The Helicase from Hepatitis C Virus Is Active as an Oligomer*

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Helicases catalyze the unwinding of dsDNA1 and RNA during such cellular processes as replication, recombination, transcription, splicing, and translation (1, 2). A large number of helicases have been identified at the present time, and, based on their amino acid sequence, they have been divided into several superfamilies (3). Helicases are also classified as DNA or RNA helicases according to their ability to unwind dsDNA, RNA, or mixed duplexes. The RNA helicases cannot be distinguished from the DNA helicases simply from their amino acid sequence. This is because RNA and DNA helicases share conserved amino acid sequence motifs and can belong to the same superfamily. The RNA and DNA helicases also show similarity in their three-dimensional structures. The HCV helicase, for example, can unwind both DNA and RNA duplexes (4, 5), and its three-dimensional structure is similar to Escherichia coli DNA helicases such as PcrA and rep (6). Therefore, the structural differences between RNA and DNA helicases are small and most likely confined to the nucleic acid binding site.

Nucleic acid unwinding by a helicase involves the processes of translocation and nucleic acid strand separation. Both of these processes are fueled by ATP hydrolysis and must occur simultaneously. Helicases are therefore DNA and RNA motor proteins that couple ATP hydrolysis to nucleic acid unwinding. The mechanism of nucleic acid unwinding is still unclear and a subject of intense study at the present time. Several models describing either strand separation, translocation, or both are currently in discussion (7–12). All of these models require multiple DNA or RNA binding sites that bind and release the nucleic acid in response to conformational changes induced by ATP binding and hydrolysis. In principle, the multiple DNA binding sites can be present on a monomer or on separate subunits of an oligomeric helicase. Many helicases function as hexamers or as dimers, in which case multiple DNA binding sites are provided by separate subunits. Recently, suggestions have been made that some helicases may act as monomers (10, 11, 13), in which case multiple DNA binding sites must be provided by domains of the same polypeptide. Different mechanisms have been proposed for helicases that unwind DNA as a hexamer or dimer or as a monomer (7, 9, 12). Therefore, the determination of the active state of the helicase protein is the first step toward understanding the mechanism of unwinding by the helicase.

The investigation of the oligomeric state of the HCV helicase is the objective of this work. An estimated 1–2% of the human population worldwide is currently infected with HCV making this virus a major human pathogen (14). Because current therapies are not very effective, the development of new drugs has become critical (15). The single-stranded RNA genome of HCV codes for a single open reading frame. The RNA translates into a polypeptide of about 3000 amino acids that is cleaved by the host and viral proteases into 10 proteins. The non-structural protein 3 (NS3) has two activities. The N-terminal domain contains the protease activity and the C-terminal domain contains the helicase activity. The two domains have been expressed as separate polypeptides and shown to retain protease and helicase activities (16, 17).

The HCV helicase has been the subject of intense studies in several laboratories. A total of three crystal structures of this protein have been reported (10, 18, 19). The crystal structure reported by Cho et al. (19) showed interactions between NS3h monomers that may indicate formation of a protein dimer. In two crystal structures, no significant intersubunit protein-protein interactions were detected (10, 18). The protein crystals were grown in the absence of magnesium and in the presence of high concentration of salt and/or polyethylene glycol, conditions under which the protein has no ATPase or helicase activity. Therefore, the crystal structures do not provide definitive information on the oligomeric state of the HCV helicase protein. In addition, weak or transient protein-protein interactions can rarely be detected by crystallographic methods. The oligomeric structure of the helicase domain of the NS3 protein has not been examined extensively by biochemical methods. In this study, we employ several biochemical methods to investigate...
the oligomeric state of the helicase. Our results show that the HCV helicase functions as an oligomer to unwind DNA.

MATERIALS AND METHODS

MOPS (free acid) and ATP were purchased from Sigma; [α-32P]ATP and [γ-32P]ATP were from Amersham Pharmacia Biotech. Protein-protein cross-linking, DNA binding, ATPase, and helicase experiments were performed in the reaction buffer containing 20 mM MOPS-NaOH, 5 mM magnesium acetate, pH 7.0. The experiments described in this paper were conducted at room temperature (24 °C), unless indicated otherwise.

**HCV NS3h Protein Purification**—The recombinant plasmid pET21b-NS3HC was kindly provided by Dr. Joonho Choe (16). The plasmid codes for the initial amino acid sequence MASMTGGQQMGRGS followed by amino acids 1183–1658 of HCV-1 open reading frame followed by KACGRTRPRPQVEHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHHHiHH HiH
in size from a dimer to a pentamer and higher were observed. In the absence of DNA, the cross-linked products were predominantly oligomers that were larger than the octamer. In the presence of 5 mM oligo(dT)₂₀, protein dimers were the major accumulated species. The cross-linked dimers formed in the absence or in the presence of DNA were represented by two bands. Without DNA, the intensities of the two bands were nearly identical. However, in the presence of oligo(dT)₂₀, the faster migrating band was more intense. Other factors such as the presence of ATP, its unhydrolyzable analog (AMP-PNP), or magnesium had no detectable effect on the efficiency or the distribution of the cross-linked species (data not shown).

