Chloroplasts contain a unique signal recognition particle (cpSRP). Unlike the cytoplasmic forms, the cpSRP lacks RNA but contains a conserved 54-kDa GTPase and a novel 43-kDa subunit (cpSRP43). Recently, three functionally distinct chromodomains (CDs) have been identified in cpSRP43. In the present study, we report the three-dimensional solution structures of the three CDs (CD1, CD2, and CD3) using a variety of triple resonance NMR experiments. The structure of CD1 consists of a triple-stranded β-sheet segment. The C-terminal helical segment typically found in the nuclear chromodomains is absent in CD1. The secondary structural elements in CD2 and CD3 include a triple-stranded antiparallel β-sheet and a C-terminal helix. Interestingly, the orientation of the C-terminal helix is significantly different in the structures of CD2 and CD3. Critical comparison of the structures of the chromodomains of cpSRP43 with those found in nuclear chromodomain proteins revealed that the diverse protein-protein interactions mediated by the CDs appear to stem from the differences that exist in the surface charge potentials of each CD. Results of isothermal titration calorimetry experiments confirmed that only CD2 is involved in binding to cpSRP54. The negatively charged C-terminal helix in CD2 possibly plays a crucial role in the cpSRP54-cpSRP43 interaction.

Chloroplasts are the site of photosynthesis and various other important metabolic processes. Most of the proteins required for the function and maintenance of the chloroplasts are encoded in the nucleus, synthesized in the cytoplasm, and imported into the stroma by general import machinery (1, 2). The routing of proteins from the stroma to the thylakoid membrane occurs via one of four different targeting pathways (3). The post-translational transport of a family of light-harvesting chlorophyll a/b-binding integral membrane proteins (LHCPs)2 from the cytoplasm, and imported into the stroma by general import machinery (1, 2), involves in binding to cpSRP54 either/or on LHCP integration into the thylakoid membrane occurs predominantly via the chloroplast signal recognition particle (cpSRP)-mediated pathway (4, 5).

CpSRP differs significantly from all other signal recognition particles systems (4–6). Unlike cytosolic signal recognition particles from eukaryotes and prokaryotes, cpSRP is not a ribonucleoprotein. It lacks RNA and contains a novel 43-kDa subunit that interacts with its substrates post-translationally. CpSRP is a heterodimer consisting of a 54-kDa subunit and a 43-kDa subunit (7). A recent study (8) revealed that the cpSRP43 interaction site is unique to cpSRP54 and is not present in the SRP54 subunit of other signal recognition particle systems.

The cpSRP43 subunit is composed of two types of structural motifs, ankyrin repeats and chromodomains (chromosome organization modifier) (4, 9, 10). Ankyrin repeats are one of the most frequently observed amino acid motifs in the protein data base (11, 12). Ankyrin repeats are a stable modular domain (of ~33 amino acids) scaffold that are tailored for molecular interaction. Ankyrin repeats have a well defined structure and canonical helix-loop-helix β-hairpin-loop folds (13). Four ankyrin repeats have been identified in cpSRP43 and have been shown to provide a binding interface for LHCPs (9). The chromodomains (CDs) are a family of highly conserved structural motifs that were discovered in the Drosophila polycomb gene-silencing proteins. Two closely spaced CDs are found at the C-terminal end of cpSRP43 (Fig. 1) (9, 10). Based on sequence alignment analysis, a third CD has been identified at the N terminus of cpSRP43 (10). CDs have also been identified in a variety of animal and plant species and are generally structural components of large macromolecular assemblies localized in the nucleus where CDs are involved in gene organization and regulation of gene activity (4, 5, 14). Interestingly, cpSRP43 is the first example of a non-nuclear protein that contains CDs.

