The RNA Helicase DbpA Exhibits a Markedly Different Conformation in the ADP-bound State When Compared with the ATP- or RNA-Bound States*

Received for publication, July 24, 2002, and in revised form, September 10, 2002
Published, JBC Papers in Press, September 24, 2002, DOI 10.1074/jbc.M207438200

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The motor enzymes that belong to the family of RNA helicases catalyze the strand separation of duplex RNA via ATP hydrolysis. Among these enzymes, Escherichia coli DbpA is a unique RNA helicase because it possesses ATPase-specific activity toward the peptidyl transferase center in 23 S ribosomal RNA. For this reason, it has been the subject of numerous biochemical and structure-function studies. The ATP-stimulated unwinding activity of DbpA toward specific and nonspecific RNA duplexes has been demonstrated. However, the underlying molecular and structural basis, which facilitates its helicase activities, is presently not known. We combined time-dependent limited proteolysis digestion, fluorescence spectroscopy, and three-dimensional structural homology modeling techniques to study the structural conformations of DbpA with respect to its binding to stoichiometric ratios of RNA and cofactors. We show that the conformational state of DbpA is markedly different in the ADP-bound state than in any other state (ATP- or RNA-bound). These results, together with structural homology studies, suggest that a hinge region located in the core domain of DbpA mediates such conformational changes.

RNA adopts specific structures that govern its biological activities in various RNA-dependent cellular processes (1, 2). The correct folding of RNA may be regulated by the ATP-dependent DEXD/H box RNA helicases (3, 4). In this process, the hydrogen bonds of the double-stranded RNA region are disrupted enzymatically by helicases in the course of the unwinding reaction (5, 6). These enzymes are found in a wide range of organisms, ranging from viruses and prokaryotes to lower and higher eukaryotes. RNA helicases participate in many essential cellular processes, such as transcription, translation, ribosome assembly, cell differentiation, cell development, RNA processing, and mRNA splicing (7, 8). Therefore, helicases may play a key role in regulating these biological processes by controlling RNA structures.

In general, these motor enzymes belong to a large family (designated as superfamily II) characterized by a DEAD/H (Asp-Glu-Ala-Asp/His) box motif in addition to eight other conserved structural motifs in their sequences (Fig. 1) (9, 10). Specifically, the DEAD/H box motif is associated with ATP binding and hydrolysis processes, and therefore, it is thought to affect the unwinding or helicase activity of these enzymes (11, 12). Interestingly, all the conserved motifs are positioned in the so-called “core region” of the protein (13). The core region is attached to N- or C-terminal extensions. These N- or C-terminal extensions in RNA helicases provide the enzyme with its substrate specificity and localization, both in the cell and in other protein-protein interactions (5). It was, therefore, proposed that with the helicase superfamily, the core domain scaffold that provides the enzymes with their mechanical activity is structurally and functionally similar (5, 14). In addition, it is thought that helicases generally execute their helicase activities on double-stranded nucleic acid substrates regardless of their sequences.

The DEAD box RNA helicase from Escherichia coli, DbpA, is a unique enzyme of superfamily II. Unlike other RNA helicases, DbpA possesses full ATPase activity in the presence of the 153-nucleotide region (bases 2454–2606) from domain V of 23 S ribosomal RNA (rRNA), also termed the peptidyl transferase center (15–17). More specifically, Pugh et al. (18) demonstrated that DbpA extensively hydrolyzes ATP upon the interaction with hairpin 92 of the peptidyl transferase center. Additionally, nonspecific interactions with single-stranded RNA (ssRNA) regions distal to the hairpin 92 could also be detected (19). Interestingly, the ability of DbpA to mediate its natural unwinding activity on duplex RNA has been questioned (18). However, using both biochemical and single-molecule imaging assays, Henn et al. (20) demonstrated that DbpA can unwind nonspecific sequences of long duplex RNA molecules. Importantly, Diges et al. (21) recently reported that DbpA requires hairpin 92 to facilitate the robust ATP-dependent unwinding activity of short 10-mer RNA oligomers positioned either 3’ or 5’ upstream or downstream from this region in the peptidyl transferase center. It was proposed that DbpA performs its specific unwinding activity by anchoring its C-terminal extension to hairpin 92, whereas its core region unwinds a proximate duplex RNA region (19). This proposal was also supported by a schematic model of DbpA, rendered from the crystal structure of a highly homologous DEAD box RNA helicase (22). Collectively, these results support the notion that the structure of DbpA contains flexible regions that are utilized by the enzyme for its unwinding activity.

