Global Conformational Transitions in Escherichia coli Primary Replicative Helicase DnaB Protein Induced by ATP, ADP, and Single-stranded DNA Binding

M aria J. J ezewska and W łodzimierz B ujalowski

From the Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1053

(Received for publication, November 30, 1995, and in revised form, December 21, 1995)

The direct evidence of dramatic conformational changes of the DnaB hexamer, induced by nucleotide binding, and the presence of multiple conformational states of the enzyme have been obtained by using analytical sedimentation equilibrium, sedimentation velocity studies, and the rigorous fluorescence titration technique. Equilibrium sedimentation measurements show that in the presence of the ATP nonhydrolyzable analog, AMP-PNP, the DnaB helicase fully preserves its hexameric structure. However, in the presence of the saturating concentration of AMP-PNP, the sedimentation coefficient of the hexamer is 11.9 ± 0.2 compared to the sedimentation coefficient 10.5 ± 0.2 of the free DnaB helicase hexamer. This large sedimentation coefficient change indicates dramatic global conformational transitions of the hexamer, encompassing all six subunits, upon binding the ATP analog. In the presence of ADP, the DnaB hexamer sedimentation coefficient is 11.4 ± 0.2, indicating that the conformation of the ADP form of the hexamer is different from the ATP form. The sedimentation coefficient of the ternary complex DnaB-(AMP-PNP)-dATP is 12.4, suggesting that the DnaB helicase undergoes further conformational changes upon binding single-stranded DNA (ssDNA). The large global structural changes correlate with the functional activities of the enzyme. In the absence of the ATP analog, the hexamer exists in a "closed" conformation which has extremely low affinity toward ssDNA. Upon binding the ATP analog, the DnaB hexamer transforms into a "tense" state which binds ssDNA with an affinity of ~4 orders of magnitude higher than in the absence of the nucleotide. In the presence of ADP, the DnaB hexamer assumes a "relaxed" conformation. The functional difference between these two conformational states is reflected in the much weaker allosteric effect of ADP on the ssDNA binding with the affinity constant ~3 orders of magnitude weaker than in the presence of the ATP analog (tense state).

DnaB is the essential DNA replication protein in Escherichia coli, originally identified based on its requirement for in vitro X174 phage replication (Wickner et al., 1973; McMacken et al., 1977). DnaB protein is the primary E. coli replicative helicase, i.e. the factor responsible for unwinding the DNA duplex in front of the replication fork. It is the only helicase required to reconstitute DNA replication in vitro from the chromosomal origin of replication (oriC) (Kornberg and Baker, 1992; LeBowitz and McMacken, 1986; Baker et al., 1987). The enzyme is involved in both the initiation and elongation stages of DNA replication (Matson and Kaiser-Rogers, 1990; Kornberg and Baker, 1992; Marians, 1992).

The native DnaB helicase forms a hexamer composed of six identical subunits (Reha-Krantz and Hurwitz, 1978; Bujalowski et al., 1994). Analytical sedimentation studies have shown that the DnaB helicase exists as a stable hexamer over a large protein concentration range with magnesium ions playing a crucial structural role in stabilizing the hexameric structure of the helicase. Hydrodynamic data indicate that six protomers aggregate with cyclic symmetry in which the protomer-protomer contacts are limited to only two neighboring subunits (Bujalowski et al., 1994).

Physiological functions of the DnaB helicase are related to the ability of the protein to interact with ss- and dsDNA under the control of ATP binding and hydrolysis (Arai and Kornberg, 1981a, 1981b; LeBowitz and McMacken, 1986). Studies of nucleotide binding to the DnaB helicase have established that the hexamer has six nucleotide binding sites, presumably one on each protomer (Arai and Kornberg, 1981b; Bujalowski and Klonowska, 1993, 1994a, 1994b). The binding process is biphasic, resulting from the negative cooperative interactions limited to neighboring subunits (Bujalowski and Klonowska, 1993, 1994a, 1994b).