There have been various reports on the structural and functional roles of individual CDs in cpSRP43. Jonas-Straube et al. (9), using yeast two-hybrid studies, suggested that CD2 and CD3, located at the C terminus of cpSRP43, act together to provide the binding surface for cpSRP54. In a recent study, Goforth et al. (10), employing GST-fused or CD deletion constructs, showed that CD2 alone contributes to the binding of cpSRP54. Deletion of CD3 was shown to have no impact on cpSRP43 binding to cpSRP54 either/or on LHCP integration into the thylakoid membranes (10). In this context, we examined the binding affinity of cpSRP43 using isothermal titration calorimetry (ITC). Results of ITC experiments reveal that cpSRP54 binds to CD2 but not to the other two CDs (CD1 and CD3) in cpSRP43. In addition, we have solved the three-dimensional structures of the three CDs in cpSRP43 at high resolution, using multidimensional NMR techniques. The overall archi-
tecture of the CDs in the cpSRP43 subunit and CDs found in nuclear chromoproteins is similar. However, the distribution of surface charges in the structures of the CDs derived from various chromoproteins is significantly different, potentially lending insight into the functional roles of individual CDs of cpSRP43.

MATERIALS AND METHODS

Expression and Purification of CD1, CD2, and CD3—Escherichia coli BL21 (DE3) cells were transformed with the expression vector pGEX 4T2 encoding the cDNA sequence of individual CDs (CD1, CD2, and CD3) of cpSRP43 and grown at 37 °C in diluted 1-liter cultures of Luria broth containing 100 μg/ml ampicillin (Ameresco). Protein expression was induced at an \( A_{600} \) of 0.50 with 1 mM isopropyl thiogalactoside, and the cells were incubated for an additional 4 h. Cultures were then harvested by centrifugation. Cell pellets were then resuspended in 25 ml of resuspension buffer (10 mM phosphate buffer, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (Sigma)) and lysed by sonication. The expression of the GST fusion proteins (GST-CD1, GST-CD2, GST-CD3) was checked by SDS-PAGE stained with Coomassie Blue.

GST-fused CDs were purified from bacterial lysates by affinity chromatography using GST-Sepharose (GE Healthcare). GST-fused CDs were eluted with 10 mM Tris buffer (pH 8.0) containing 5 mM reduced glutathione (Sigma). The affinity tag (GST) was removed by cleavage with thrombin (Sigma), and the CDs were purified on a GST-Sepharose column using 100 mM phosphate buffer (pH 6.8) containing 100 mM NaCl. The authenticity of the purified samples was checked by SDS-PAGE and electrospray ionization mass spectroscopy.

Preparation of Isotope (\( ^{15}N \) or \( ^{13}C \))-enriched CD Samples—Uniformly \( ^{15}N \)-labeled CDs were prepared by growing \( E. \) coli in M9 medium containing 0.5 g/liter \( ^{15}NH_{4}Cl \) (Cambridge Isotope Labs, \( ^{15}N >98\% \)) as the sole source of nitrogen (pH 6.8). \( ^{13}C \) and \( ^{15}N \) doubly labeled CD samples were prepared in M9 medium containing 4 g/liter \( ^{13}C \)glucose (Cambridge Isotope Labs, \( ^{13}C >98\% \)) and 0.5 g of \( ^{15}NH_{4}Cl \) as the sole sources of carbon and nitrogen, respectively. The expression and purification protocols for the isotope-labeled CD samples were the same as those used for the unlabeled samples.

Circular Dichroism Measurements—Circular dichroism spectra were recorded on a Jasco J 720 spectropolarimeter with a cuvette of 0.1-cm path length. The scan speed was 20 nm/min, the bandwidth 1.0 nm, and the response time was 1 s. The protein concentration used was 0.5 mg/ml in 100 mM phosphate buffer (pH 6.8) containing 100 mM NaCl. All circular dichroism measurements were made at 25 °C.