These results prompted us to investigate the conformational changes induced in the protein during the formation of the productive complexes of DbpA with ssRNA and cofactors (ATP, S and ADP). The high sequence and structural similarities among RNA helicases and other RNA and DNA
Conformational States of the RNA Helicase DbpA

helicases (7, 10, 19, 22, 23) suggest that they all share common themes regarding their mode of action. Nevertheless, the details of this mechanism are not fully understood. Combining the techniques of time-dependent limited-proteolysis digestion, fluorescence spectroscopy, and modeling studies, we mapped the conformational changes in DbpA that are induced upon its binding to ssRNA and cofactors.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemical reagents were of molecular biology grade (Sigma); ADP, trypsin, and chymotrypsin were of sequencing grade (Sigma); ADP, trypsin, and chymotrypsin were of sequencing grade, respectively. The reaction was performed in quartz cells with a light manual slit. Excitation and emission bandwidths were 8 and 4 nm, respectively. The reaction was performed in quartz cells with a light manual slit. Excitation and emission bandwidths were 8 and 4 nm, respectively. The reaction was performed in quartz cells with a light manual slit. Excitation and emission bandwidths were 8 and 4 nm, respectively.

**METHODS**—Limited Proteolysis Digestion—The reaction buffer contained 20 mM HEPES, pH 7.5, 50 mM NH₄Cl, 2 mM MgCl₂. The reaction mixture contained 4 mM DbpA, and in stoichiometric ratio, ssRNA, ADP-Mg²⁺, or ATP-S-Mg²⁺. Incubation was performed at 20 °C for 10 min followed by the initiation of limited proteolysis digestion by adding the specific protease at 0.4 μM and performing cleavage at 37 °C. Aliquots (10 μL) were removed at various times, quenched in 2% SDS, 0.1% bromphenol blue, 10% glycerol, 50 mM Tris-Cl, pH 6.8, 10 mM dithiothreitol, and 1 mM iodoacetamide. The reaction was performed in quartz cells with a light manual slit. Excitation and emission bandwidths were 8 and 4 nm, respectively. The reaction was performed in quartz cells with a light manual slit. Excitation and emission bandwidths were 8 and 4 nm, respectively.

**RESULTS**—Limited proteolysis experiments were performed to detect and characterize the structural conformational changes induced at the protein level during DbpA catalysis. The enzymatic cleavage pattern can be predicted by the accessibility of the protease to its specific target sequences. Therefore, limited proteolysis can be used to determine distinct conformational changes of intact proteins and complexes. We examined the cleavage patterns of DbpA bound to ssRNA, ADP, and ATP by using two different proteases: trypsin and chymotrypsin. These studies were conducted following the method of a previously reported experiment (25). Briefly, ssRNA, ADP, and ATP were used in a 1.25 molar excess over protein to saturate all the RNA- and nucleotide-binding sites of the enzyme. We used ATP as a non-hydrolyzable analog of ATP since the affinity of the alternative analog AMPPNP to DbpA is greatly reduced by more than 30-fold when compared with the binding of ATP (26). Each complex was incubated at 20 °C for

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**Fig. 1.** Sequence alignment of the conserved structural motifs of helicase from super families I and II. The conserved motifs are designated by black boxes and numbered according to Gorbalenya et al. (11). The alignment was performed with the CLUSTALW program and was used to construct the three-dimensional structural model by homology building. The sequence homology of the DEXD/H box family extends from bacteria to eukaryotes.

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| Helicases | Materials | Methods | Results |
|-----------|-----------|---------|---------|
| Helicases | Helicases | Helicases | Helicases |

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**Table 1.** Conformational States of the RNA Helicase DbpA

| Conformational State | DBPA Binding | RNA Binding | ATP Binding |
|----------------------|--------------|-------------|-------------|
| Native helicase      | +            | -           | -           |
| Denatured helicase   | -            | +           | +           |
10 min before subjecting the complex to the enzymatic proteolysis at various time intervals. The resulting protein fragments were analyzed on high resolving gradient gels. The desired bands from the gel were further analyzed by N-terminal amino acid sequencing (Table I). We mapped the fragments resulting from the proteolytic cleavages according to their N terminus amino acid, identified by sequencing analysis. These fragments are designated as D1–8 in Table I.