Interactions of the DnaB helicase with ssDNA and the structure of the formed complexes have only recently been quantitatively studied (Bujalowski and J ezewska, 1995). On the basis of thermodynamically rigorous fluorescence titrations, we have established that in the presence of the ATP nonhydrolyzable analog, AMP-PNP, stoichiometry of the DnaB hexamer complex with the polymer ssDNA (site size) is 20 ± 3 nucleotides. Binding studies performed with ssDNA oligomers have shown that the hexamer has only a single, strong ssDNA binding site. Moreover, photo-cross-linking experiments indicate that only a limited set of subunits, most probably only one, is engaged in the complex with the nucleic acid. These results indicate that long-range allosteric interactions occur on the level of the quaternary structure of the hexameric enzyme, leading to the
Multiple Conformations of Replicative Helicase

Experimental Procedures

DnaB Protein and Nucleic Acids—The E. coli DnaB protein was purified, as described previously (Bujalowski and Klouowska, 1993; 1994). Poly(dA) and dA(pA)19 were purchased from Midland Certified Reagents (Midland, TX) and converted into their fluorescent derivative (etheno-A) by modification with chloroacetaldehyde (Bujalowski et al., 1994). Buffer T2 is 50 mM Tris adjusted to pH 8.1 with HCl, 5 mM MgCl2, and 10% glycerol.

Fluorescence Measurements—All steady-state fluorescence measurements were performed using the SLM 48000S spectrofluorometer, as described previously (Bujalowski and Klouowska, 1993, 1994a, 1994b; Bujalowski and J. eekewska, 1995).

Sedimentation Velocity Measurements—Equilibrium sedimentation and sedimentation velocity experiments were performed using a Spinco Model E analytical centrifuge, and the analysis of the sedimentation runs was performed, as we described previously (Bujalowski et al., 1994). The total concentration at radial position r is defined by Equation 1 (Cantor and Schimmel, 1980)

\[ c = c_0 \exp\left[1 - \left(1 - \frac{r}{r_c}\right)^2\right] \]  

where \( c_0 \), \( r_c \), and \( M \) are the concentration (absorption) at the bottom of the cell, partial specific volume, and molecular weight of the protein, respectively, \( r \) is the density of the solution, \( \omega \) is the angular velocity, and \( b \) is the base line error term. Equilibrium sedimentation profiles were fitted to Equation 1 with \( M \) and \( b \) as fitting parameters. The reported values of the sedimentation coefficients were corrected to the standard conditions, \( s_{20,w} \), for solvent density and viscosity (Cantor and Schimmel, 1980).

Analysis of the DnaB Helix-Polymer ssDNA Binding Isotherm—McGhee and von Hippel (1974) derived two explicit equations for non-cooperative and cooperative binding of a large ligand to a one-dimensional, homogenous lattice, with overlapping potential binding sites. Previously, we obtained a single generalized equation for the McGhee-von Hippel model which can be applied to both cooperative and noncooperative binding (Bujalowski et al., 1989). The Scatchard form of the generalized equation is described by

\[ R = K(1 - n)\left[\frac{[2\omega(1 - n)]}{[2\omega - 1 - (1 - n) + \frac{n}{\omega} + R]}\right]^{(n-1)} \left[\frac{1 - (1 + \frac{n}{\omega} + R)\left(1 - n\right)}{2(1 - n^2)}\right] \]  

where \( K \) is the intrinsic binding constant, \( n \) is the number of nucleotides covered by the protein in the complex (site size), \( \omega \) is the parameter characterizing cooperativity, and \( R = (1 - (1 + \frac{n}{\omega} + R)\left(1 - n\right))\frac{n}{\omega} \).

Analytical Sedimentation Studies of the DnaB Helicase—Experimental-ultracentrifuge experiments provide direct information about the global conformational properties of a protein which are reflected in the hydrodynamic properties of the macromolecule (Cantor and Schimmel, 1980). Sedimentation velocity profiles of the DnaB helicase in buffer T2 (pH 8.1, 20 °C) containing 50 mM NaCl and 5 \times 10^{-2} M AMP-PNP (monitored at 292 nm) are shown in Fig. 2a. At this concentration of the ATP analog, all six ATP binding sites of the DnaB hexamer are saturated with the nucleotide (Bujalowski and Klouowska, 1993, 1994a, 1994b). There is a single, well-defined moving boundary throughout the sedimentation process which has a sedimentation coefficient of \( s_{20,w} = 119 \pm 0.7 \).