Isothermal Titration Calorimetry Measurements—Titration calorimetry measurements were performed using a Microcal VP titration calorimeter (Northampton, MA). Samples were centrifuged prior to the titration and were examined for precipitates, if any, after the titration. A typical titration consisted of injecting 5–10-μl aliquots of 1 mM ligand (CD) solutions into 0.25–0.3 mM cpSRP54 domain solution every 3.5 min to ensure that the titration peak returned to the base line prior to the next injection. Aliquots of ligand solutions were also injected into the reaction buffer (100 mM phosphate buffer (pH 6.8) containing 100 mM NaCl) to measure the heats of dilution of the ligand.

NMR Spectroscopy—All NMR measurements were performed on a Bruker AVANCE DMX-700 MHz spectrometer at 25 °C. A 5-μm triple resonance inverse cryoprobe with z axis field gradient was used. Samples for NMR measurements were prepared by dissolving the proteins (−0.5 mM) in 100 mM phosphate buffer (pH 6.8 containing 100 mM NaCl) prepared in 90% H2O and 10% D2O. Two-dimensional \( ^{1}H-^{15}N \) HSQC spectra were acquired with the pulse sequence of phase-sensitive echo/antiecho TPP1 gradient selection (15). The sequential backbone assignments were made using standard three-dimensional triple-resonance HNCO (16), HNCA (17), HN(CO)CA (18), HNCACB (19), CBCA(CO)NH (20) experiments. Side-chain resonances were assigned using two-dimensional \( ^{1}H \) TOCSY and three-dimensional \( ^{15}N \) TOCSY.
HSQC experiments. NOE-derived distance restraints were obtained from two-dimensional $^1$H-NOESY and three-dimensional $^{15}$N NOESY-HSQC experiments, with a mixing time of 250 ms. The $^3$(H$^N$,$^1$H$^{'})$^1$H coupling constants were measured from a three-dimensional HNHA spectrum (21). $^1$H and $^{13}$C chemical shifts were referenced to tetramethylsilyle propionate (sodium salt, TSP-$d_4$) at 0 ppm, and $^{15}$N resonances were referenced indirectly (22). All NMR spectra were processed using the XWINNMR and Sparky (23) software.

Structure Calculations—Structures of the CDs were calculated by a distance geometry-simulated annealing protocol using crystallography NMR software (24). Distance restraints obtained by converting NOE peak intensities into distance upper limits with $d$\text{(strong)} = 3 Å, $d$\text{(medium)} = 4.0 Å, and $d$\text{(weak)} = 5.0 Å. A total of 100 random structures were calculated and subjected to energy minimization. The 20 lowest energy structures for each CD were selected to represent the three-dimensional folds. Structural quality was analyzed using PROCHECK-NMR (25). Graphical images were prepared with MOLMOL (26).

RESULTS AND DISCUSSION

Secondary Structure of the CDs of cpSRP43

The CDs of cpSRP43 are ~50 amino acids long (Fig. 1). The average secondary structures of the three cpSRP43 CDs were determined using far-UV circular dichroism spectroscopy. The far-UV circular dichroism spectrum of CD1 shows a positive ellipticity band centered at 228 nm and negative ellipticity extrema near 205 nm (data not shown). These spectral features are characteristic of proteins with $\beta$-barrel architecture (27). Interestingly, the structure of CD1 does not appear to contain a helical segment(s) that is typical of CDs derived from nuclear chromoproteins. The far-UV circular dichroism spectra of both CD2 and CD3 show negative ellipticity bands near 208 and 222 nm suggesting that portions of the backbone of these domains are in helical conformation(s) (data not shown).