The Proteolysis Pattern of Free DbpA and DbpA Bound to Cofactors (ADP and ATPyS)—Fig. 2 shows the limited proteolysis profile, by trypsin, of DbpA, DbpA-ADP, and DbpA-ATPyS. Digestion of free DbpA with trypsin produced 11 polypeptide products. The designated D1–4 polypeptides were identified by N-terminal amino acid sequencing analysis. The N-terminal residue of the D1 product was mapped to Asp^117 (Table I), referring to the 40-kDa fragment (Fig. 2). Asp^117 is positioned between the ATP-binding and hydrolysis sites, designated as Walker A and Walker B (Fig. 1). The N-terminal residue of the D2 product was mapped to Met^1 with an apparent molecular mass of 37 kDa. Presumably, this fragment resulted from cleavage of trypsin at the C-terminal extension of DbpA, which is rich in Lys and Arg. The D3 polypeptide fragment that was mapped to Val^198 is located 10 amino acid residues after the SAT motif, which is directly involved in the unwinding activity of helicases (Fig. 1). The D4 fragment that was mapped to Val^198 refers to the 25-kDa fragment (Table I and Fig. 2). Val^198 is located near the Walker A motif (Fig. 1). Because of this highly specific cleavage site, the D4 product can serve as a marker for the conformational changes at the nucleotide-binding site. In this way, the trypsin fragmentation of DbpA provides a fingerprint of the free enzyme conformation.

A comparison of the proteolysis profiles of free DbpA with DbpA-ADP revealed a distinct change in the cleavage at Val^198 and Val^195 (designated as D3 and D4). More specifically, D3 has a different cleavage pattern in the presence of ADP (Fig. 2, lanes 5–7), whereas the D4 product is not formed upon the binding of ADP to DbpA. The latter might occur either as a result of induced structural conformational changes in the enzyme or by masking Val^195 by ADP. Nevertheless, in Fig. 2, lanes 5–7 show that in addition to the absence of the D4 fragment in the ADP-bound state, the intensity of the D2 fragment is more enhanced than in both the free and the ATPyS-bound states. These results suggest that the changes in the cleavage patterns of the D2, D3, and D4 fragments are due to structural rearrangements of the overall enzyme conformation, which originate from the interaction of ADP with the nucleotide-binding site. Such conformational changes do not occur upon the binding of ATPyS.

The Proteolysis Pattern of the Productive Ternary Complex of DbpA-ssRNA-ADP/ATPyS—The time-dependent limited proteolysis of DbpA bound to ssRNA and nucleotides by both the trypsin and chymotrypsin proteinases is shown in Fig. 3, A and B, respectively. The binding of ssRNA to the nucleotide-bound states of DbpA resulted in an enhancement in cleavage (over time) by trypsin (Fig. 3A) when compared with the free ssRNA complexes of DbpA (Fig. 2). This suggests that the enzyme polypeptide chains are more susceptible to the proteinases, presumably because of large conformational changes induced by the binding of ssRNA to DbpA. Such conformational changes are manifested by changes in the cleavage patterns of the D5 and D6 polypeptide fragments (Fig. 3A and Table I). Interestingly, these fragments were not observed in the absence of ssRNA. Similarly to the data presented in Fig. 2, distinct differences in the band intensities (Fig. 3A, lanes 6, 3, and 9) were detected between the complex of DbpA-ssRNA-ADP and the complexes of both DbpA-ssRNA and DbpA-ssRNA-ATPyS. The N-terminal residue of both the D5 and D6 polypeptides (with an apparent molecular mass of 15 and 10 kDa, respectively) was identified and mapped to Arg^116.

