIGURE 6. Residues in CTD I involved in substrate binding. A, co-chaperone activity of CbpA(192, 194, 196). Reactions were carried out as described under “Experimental Procedures” with wild type CbpA (blue circles) or CbpA(192, 194, 196) (red squares). B, DNA binding activity of CbpA(192, 194, 196). Experiments were carried out as described under “Experimental Procedures” with wild type CbpA (blue circles) or CbpA(192, 194, 196) (red squares). C, substrate binding by wild type and mutant CbpA. CbpA-RepA binding experiments were performed as described under “Experimental Procedures” using 100,000 MWCO filters with [3H]RepA and wild type CbpA (blue circles), CbpA(104–107) (pink triangles), CbpA(192, 194, 196) (red squares), or CbpA(H33Q) (black squares). Results shown are means (± S.E.) of three or more experiments.

DNA Binding Activity of CbpA Is Separable from Co-chaperone Function—We next asked whether the DNA binding function of CbpA is distinct from the co-chaperone function. The alanine substitution mutants, that were used above to identify residues important for DNA binding, were tested for
co-chaperone function by measuring RepA activation. CbpA(104–107) and CbpA(133–136) retained wild type levels of co-chaperone activity, although they were defective in DNA binding (Fig. 5, C and A). CbpA(87–90) was defective in co-chaperone activity as well as DNA binding activity (Fig. 5, C and A). Other CbpA mutants in the 79–202 region that were active in DNA binding possessed wild type co-chaperone activity, including CbpA(77–80), CbpA(82–85), and CbpA(156, 162) (data not shown). Thus, some DNA binding defective mutants retain full co-chaperone activity pointing out that the two activities are separable and involve distinct amino acid residues.

**Substrate Binding Is Separable from DNA Binding**—Next we tested whether the DNA binding site and substrate interaction overlap. The location of the peptide binding site of a type II DnaJ homolog, Ydj1, is known from crystallographic studies (7). In addition, the peptide binding site in yeast Sis1 has been identified by genetic and biochemical studies (10, 27). We made an alanine substitution mutant in CbpA in the region predicted to interact with substrates, amino acids Leu192, Leu194, and Ile196 (Fig. 2, B and C). When we tested the mutant protein in vitro, we found that it lacked detectable co-chaperone activity (Fig. 6A) but retained DNA binding activity (Fig. 6B).

We directly measured the interaction of the CbpA(192, 194, 196) with RepA by incubating labeled RepA with wild type or mutant CbpA. Complexes were then collected by centrifugation through 100-kDa cut-off filters, and radioactivity in the retentates was measured. CbpA(192, 194, 196) bound RepA with ~2-fold lower affinity than wild type CbpA (Fig. 6C). CbpA(H33Q), which has a mutation in the conserved tripeptide (His, Pro, Asp) and lacks detectable co-chaperone activity but binds DNA with similar affinity to wild type (Fig. 5 and data not shown), bound RepA with similar affinity to wild type (Fig. 6C). This observation is consistent with the known involvement of the conserved tripeptide in interaction with DnaK. CbpA(104–107), that has wild type levels of co-chaperone activity but is defective in DNA binding (Fig. 5, A and C), also bound RepA with similar affinity to wild type (Fig. 6C). These results suggest that CbpA(192, 194, 196) is partially defective in substrate binding. In summary, although both DNA binding and substrate interaction require participation of CTD I, the two activities involve different residues.

**CTD II Domain Is Required for Dimerization**—To identify the portion of CbpA that is involved in dimerization, chemical cross-linking experiments were performed. Bis(sulfosuccinimidyld) suberate, BS3, was reacted with the CbpA domain mutants, and then the proteins were separated by SDS-PAGE. The results suggested that CbpA(CTDII) is a dimer (Fig. 7, lanes 8–9). CbpA(CTDII) migrated with an apparent molecular mass...
of 8.7 ± 0.4 kDa in the absence of cross-linking and 25.7 ± 1.0 kDa in the presence of cross-linking reagent. In agreement with this observation, the calculated molecular mass of the CbpA(CTDII) dimer is 25.7 kDa. The apparent molecular masses of CbpA(J) and CbpA(J,L,CTDI) did not change following BS3 treatment, suggesting that those mutants existed as monomers (Fig. 7, lanes 4–7). As a control, cross-linked CbpA migrated with an apparent molecular mass of 63 kDa, whereas untreated CbpA migrated with a molecular mass of 35 kDa (Fig. 7, lanes 2 and 3), consistent with the known dimeric structure of CbpA (9). Thus, the dimerization domain is in CTDII. In combination with the results showing that CbpA(J,L,CTDI) binds DNA, this observation additionally demonstrates that CbpA monomers are able to bind DNA.

**DISCUSSION**

We have identified regions of CbpA that make it a unique DnaJ homolog. The results from both the deletion mutants and site-directed mutants are compiled in Table I. The following is a summary of our findings. (i) The CbpM interacting region is within the J-domain. This is somewhat surprising in that the J-domain is highly conserved between *E. coli* DnaJ and CbpA, yet CbpM interacts specifically with CbpA and not DnaJ. (ii) Residues essential for DNA binding are C-terminal to the J-domain, including the linker region and CTD I, with some contribution from CTD II. (iii) The co-chaperone, DNA binding, and CbpM interaction functions of CbpA are separable. (iv) Dimerization of CbpA is through CTD II. It is likely that the dimerization region is at the C-terminal portion of CTD II, as has been found for yeast Sis1 and *E. coli* DnaJ, where deletion of 15 and 45 amino acids, respectively, results in monomeric proteins (26, 28).

The ability of CbpM to interact with CbpA and inhibit the activities of CbpA is intriguing. The inhibition of CbpA by CbpM appears to be the direct result of the binding of CbpM to the J-domain of CbpA. This very likely interferes with the DNA binding site and explains the inhibition of DNA binding by CbpM. By binding to the J-domain, CbpM may also block the substrate binding site, the DnaK interaction site, or disrupt communication between the CbpA domains and thereby inhibit co-chaperone activity.

Interestingly, in a diverse group of bacteria, including *Shigella flexneri*, *Pseudomonas fluorescens*, *Coxiella burnetii*, *Streptomyces avermitilis*, and *Geobacter sulfurreducens*, *cbpM*-like genes are downstream of *dnaJ*-like genes. This similarity in gene organization suggests that the mechanism of regulation of CbpA by CbpM is conserved. Thus, CbpM may well be the founding member of a new family of DnaJ regulators. The puzzle remains as to why genes for the effector and inhibitor proteins are co-transcribed. One could imagine that during part of the cell cycle, CbpA activity is kept in check by its physical interaction with CbpM. At other times, CbpA is activated for DNA binding and/or for co-chaperone activity perhaps by inactivation or degradation of CbpM.

Another unique function of CbpA is its DNA binding activity and possible role in chromosome structure. The observation that residues in CTD I are involved in DNA binding and substrate interaction is consistent with the finding that the co-chaperone function of CbpA is not active in vitro when CbpA is bound to DNA (9). The results suggest that DNA binding occludes the substrate binding site or blocks communication between the domains and argues against a model in which DNA binding by CbpA recruits DnaK to act on protein-DNA complexes. In conclusion, this work provides new understanding of the biochemical properties of CbpA and its interaction with CbpM and DNA.

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