Assignment of Resonances and Identification of Secondary Structure Elements in CDs of cpSRP43

Resonance assignments in CD1, CD2, and CD3 were achieved using a variety of triple resonance experiments. $^1$H-$^{15}$N HSQC spectra of the CDs of cpSRP43 are well dispersed suggesting that these domains are structured (Fig. 2). Assignments of the chemical shifts of $^1$H, $^{13}$C, and $^{15}$N nuclei in the backbone of proteins were made using a combination of three-dimensional HNCA (17), HN(CO)CA (18), CBCA(CO)NH (20), and HNCACB (19) experiments. Aliphatic side-chain proton resonances were assigned using two-dimensional $^1$H-TOCSY and three-dimensional $^{15}$N HSQC-TOCSY data. Long and medium range distance constraints were derived from two-dimensional $^1$H-NOESY and three-dimensional $^1$H-$^{15}$N HSQC-NOESY experiments. The elements of sec-
Structures of Non-nuclear Chromodomains

**TABLE ONE**

<table>
<thead>
<tr>
<th>Structural statistics for chromodomains of cpSRP43</th>
<th>CD1</th>
<th>CD2</th>
<th>CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>CNS</em> energies (kcal mol (^{-1}))</em>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E_{\text{tot}})</td>
<td>(-486 \pm 9.2)</td>
<td>(-762 \pm 9.7)</td>
<td>(-755 \pm 10.2)</td>
</tr>
<tr>
<td>(E_{\text{bound}})</td>
<td>35 (\pm) 5.6</td>
<td>39 (\pm) 9.5</td>
<td>30 (\pm) 4.1</td>
</tr>
<tr>
<td>(E_{\text{imp}})</td>
<td>70 (\pm) 5.8</td>
<td>34 (\pm) 9.1</td>
<td>29 (\pm) 5.5</td>
</tr>
<tr>
<td>(E_{\text{vdw}})</td>
<td>67 (\pm) 8.9</td>
<td>83 (\pm) 9.2</td>
<td>20 (\pm) 2.3</td>
</tr>
<tr>
<td>r.m.s. deviation from mean coordinates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backbone all atoms (N, C(_\text{alpha}), C) (Å)</td>
<td>1.13 (\pm) 0.1</td>
<td>0.85 (\pm) 0.1</td>
<td>1.23 (\pm) 0.1</td>
</tr>
<tr>
<td>Backbone, secondary structure (Å)</td>
<td>0.55 (\pm) 0.1</td>
<td>0.46 (\pm) 0.1</td>
<td>0.54 (\pm) 0.1</td>
</tr>
<tr>
<td>All heavy atoms (Å)</td>
<td>1.68 (\pm) 0.1</td>
<td>1.52 (\pm) 0.1</td>
<td>2.09 (\pm) 0.1</td>
</tr>
<tr>
<td>Ramachandran plot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residues in favorable regions</td>
<td>86.9%</td>
<td>91.2%</td>
<td>89.1%</td>
</tr>
</tbody>
</table>

* CNS, crystallography NMR software.
* No NOE distance and dihedral angle restraints was violated by more than 1 Å.
* r.m.s., root mean square.
* Mean coordinates were obtained by averaging coordinates of the 20 calculated structures.
* This reflects residues in both most favored and additionally favored regions.

### Quality of the Three-dimensional Structure of the CDs of cpSRP43

The three-dimensional structures of the CDs were computed based on the distance geometry calculation and energy minimization. Ensembles of 20 structures were calculated for each of the CDs. The average NMR structure represents a good fit of the experimental data, with very small residual constraint violations (<0.1 Å for distance constraints and >1 Å for the dihedral angle constraints). A summary of the structural statistics for the final 20 structures of each CD is listed in TABLE ONE. The high quality of the three chromodomain structures is evident from the results of PROCHECK analysis, which reveals that the majority of the residues are in the most favored region of the Ramachandran plot.