To better refine these structural differences, we conducted the same experiments in the presence of chymotrypsin. The cleavage pattern by chymotrypsin revealed 14 distinct polypeptide products in the free protein. Analysis of the proteolysis cleavage pattern of free DbpA with DbpA-ADP and DbpA-ATPyS did not reveal any appreciable changes in the conformational states of the enzyme (data not shown). Fig. 3B shows chymotrypsin proteolysis of the DbpA-ssRNA, DbpA-ssRNA-ADP, and DbpA-ssRNA-ATPyS complexes. Interestingly, changes in the rate of cleavage of DbpA-ssRNA-ATPyS when compared with the DbpA-ssRNA and DbpA-ssRNA-ADP complexes could be detected. This observation is based on the disappearance of the upper band in Fig. 3, lanes 8–10. Yet the overall cleavage patterns of DbpA-ssRNA and DbpA-ssRNA-ATPyS remain the same. The designated D7 band appears with the complex of DbpA-ssRNA-ADP and the complexes of both DbpA-ssRNA and DbpA-ssRNA-ATPyS. The N-terminal residue of both the D5 and D6 polypeptides (with an apparent molecular mass of 15 and 10 kDa, respectively) was identified and mapped to Gly^113.

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**Table I**

| Fragment Name | N-terminal Residue (first amino acid) | Apparent molecular mass (kDa) | Approximate C-terminal Residue |
|---------------|--------------------------------------|------------------------------|-------------------------------|
| D1            | Asp^117                              | 38                           | Lys^557                       |
| D2            | Met^1                                | 36                           | Ala^233                       |
| D3            | Val^198                              | 31                           | Lys^257                       |
| D4            | Val^195                              | 25                           | Gln^278                       |
| D5            | Asp^117                              | 15                           | Thr^250                       |
| D6            | Asp^117                              | 10                           | Gln^199                       |
| D7            | Ser^184                              | 32                           | Lys^257                       |
| D8            | Gly^113                              | 31                           | Met^295                       |

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**Fig. 2.** Pattern of trypsin cleavage of DbpA in the presence of ADP and ATPyS. Gradient (4–16%) SDS-PAGE analysis showing the pattern of trypsin digestion of DbpA as a function of time is shown. DbpA (4 μg) was digested with 400 nM of trypsin. The reaction mixture was preincubated for 10 min at 20 °C followed by time-dependent proteolysis. The time scale is in minutes as indicated above.
cases used in the alignment were required to form the basis for constructing the three-dimensional model of DbpA. The first 360 amino acids of DbpA exhibit a high degree of homology to several solved structures, which are also designated as the core domain of the helicase superfamily (see “Experimental Procedures”). The remaining 100 amino acids, termed the C-terminal extension of DbpA, have no homology to any known structure in the PDB. However, the predicted secondary structure of this domain includes a long loop terminated by a β-sheet-a-helix motif.

Two independently folding units, termed the N and C domains, comprise a core region of the crystal structures of helicases that resemble DbpA by their sequence homology. A distinct linker that governs their orientation in each of the structures connects these two domains. To construct the three-dimensional model of DbpA, we performed homology modeling of the separate domains. The N domain of the core region was built from three homologous regions obtained from MjDEAD (PDB: 1HV8) and eIF4A (PDB: 1FUU, 1QVA). The overall average root mean square deviation for all the structures was 0.71. The basis of the model of DbpA consisted of the highest sequence homology segments identified in each of the known crystal structures. Loops and surfaces that did not match by sequence homology or length were constructed by using the homology module (Insight II® software). The model of the N domain of the DbpA core region was refined by an energy minimization procedure by using the discovery module (Insight II® software, see “Experimental Procedures”). The C domain of the DbpA core region was built using the same procedure described above. Specifically, we used the structures of MjDEAD-C (PDB: 1HV8), eIF4A-C (PDB: 1FUU), and UvrB (PDB: 1D9Z). The average root mean square deviation among these structures is 2.03. Constructing the overall three-dimensional model required determining the correct orientation of the N and the C domains of the core region. Therefore, each of the constructed domains of DbpA was superimposed on the structure of MjDEAD. The missing linker region in the model of DbpA was constructed from the analogous segment in MjDEAD followed by an energy minimization procedure (see “Experimental Procedures”).