It should be pointed out that in our studies the error in the calculation of the sedimentation coefficients, each for a particular system, is a standard deviation determined from 8 to 12 independent sedimentation velocity experiments using two different protein concentrations. The obtained value of \( s_{20,w} \) of the DnaB-(AMP-PNP) complex is ~14% higher than the sedimentation coefficient of \( s_{20,w} = 105 \pm 0.2 \) of the free DnaB hexamer (Bujalowski et al., 1994). The magnitude of this increase of the sedimentation coefficient can be realized by recalling that a simple dimerization of two hexamers into a dodecamer would not.
lead to an ~58% increase of its sedimentation coefficient (Cantor and Schimmel, 1980). To exclude the possibility that the nucleotide binding affects the oligomeric state of the DnaB hexamer, we performed sedimentation equilibrium measurements of the DnaB helicase in the presence of saturating concentrations of AMP-PNP. A set of DnaB protein concentration profiles (recorded at two different wavelengths, 285 and 292 nm) as a function of the square of the radius at sedimentation equilibrium, in buffer T2 (pH 8.1, 10°C) containing 50 mM NaCl and 5 × 10^{-4} M AMP-PNP. The concentration of the DnaB protein is 3 × 10^{-9} M (hexamer); 8-min intervals, 30,000 rpm. b, sedimentation equilibrium absorption profiles of the DnaB helicase at two different wavelengths, 285 nm (□) and 292 nm (○), in buffer T2 (pH 8.1, 10°C) containing 50 mM NaCl and in the presence of 5 × 10^{-4} M AMP-PNP. Solid lines are nonlinear least-squares fits to Equation 1, with a single sedimenting species having molecular weights of 303,000 (□) and 301,000 (○), respectively. The DnaB protein concentration is 5.59 × 10^{-9} M (hexamer); 9,000 rpm.

It is very interesting to determine how the binding of the ssDNA affects the conformational transitions of the DnaB hexamer induced by nucleotide binding. Because the etheno-derivatives of the nucleic acids have significant absorption above 310 nm, where there is practically no contribution of the protein spectrum, this allows us to monitor only one component, the nucleic acid, during the sedimentation process. Sedimentation velocity profiles of the mixture of the DnaB helicase and deA(peA)_{19} in the 1.25:1 molar excess of the enzyme over the nucleic acid are shown in Fig. 3. Recall, the 20-mer exactly spans the size of the DnaB helicase-ssDNA complex (Bujalowski and Jezewska, 1995). Because the [DnaB] and [deA(peA)_{19}] are >1/K_{D}, all the nucleic acid should be complexed with the helicase (Bujalowski and J. Jezewska, 1995). As a result, only a single, well-defined boundary of the complex is observed throughout the entire sedimentation process and has a sedimentation coefficient of s_{20,w} = 12.4 ± 0.2. This value is ~17% higher than the sedimentation coefficient of the free DnaB hexamer and ~3 and ~9% higher than the sedimentation coefficient of the DnaB helicase, when saturated with the ATP analog or ADP. This suggests that the binding of ssDNA, most probably, introduces additional conformational changes in the ternary complex, DnaB-(AMP-PNP)_{e}A(peA)_{19} (see “Discussion”).

**DISCUSSION**

Binding of the ATP Analog, AMP-PNP, and ADP Induces Major Global Conformational Changes in the DnaB Protein Hexamer—Helicases are the essential enzymes which catalyze the vectorial unwinding of the dsDNA to provide a single-stranded intermediate in the reaction which is coupled to ATP hydrolysis (Hill and Tsuchiya, 1981; Lohman, 1993). The plausible model of this process, supported by the binding studies of
nucleic acids in the presence of nucleotide cofactors, is that the enzyme cycles in a vectorial fashion through a number of conformational states controlled by ATP binding and hydrolysis in which its affinity for ss- and dsDNA dramatically changes (Wong and Lohman, 1993). Yet, evidence of the physical existence of different conformations or intramolecular transitions within the oligomeric structure of the hexameric enzyme, induced by nucleotide binding, so far has not been obtained.