### Description of the Three-dimensional Structures of CDs of cpSRP43

**Three-dimensional Structure of CD1**—CD1 contains the tryptophan pair (Trp\(^{25}\) and Trp\(^{29}\)) that is conserved in all CDs derived from both nuclear and non-nuclear chromoproteins (14) (Fig. 1). Although no protein binding partners for CD1 have yet been identified, CD1 has been shown to play an important role in the integration of LHCP into the thylakoid membrane (10). Recently, Goforth et al. (10) showed that deletion of CD1 from cpSRP43 resulted in a 4-fold increase in the rate at which GTP is hydrolyzed by cpSRP54/FtsY. Therefore, it is proposed that CD1 acts as a negative regulator of GTP hydrolysis (10).

The solution structure of CD1 shows three antiparallel β-strands (strand I, residues 10–14; strand II, residues 18–22; and strand III, residues 32–35) organized to form a triple-stranded β-barrel structure. The C-terminal α-helix, which is a characteristic feature of CD structures, is conspicuously absent in CD1 (Fig. 3). These observations are consistent with the far-UV circular dichroism data. In this context, it would be interesting to note that recently a putative nuclear CD from MOF, a member of the histone acetylating transferase from Drosophila, has been shown to have a β-barrel structure without the C-terminal helix conventionally found in the structures of CDs (28). Hydrogen-bonding interactions between residues located in β-strands I and III (Thr\(^{13}\)-NH, strand I) to Val\(^{34}\)-CO (strand III) and Val\(^{36}\)-NH (strand III) to Ser\(^{11}\) (strand I) stabilize the β-barrel structure of CD1. Similarly, a hydrogen bond between the amide proton of Asp\(^{27}\) and the carbonyl group of Val\(^{19}\) links the flexible N-terminal end with residues located in the loop region situated between strands II and III (Fig. 3). Several long range NOEs from the indole ring of Trp\(^{25}\) could be detected in the two-dimensional H-NOESY and three-dimensional 15N HSQC-NOESY spectra of CD1. These long range interactions aid in the positioning of the indole ring in the central core of the CD1 molecule. The structure of CD1 is characterized by large clusters of hydrophobic residues (Fig. 4). Isolated charged residues are located at the periphery of the hydrophobic core. The large areas of non-polar surfaces found in CD1 may be important for its negative regulatory role on the cpSRP54/CPFTS5-mediated GTPase activity (10). Non-polar surfaces in CD1 might help in the exclusion of water from the GTP-binding site in SRP54/FtsY and consequently prevent nonproductive GTP hydrolysis.

**Three-dimensional Solution Structure of CD2**—The solution structure of CD2 has been determined at high resolution (TABLE ONE). The secondary structural elements in the protein include three β-strands (strand I, residues 14–18; strand II, residues 22–26; and strand III, residues 36–39) and a C-terminal α-helix (residues 44–56). The α-helix in CD2 is positioned perpendicular to the plane of the triple-stranded, antiparallel β-sheet (Fig. 3). This structural feature is also shared by CDs from nuclear chromoproteins. The structure of CD2 is characterized by the presence of β-bulge structures, and this aspect possibly reflects their evolution from a common ancestral gene. The β-bulge at Asp\(^{11}\) facilitates long range interactions of the amide proton of Glu\(^{15}\) (in β-strand I) with residues in the α-helix segment. Similarly, the β-bulge located at Leu\(^{26}\) aids in the accommodation of the large indole ring of Trp\(^{29}\) within β-strand II. There are strong NOEs in the two-dimensional H-NOESY and three-dimensional 15N HSQC-NOESY spectra that define the interactions of the C-terminal helical segment with other portions of the structure of CD2. These interactions include the α-proton of Glu\(^{56}\) with the amide proton of Val\(^{5}\), the amide proton of Tyr\(^{51}\) with the indole ring proton of Trp\(^{29}\), and the α-proton of Tyr\(^{51}\) with the amide proton of Glu\(^{15}\). These long range interactions are primarily responsible for the perpendicular alignment of the C-terminal helix with the plane of the triple-stranded β-sheet segment. An indirect structural consequence of the occurrence of the two β-bulges in CD2 is...
the formation of two distinct hydrophobic clusters that are enclosed between the α-helix and β-strand I (Fig. 4). Such hydrophobic cores have been shown to be protein-protein interaction sites in CDs of nuclear chromoproteins (29, 30). The two non-polar clusters are organized around each of the conserved tryptophan residues (Trp29 and Trp37). Cluster-1 is formed by non-polar residues (Val23, Tyr25, Leu26, and Val27) surrounding Trp29. The other isolated hydrophobic core consists of Trp37, Val38, Val41, and Ala42. The hydrophobic cluster built around Trp29 shows an interesting arrangement. This cluster is enclosed by two layers of charged residues. The layer bordering the hydrophobic core is comprised of positively charged residues.