Fig. 4A depicts a ribbon diagram of the three-dimensional model of the core region of DbpA (amino acid residues 1–360). The N domain of the DbpA core region consisted of an α/β structure with a seven-strand parallel β-sheet sandwich between the α helices. The C domain of the core region consists of a six-strand parallel β-sheet sandwich between the α helices. The two domains create a deep cleft connected by a linker. Similar to other helicase structures, the conserved motifs in DbpA are positioned within the cleft (29). The designated D1, -3, -5, and -6 cleavage sites were detected by trypsin digestion experiments, independently of the presence of cofactors or ssRNA. Consistent with our proteolysis studies, these sites were located on the exposed surface of the DbpA model and thus were susceptible to cleavage. Moreover, these results demonstrated the high correlation between the observed cleavage patterns and the constructed model of DbpA. In contrast to the observed D1, -3, -5, -6, and -8 fragments, the D4 and in part, the D7 cleavage sites are sensitive to their interaction with ATP·S and ssRNA, respectively. This suggests that these motifs undergo conformational changes during the interaction of DbpA with cofactors and ssRNA. These sites reside within the Walker A and the SAT motifs (Fig. 4B). Both the Walker A and the SAT motifs are located in two loops in our model. Interestingly, these loops are highly conserved through-

![Fig. 3. Pattern of trypsin and chymotrypsin cleavages of the DbpA-ssRNA complex in the presence of ADP and ATP·S as a function of time. DbpA-ssRNA in a 1:1 ratio and DbpA-ssRNA-ADP/ATP·S·S in 1:1:25:1.25 ratios were incubated and treated as indicated in Fig. 2A, trypsin digestion pattern. Fragment D4 is formed in the complexes of DbpA-ssRNA and DbpA-ssRNA-ATP·S and is absent in DbpA-ssRNA-ADP. The D5–6 fragments appear to be more exposed to cleavage in the DbpA-ssRNA-ADP complex, and the N-terminal amino acid of the D5–6 fragments was shown to be identical to the D3 fragment. B, chymotrypsin digestion pattern. The D7 fragment appeared in all complexes, but fragment D8 appears only in the DbpA-ssRNA complex and, to a lesser extent, in the DbpA-ssRNA-ADP complexes.](http://www.jbc.org/)

Importantly, Ser184 is located at the SAT motif (Fig. 1), which was shown to couple ATP hydrolysis to the unwinding activity in eIF-4A (27). Therefore, the D7 fragment can be used as an intrinsic marker to detect enzymatic conformational changes taking place at or near the SAT motif. Fig. 3B shows that the binding of ssRNA to DbpA results in the exposure of the SAT motif. Similar to the trypsin digestion studies (Figs. 2 and 3A), the interaction of DbpA with ssRNA and cofactors detects two main conformational states. This structural rearrangement originates at the core domain of the enzyme. To further analyze the conformational changes in DbpA observed by these proteolysis studies, we constructed the three-dimensional structure of DbpA by homology modeling.

**Multiple Sequence Alignment of the DbpA Core Region and Three-dimensional Homology Modeling Studies**—To structurally assign the observed conformational changes in DbpA upon substrate binding, we compared its sequence with both DNA and RNA helicases and constructed its three-dimensional homology model. Individual members of the DEAD box RNA helicase family display high sequence homology (8, 12, 28). Specifically, a pronounced similarity was detected in the various conserved motifs associated with the ATPase and helicase activities. The spacing and the linear arrangement of the conserved motifs within the core region show that these enzymes might carry out their unwinding activity by similar molecular mechanisms (4). Fig. 1 demonstrates the high homology of DbpA when compared with other members of the helicase superfamilies I and II. We performed multiple sequence alignment of several DNA and RNA helicases with the crystal structures available in the PDB. The various DNA and RNA helicases used in the alignment were required to form the basis for constructing the three-dimensional model of DbpA. The first 360 amino acids of DbpA exhibit a high degree of homology to several solved structures, which are also designated as the core domain of the helicase superfamily (see “Experimental Procedures”). The remaining 100 amino acids, termed the C-terminal extension of DbpA, have no homology to any known structure in the PDB. However, the predicted secondary structure of this domain includes a long loop terminated by a β-sheet-a-helix motif.
out the helicase superfamily, suggesting that they possess a similar structure and function during unwinding catalysis.