Hydrodynamic properties, including the sedimentation coefficient, are directly sensitive to the global conformational properties of the macromolecules (Cantor and Schimmel, 1980). Using analytical sedimentation velocity experiments and the rigorous fluorescence titration technique, we present direct evidence that the E. coli primary replicative helicase DnaB protein undergoes dramatic conformational transitions induced by binding of the ATP nonhydrolyzable analog, AMP-PNP, and ADP. The sedimentation coefficient of the free DnaB hexamer increases from $s_{20,w} = 10.5$ to $s_{20,w} = 11.9$ for the hexamer in the presence of $5 \times 10^{-6}$M AMP-PNP and to $s_{20,w} = 11.4$ in the presence of $5 \times 10^{-4}$M ADP. At these concentrations of AMP-PNP and ADP, all six binding sites are saturated with the nucleotide (Bujalowski and Klönowska, 1993).

The very large increase of the sedimentation coefficient does not result from the dimerization of the DnaB hexamer induced by nucleotide binding. Equilibrium sedimentation measurements show that, in the presence of the saturating concentration of AMP-PNP, the hexameric structure of the enzyme is fully preserved (Fig. 2b). In the absence of oligomerization, the sedimentation coefficient of the protein can change as a result of changes of its partial specific volume or frictional coefficient, or both. Partial specific volume of the ATP molecule is $\nu = 0.44$ ml/g compared with $\nu = 0.732$ ml/g of the DnaB protein (Howlett and Schachman, 1977; Bujalowski et al., 1994). However, the six bound nucleotide molecules constitute only $\sim 1\%$ of the molecular weight of the hexamer and the trivial effect of a lower $\nu$ of the bound nucleotides on the measured $s_{20,w}$ of the hexamer would amount to $\sim 0.004$; thus, it is negligible. Moreover, the additional data suggest that the global conformational changes are predominantly induced by the binding of only the first three nucleotide molecules to the three high affinity binding sites of the hexamer. Therefore, regardless of the nature of the observed conformational transitions, the large increase of the sedimentation coefficient indicates large global structural changes of the DnaB hexamer, which are induced by ATP analog binding, and these structural transitions lead to intrinsic changes of the partial specific volume of the hexamer and/or its frictional coefficient. Although the exact nature of the conformational transition induced by nucleotide binding is still unknown, it should be mentioned that dynamic light scattering data indicate that the diffusion coefficient of the hexamer is increased in the presence of the saturating concentration of AMP-PNP, indicating that the frictional coefficient of the hexamer is decreased, in agreement with the sedimentation data reported here.

Binding of ADP to the DnaB helicase also induces global conformational changes in the protein hexamer. The sedimentation coefficient, $s_{20,w} = 11.4$, is increased by $\sim 9\%$ when compared to the free DnaB hexamer; however, this value is smaller than the 11.9 obtained in the presence of AMP-PNP. The difference is larger than the error of the determination of $s_{20,w}$ (±0.2) estimated using multiple scans at different protein concentrations (see above). Thus, the sedimentation data suggest that the conformation of the hexamer-ADP complex differs from that of the free DnaB protein and is also different from the DnaB helicase-(AMP-PNP) complex. As a result, the affinity of the hexamer toward ssDNA drops by $\sim 3$ orders of magnitude when compared to the affinity of the DnaB-(AMP-PNP) complex (see above). The difference between the effect of AMP-PNP and ADP does not result from the weaker binding of ADP to the helicase. As we determined previously, using a series of fluorescent nucleotide analogs, the ADP and AMP-PNP analogs bind with higher affinity to the DnaB helicase than the ATP analogs (Bujalowski and Klönowska, 1993, 1994a, 1994b). Thus, the allosteric effect of AMP-PNP on the DnaB global conformation, which leads to the dramatically increased affinity of the helicase to ssDNA as opposed to the dramatic drop in the affinity in the presence of ADP, must result from the specific interactions of $\gamma$-phosphate in the nucleotide binding site.