Structure of the CD3 Domain—The three-dimensional solution structure of CD3 is characterized by the presence of three antiparallel β-strands (Fig. 3) (strand I, residues 4–9; strand II, residues 20–25; and strand III, residues 29–33) and a C-terminal α-helix (residues 40–50). Unlike CD2, the axis of the α-helix in CD3 is parallel to the plane of the triple-stranded β-sheet (Fig. 3). Interestingly, the structure of CD3 represents the first example of a CD in which the α-helix is positioned parallel to the plane of the β-sheet. As no functional role has been assigned to CD3 and cpSRP43 lacking CD3 is fully functional in assays that reconstitute LHCP integration into the thylakoids, it is difficult to comprehend the functional significance of the unusual spatial orientation of the α-helical segment. β-Strand I and the C-terminal α-helix in CD3 are held together by a long range hydrogen bond between the amide proton of the Val8 and the main-chain carbonyl group of Gln27. A large cluster of hydrophobic residues is enclosed between β-strand I and the α-helix (Fig. 4). The hydrophobic cluster is built around the indole rings of the conserved pair of tryptophan residues located at positions 24 and 32. The large core of hydrophobic residues not only appears to confer structural stability to the protein but also could play a vital role in interactions of CD3 with partners that are yet to be identified. The surface charge potential of the triple stranded β-sheet in CD3 is mostly neutral, but the α-helical segment carries a positive electrostatic potential. The positive surface charge potential of the α-helix may be functionally important because many CDs are shown to interact with their protein partners through residues located in the conserved α-helix (31, 32).

Surface Charge Distribution in CDs Are Distinctly Different—Protein-protein interactions are frequently highly specific; for example, it is often the case that only one member of a particular protein family will specifically recognize one binding partner despite the scenario that all other members of the family adopt an identical fold (33). As CDs present in various chromoproteins have different interaction partners, analysis of the surface charge distribution in the three-dimensional structures of CDs is likely to provide useful insights into the molecular forces that govern protein-protein interactions involving CDs. For example, the exterior of the triple-stranded β-sheet domain of Sac7d is highly positive (30). This is consistent with the fact that Sac7d binds nonspecifically.
to the major groove of DNA (30). In marked contrast, the electrostatic potential of the CD of HP1 is highly negative suggesting that unlike Sac7d, this class of CD would not by itself bind to DNA or RNA. The high density of negative surface charge plays an important role in the recognition and binding of Lys9-methylated histone H3 by the CD of HP1. There is wide variation in the surface charge potentials of the CDs of cpSRP43. The surface charge potential of the CD1 is nearly zero. In CD3, the exterior of the triple stranded /H9252-sheet is neutral, but the surface charge potential of the α-helix is positive. In marked contrast, the surface charge potential of CD2 is highly negative because of the uniform distribution of acidic residues like aspartic acid and glutamic acid.

**Defining the cpSRP43-cpSRP54 Binding Interface**—Isothermal titration calorimetry is a useful technique to measure the binding affinity of a protein to its ligand (34). In addition, ITC experiments can also provide valuable information on the thermodynamics of protein-ligand interactions (35). It has been suggested previously (9) that CD2 and CD3 together provide the binding interface for cpSRP54. However, Goforth et al. (10), using a variety of CD deletion constructs of cpSRP43, showed that CD2 is the primary binding site for cpSRP54. In this context, we performed ITC experiments to investigate the relative binding affinities of each of the CDs (in cpSRP43) to cpSRP54.