**Fluorescence Spectroscopy Studies**—The proposed conformational changes of DbpA, induced by its binding to cofactors and ssRNA, were examined by intrinsic protein fluorescence spectroscopy. Briefly, we measured the emission spectra of the protein in various complexes after excitation at 280 and 295 nm (Fig. 5). Emission after excitation at 280 nm reflects the contribution of Trp and the energy transfer from Tyr to Trp, but the emission after excitation at 295 nm reflects only changes in the contribution of the Trp residues. Analysis of the DbpA primary amino acid sequence shows seven relevant aromatic residues including Tyr23, Tyr221, Tyr426, Trp187, Trp320, Trp366, and Trp 438. The three-dimensional structural model of the DbpA core region can be used to approximate the relative positions of the aromatic residues. The model of DbpA shows that Tyr23 is located in the catalytic cleft of the core domain and that Trp 187 resides on the loop of the SAT motif.

Fig. 5A shows the effect on the emission spectra of the protein (excited at 280 nm) of ATPγS binding to both DbpA and DbpA-ssRNA. The binding of ATPγS resulted in no appreciable change in the spectrum of free DbpA. In contrast, the binding of ssRNA to DbpA resulted in a large increase in the fluorescence of the protein. Remarkably, the binding of ATPγS to the DbpA-ssRNA complex did not affect the fluorescence spectra of the complex. This is consistent with our limited proteolysis digestion studies indicating that the binding of ATPγS to free

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**Fig. 4.** Three-dimensional structure modeling of the DbpA core region (1–360 amino acid). A, oriented according to the loop position as in the structure of MjDEAD. Structural homology modeling of the DbpA core region was constructed from three structures: MjDEAD (PDB: 1HV8), cIF4A (PDB: 1FUU), and some small stretches taken from the structure of UvrB (PDB: 1D9Z). The color ribbons denote the conserved motifs of the DEAD/H box RNA helicase superfamily II found in DbpA, as follows: yellow, motif I (Walker A); cherry, motif II (Walker B); pink, motif III (SAT); red, motif IV; lime, motif V; olive green, motif VI. B, mapping the N terminus positions of the fragments identified by time-dependent proteolysis.

**Fig. 5.** Fluorescence emission spectra of the DbpA, DbpA-ssRNA, and DbpA-ssRNA-ATPγS/ADP complexes. Reaction mixtures of 400 μl with 1:1 protein to the ratio of the substrate were incubated at 20 °C for 5 min. A, binding of ATPγS to the free enzyme or to the DbpA-ssRNA complex. Intrinsic protein fluorescence was measured after excitation at 280 nm. AU, arbitrary units. B, binding of ADP to the free enzyme or to the DbpA-ssRNA complex. Intrinsic protein fluorescence was measured after excitation at 280 nm. C, binding of ADP to the free enzyme or to the DbpA-ssRNA complex. Intrinsic protein fluorescence was measured after excitation at 295 nm. Collectively, the intrinsic fluorescence of DbpA suggests two main conformational states of the enzyme upon its interaction with its substrates. The interaction of DbpA with ATPγS did not show significant changes in the fluorescence signals when compared with the free enzyme.
DbpA or DbpA-ssRNA does not change the original cleavage pattern.

The effect of ADP binding to DbpA and DbpA-ssRNA is shown in Fig. 5, B and C. The binding of ADP to both states of the enzyme resulted in a marked change in protein fluorescence. Together with the reported limited proteolysis digestion (Figs. 2 and 3), these results further support that the binding of ADP induces distinct structural conformational changes in DbpA. To determine whether the fluorescence spectrum results from Tyr or Trp residues, we measured the emission spectra after excitation at 295 nm. Fig. 5C shows that the binding of ssRNA reduces the contribution of Trp fluorescence, while exposing tyrosine residues (Fig. 5B). This may result from the binding of the ssRNA to the C-terminal extension of DbpA, which contains the Trp366 and Trp438 residues. Interestingly, the binding of ADP affects both Trp and Tyr fluorescence. At this stage, it is difficult to assign this spectral contribution to specific residues in the core region of DbpA.

**DISCUSSION**

It is now recognized that structural flexibility in motor proteins plays an important role in performing their action (10, 29, 30). However, the relationship between this dynamic structural ability and the reaction mechanism remains to be elucidated. In helicases, the unwinding of double-stranded nucleic acids is correlated with the binding and hydrolysis of ATP (31). Structural and biochemical studies of the DNA helicase PcrA (32) and Rep (33) have advanced our basic understanding of the mechanistic aspects of DNA unwinding catalysis. Yet for DEAD/H box RNA helicases, the “molecular motor” mechanisms that drive the helicase activities have not been fully characterized (29). This work focused on studying the conformational protein dynamics as well as the structure-function relationships by which the unique DEAD box RNA helicase DbpA interacts with ssRNA and cofactors.