The sedimentation coefficient of the DnaB-(AMP-PNP)- ssDNA ternary complex, $s_{20,w} = 12.4 \pm 0.2$, indicates that conformational changes induced by AMP-PNP binding are preserved in the ternary complex. It should be noted that the increase of the sedimentation coefficient of the ternary complex exceeds the value expected from the combined trivial effects of the different partial specific volume of the nucleic acid and the higher molecular weight of the DnaB hexamer-20-mer complex. The bound 20-mer constitutes only an additional $\sim 2\%$ of the molecular weight of the complex. The trivial effect of the lower partial specific volume of the nucleic acid molecule ($\nu = 0.531$ ml/g) (Pearce et al., 1975) and the increased molecular weight of the complex would contribute $\sim 0.008$ and $\sim 0.2$ to the measured sedimentation coefficient, respectively (Cantor and Schimmel, 1980). Moreover, binding of the shorter 10-mer ssDNA fragment (data not shown) causes the same increase of the sedimentation coefficient of the hexamer ($s_{20,w} = 12.5 \pm 0.2$) as the 20-mer, although it has 10 less nucleotide residues than the 20-mer and constitutes only $\sim 1\%$ of the molecular weight of the hexamer. If the trivial effects were mainly responsible for the observed increase of $s_{20,w}$ of the ternary complex, then the effect of the 10-mer should be half of the one observed for the 20-mer, but this is not what is experimentally observed. Thus, the increased value of $s_{20,w}$ of the ternary complex suggests that, most probably, the DnaB helicase undergoes further conformational changes upon binding ssDNA, although the obtained hydrodynamic data indicate that the major conformational transition of the helicase is induced by AMP-PNP or ADP binding.

Multiple Conformational States of the DnaB Hexamer—Hydrodynamic studies reported in this work provide the first direct evidence of the dramatic global conformational changes of the DnaB helicase hexamer, induced by nucleotide binding, and the existence of the multiple, structural states of the enzyme. Moreover, the different conformations correlate well with the functional properties of the enzyme, i.e. its interactions with DNA, as determined by thermodynamic studies. In the absence of the nucleotide cofactors, the DnaB hexamer, built of six chemically identical subunits, has a very low affinity toward ssDNA. In this “closed” state, all subunits of the hexamer are equivalent and capable of initiating binding of ATP and ADP (see above, Bujalowski and Klönowska (1993)). Upon binding the ATP analog, the global rearrangement of the protomers within the hexamer is induced, resulting in a “tense” state with a high affinity for ssDNA. The transition leads to the selection of only a limited set of subunits, most probably only one, as a binding site for ssDNA (Bujalowski and Jezewska, 1995). The large increase of the sedimentation coefficient suggests a more compact structure of the hexamer in a tense state. Although ADP also induces similar large global changes in the structure of the DnaB hexamer, this state of the enzyme is different from the one induced by ATP. A smaller value of $s_{20,w}$

---

2 M. J. Jezewska and W. Bujalowski, manuscript in preparation.
suggests a less compact structure of the hexamer in this “relaxed” state as compared to the tense state.

The mechanism of the dsDNA unwinding by the replicative helicase and the mechanism of the enzyme translocation on the nucleic acid lattice is still unknown. In general, after breaking hydrogen bonds between the base pairs of the duplex DNA in the replication fork, the enzyme must be released from the formed ss nucleic acid and move, in an unidirectional translocation event, toward dsDNA (Hill and Tsuchiya, 1981). Our recent results show the existence of only a single, strong binding site and a very low stoichiometry of the DnaB-ssDNA complex. These results are not compatible with the models of hexameric helicase translocation along the nucleic acid lattice in which all six protomers and/or multiple binding sites are involved in ssDNA binding (Bujalowski and Jezewska, 1995).