The binding curve representing CD2-cpSRP54 interaction is sigmoidal (Fig. 5, middle panel). The binding is exothermic and proceeds with evolution of heat. The interaction of CD2 with cpSRP54 is characterized by large negative enthalpy (∆H = −13.7 kcal/mol) indicating that binding is predominantly mediated through charge interactions. The binding isotherm can be best fit to a single binding site model yielding a dissociation constant of about 190 nM. The stoichiometry of CD2 to cpSRP54 binding is estimated to be nearly 1:1. The binding isotherms representing CD3-cpSRP54 and CD1-cpSRP54 interactions are nearly flat, suggesting that these CDs do not interact with cpSRP54 (Fig. 5, left and right panels). Therefore, ITC data clearly indicate that only residues in CD2 interact with cpSRP54. In summary, our ITC data support the conclusion of Goforth et al. (10) and unequivocally suggest that only CD2 is involved in the cpSRP43-cpSRP54 interactions.

**Comparison of the Three-dimensional Structures of CDs**—In the absence of knowing the structure of cpSRP43, the possibility remains that each of the domain structures presented here may differ from their structures in the full-length protein. However, ITC data showing that CD2 alone binds cpSRP54 supports domain deletion studies, which demonstrate that CD2 is solely responsible for binding cpSRP54 (10). Furthermore, conservation of the overall backbone fold of CD1, CD2, and CD3 also strongly suggests proper folding of the isolated domains. Given this, it would be interesting to understand why CD2 alone is involved in interactions with cpSRP54 even though the three-dimensional structures of all the CDs in cpSRP43 are grossly similar. Prominent differences between the structures of CDs in cpSRP43 include: 1) absence of a helical segment in CD1, 2) difference in the orientation of the C-terminal helix in CD2 and CD3, and 3) differences in the surface charge potential of the CDs. The helical segment in CD2 is populated by a number of negatively charged residues. These negatively charged residues in the helix and those located in -strand I form a dense and continuous negatively charged surface. In marked contrast, the C-terminal helix in CD3 exhibits a small positive surface potential and is aligned parallel to the positively charged N-terminal β-strand 1 spanning residues 4–8. It appears that the highly negative surface potential in CD2 plays an important role in the interaction of cpSRP43 with cpSRP54. To evaluate the contribution of the C-terminal helix in CD2 toward the cpSRP43-cpSRP54 interaction, we generated a CD2 chimera in which the helix in CD2 was substituted with that of CD3 (residues 40–54 in CD3). The CD2 chimera fails to bind to cpSRP54 suggesting that the C-terminal helix is involved in binding with cpSRP54 (data not shown).
shown). In this context, it is reasonable to assume that the absence of a helix in CD1 renders it incapable of binding with cpSRP54. The failure of CD3 to interact with cpSRP54 suggests that both the orientation and the negative charge distribution on the helix play a crucial role in the interaction of CD3 to interact with cpSRP54. The absence of a charged segment in cpSRP54 consisting of residues 530–539 constitutes the cpSRP54 binding site. Mutational analysis revealed that the negative charge distribution on the helix play a crucial role in the orientation of Arg539 to Gly539 resulted in a reduction of binding by about 45%.

Taken together, it appears that specific interaction of cpSRP54 is primarily facilitated by the highly negative surface potential of CD2. Currently, work is under way to determine the three-dimensional structure of the CD2-cpSRP54 peptide binary complex to further understand the molecular forces governing the cpSRP43 and cpSRP54 interaction.

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Three-Dimensional Solution Structures of the Chromodomains of cpSRP43
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doi: 10.1074/jbc.M507077200 originally published online September 23, 2005

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