Using time-dependent limited proteolysis digestion studies, we probed amino acid residues, and thus, specific domain regions that undergo pronounced conformational changes that are induced by the binding of ssRNA and cofactors to the enzyme. We have shown that the binding of ssRNA induces large conformational changes, which result in the enhancement of protein proteolysis and fluorescence (Figs. 3 and 5). In addition, we have demonstrated that the binding of ADP to the DbpA or DbpA-ssRNA complex induces conformational changes originating at the catalytic site of DbpA (Walker A and SAT motifs) as well as in more distal domains (fragments D3, D5, D6, and D8). Interestingly, the binding of ATPγS maintains the original conformation of these complexes. This suggests that the rebinding of ATP closes a conformational cycle during catalysis. The binding of ADP to form a distinct conformational state is further supported by the reported fluorescence spectroscopy studies (Fig. 5, B and C). Importantly, our modeling studies suggest that a 5-residue linker (designated as a hinge region) located between the two main domains of the enzyme core may support such dynamic conformational changes (Fig. 4A).

Similar proteolysis studies on the DEAD box RNA helicase eIF4A show that, unlike in DbpA, the binding of both ADP and AMPPNP induces different structural conformational states in the enzyme (25, 34). Although high structural homology exists between eIF4A and DbpA in their core domains, apparently the molecular mechanism by which these enzymes carry out their catalysis is different. This may be related to the relatively long linker (11 residues) present between the two domains of the core region of eIF4A versus the short linker of DbpA (5 residue) (35). This may also be correlated with the rates of ATP hydrolysis observed for these enzymes, 600 min⁻¹ for DbpA versus 3 min⁻¹ for eIF4A (34).

Based on our proteolysis and modeling studies, the masking of Walker A and the enhancement cleavage of the SAT motifs (Fig. 3, D4 and D7 fragments, respectively), observed upon the binding of ssRNA or ADP, require considerable movement of one of the core domains with respect to the other. Such rotational movement can be mediated by the linker, which serves as a hinge region (Ser309 to Leu313) located between the two domains of the core region. Evidence for this mechanism may be supported by the significant changes in protein fluorescence observed upon the binding of DbpA to ssRNA and to ADP. The involvement of the hinge region in the unwinding reaction mechanism was proposed by Korolev et al. (33) for the E. coli
DNA helicase Rep. Specifically, a large rotation corresponding to a swiveling of 130° around a hinge region takes place between two domains, namely 2A and 2B of Rep, which was observed by examining the crystal structures of the Rep-ssDNA and Rep-ssDNA-ADP complexes. In contrast, such a domain-swiveling mechanism was not supported by the crystal structures of PcrA DNA helicase with DNA and with the ATP analog ADPPNP complexes (32).

To test whether the hinge region in DbpA can adopt different conformations required for the proposed domain rotation mechanism, we superimposed the C domain of the core regions in UvrB (36) and DbpA. This procedure revealed that the N domains of UvrB and DbpA could not be superimposed simultaneously because their hinge regions occupy different positions in space. Superimposition of the N domains of the core region of both enzymes, although anchoring their C domains, resulted in a pronounced rotation of the hinge region of DbpA (Fig. 6A). This model supports the observed conformational dynamics required for both ATPase and unwinding activities. In this respect, the hinge region located at the bottom of the cleft of the core region plays a central role in executing the helicase reaction.

On the basis of our results, we propose a general schematic model for DbpA motor activities. Fig. 6B describes two major catalytic states of DbpA during unwinding catalysis. Specifically, the “open complex” represents an open conformational state that results from the interaction of DbpA with ssRNA and ATP. In contrast, the “closed complex” represents a rather closed conformational state evolving by the release of the yP product. These dynamic protein conformations formulate the tracking movement of one domain with respect to the other during the unwinding catalysis. Note that this dynamic motor activity is associated with the core region of DbpA and that the C-terminal extension may be used to target this enzyme to its specific substrates in vivo as proposed previously (19, 21).

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