The existence of different conformational states of the hexameric helicase described in this work strongly suggests that the mechanism of translocation and nucleic acid unwinding might rely on global, not local, conformational changes in the hexamer which are induced by the ATP/ADP switch and/or nucleic acid binding. Such conformational transitions were postulated as necessary elements of the helicase mechanism by Hill and Tsuchiya (1981). Thus, because the transition from the tense to the relaxed state of the helicase must accompany each ATP hydrolysis step, when the ATP/ADP switch takes place in the nucleotide binding site, this transition is most probably responsible for the partial release of the ssDNA from the nucleic acid binding site (Arai and Kornberg, 1981b). After the release of ADP, the global rearrangement of the protomers within the entire DnaB hexamer, from a closed conformation to a tense one upon rebinding ATP, would allow the enzyme to translocate and encompass the subsequent fragment of dsDNA, within the active site of the helicase.

Acknowledgments—We thank Dr. James C. Lee for use of the Model E analytical centrifuge and comments on the manuscript, Drs. Edmund W. Czerwinski and T. M. Lohman for reading and comments on the manuscript, and Gloria Drennan Davis for her help.

REFERENCES
Arai, K., and Kornberg, A. (1981a) J. Biol. Chem. 256, 5253–5259
Arai, K., and Kornberg, A. (1981b) J. Biol. Chem. 256, 5260–5266
Baker, T. A., Funnell, B. E., and Kornberg, A. (1987) J. Biol. Chem. 262, 6877–6885
Bujalowski, W., and Klonowska, M. M. (1993) Biochemistry 32, 5888–5900
Bujalowski, W., and Klonowska, M. M. (1994a) Biochemistry 33, 4682–4694
Bujalowski, W., and Klonowska, M. M. (1994b) J. Biol. Chem. 269, 31393–31371
Bujalowski, W., and Jezewska, M. J. (1995) Biochemistry 34, 8513–8519
Bujalowski, W., Lohman, T. M., and Anderson, C. F. (1989) Biopolymers 28, 1637–1643
Bujalowski, W., Klonowska, M. M., and Jezewska, M. J. (1994) J. Biol. Chem. 269, 31350–31358
Cantor, C. R., and Schimmel, P. R. (1980) Biophysical Chemistry, Part II, pp. 591–641, Freeman, New York
Hill, T. L., and Tsuchiya, T. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4796–4800
Howlett, G. J., and Schachman, H. K. (1977) Biochemistry 16, 5077–5083
Kornberg, A., and Baker, T. A. (1992) DNA Replication, pp. 355–378, Freeman, San Francisco
LeBowitz, J. H., and McMacken, R. (1990) J. Biol. Chem. 265, 4738–4748
Marians, K. J. (1992) Annu. Rev. Biochem. 61, 673–719
Matson, S. W., and Kaiser-Rogers, K. A. (1990) Annu. Rev. Biochem. 59, 289–329
McGhee, J. D., and von Hippel, P. H. (1974) J. Mol. Biol. 86, 469–489
McMacken, R., Ueda, K., and Kornberg, A. (1977) J. Biol. Chem. 253, 3313–3319
Pearce T. C., Rowe, A. J., and Turnock, G. (1975) J. Mol. Biol. 105, 383–407
Reha-Krantz, L. J., and Hurwitz, J. (1978) J. Biol. Chem. 253, 4051–4057
Wickner, S., Wright, M., and Hurwitz, J. (1973) Proc. Natl. Acad. Sci. U. S. A. 71, 783–787
Wong, I., and Lohman, T. M. (1993) Science 256, 350–355
Global Conformational Transitions in *Escherichia coli* Primary Replicative Helicase DnaB Protein Induced by ATP, ADP, and Single-stranded DNA Binding: MULTIPLE CONFORMATIONAL STATES OF THE HELICASE HEXAMER

Maria J. Jezewska and Wlodzimierz Bujalowski

*J. Biol. Chem.* 1996, 271:4261-4265.
doi: 10.1074/jbc.271.8.4261

Access the most updated version of this article at [http://www.jbc.org/content/271/8/4261](http://www.jbc.org/content/271/8/4261)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 21 references, 9 of which can be accessed free at [http://www.jbc.org/content/271/8/4261.full.html#ref-list-1](http://www.jbc.org/content/271/8/4261.full.html#ref-list-1)