The protein-protein cross-linking experiment showed that NS3h protein was capable of forming oligomers, but the NS3h oligomeric state was not of a defined state. NS3h dimers accumulated in the presence of DNA, but higher oligomers were also present. The overall efficiency of cross-linking the NS3h protein was low. Even after prolonged incubation, all the monomers were not converted to cross-linked oligomers. Thus, either the NS3h does not form stable oligomers or the cross-linking of NS3h with the BS³ reagent is not efficient.

Protein Concentration Dependence of the ATPase Activity—If NS3h oligomers are more active than the monomers, then the ATPase and helicase activities of the NS3h protein should increase with increasing protein concentration. Increasing NS3h protein concentration should shift the equilibrium toward formation of oligomers. The ATPase activity was measured in the presence of oligo(dT)₂₀ DNA. The apparent $K_p$ for dT₂₀ (70 ± 20 nM) was determined by measuring the ATPase rates at increasing (dT)₂₀ concentrations, at saturating ATP and NS3h protein concentrations. The $K_m$ for ATPase was determined to be 120 ± 30 μM. The ATPase activity was then measured at increasing [NS3h] under saturating ATP (2–5 mM) and (dT)₂₀ (4 μM) concentrations. Under these conditions, the ATPase rates correspond to the $k_{cat}$ values. As shown in Fig. 3, the ATPase $k_{cat}$ increased about 100 times as NS3h protein was increased from 5 nM to 1000 nM. This result indicates that the NS3h self-assembles, and the oligomers hydrolyze ATP at a faster rate than the monomers. A similar increase in the $k_{cat}$ was also observed in the presence of poly(U) RNA, or oligodeoxyadenylic acids of different sequences, or at a higher temperature (37 °C) (data not shown).

Protein Concentration Dependence of the Helicase Activity—The helicase activity of NS3h protein was measured using the strand displacement helicase assay with the synthetic DNA substrate shown in Fig. 4A. To minimize reannealing of ssDNA reaction products, the concentration of the DNA substrate was kept at 2 nM in the reaction. An autoradiogram of a native gel with resolved ssDNA and dsDNA is shown in Fig. 4B. It demonstrates that the original dsDNA substrate (lane 1) can be
efficiently converted into ssDNA by the NS3h protein (lanes 2–5). Minor bands that did not correspond to either ds- or ssDNA were present on the native gels in some of our experiments. These bands contributed to about 10% of the radioactivity and most likely represent DNAs with alternative secondary structure. Since a large excess of NS3h protein over the DNA substrate was used in all the experiments, the minor bands did not affect the quality of the data, and they were not used in gel quantitation.

The helicase activity of NS3h protein was measured at four different NS3h protein concentrations. The time courses of the helicase reactions are shown on Fig. 5A. The unwinding kinetics were fit to a single exponential, which provided the unwinding rates. The unwinding rates increased from 0.01 to 0.35 s⁻¹ as NS3h protein was increased from 50 to 1000 nM (Fig. 5B).

**TABLE I**

| NS3h protein | ATPase rate | Apparent $K_d$ of NS3h · DNA fork |
|--------------|-------------|----------------------------------|
|              | (ADP) × [NS3h]−1 × s⁻¹ | nm | −ATP | +ATP |
| wt | 11.4 ± 0.04 | 160 ± 15 | 406 ± 80 |
| D1316A | <0.001 | 160 ± 20 | 260 ± 50 |
| K1236A | <0.001 | |

**Fig. 5.** Dependence of the helicase activity on the NS3h protein concentration. The helicase activity was measured by strand separation assay. A shows the time courses of the helicase reaction containing 50 (○), 200 (□), 400 (△), and 1000 (○) nM NS3h protein. The concentration of the ssDNA released as the product of the helicase reaction was plotted versus time. The solid lines represent fit of the data to the exponential equation. B shows the exponential rates of the helicase reactions plotted versus the concentration of NS3h protein. The error bars represent the standard errors obtained from the curve fitting.

**Fig. 6.** Conserved amino acid sequence motifs of NS3h protein and the amino acid residues mutated.

**Fig. 7.** Equilibrium DNA binding to the wt NS3h and mutant NS3h protein. A constant amount of radiolabeled fork DNA (20 nM) was titrated with increasing concentration of either the wt NS3h (●) or D1316A mutant (○) proteins. DNA binding was measured by nitrocellulose-DEAE membrane binding assay. The concentration of protein-bound DNA was plotted versus the total protein concentration. A shows DNA binding in the absence of ATP, and B shows DNA binding in the presence of 2 mM ATP. The data were fit to a hyperbola to obtain the equilibrium values.
activities of the wt protein are inhibited by the inactive mutant proteins, it will provide evidence for cooperativity between the NS3h subunits for the ATPase and/or the helicase activity.

Two mutants of the NS3h protein with impaired ATPase activity were created by site-directed mutagenesis. It is known that specific amino acids in the helicase conserved motifs I and II play an important role in the ATPase activity of the enzyme (21, 22). We made the following single amino acid substitutions in the conserved motif I: GSGK(A)STK, K1236A, and in the conserved motif II: D(A)ECH, D1316A (Fig. 6) with the intention of preparing an ATPase-defective mutant. As expected, neither of the mutants showed detectable ATPase activity (Table I).

The DNA Binding Activity of the ATPase-deficient Mutants Was Unchanged from That of the wt Protein—We compared the DNA binding properties of the wt and the D1316A mutant of the NS3h protein using the nitrocellulose-DEAE membrane binding assay (20, 23). A constant amount of radiolabeled partially dsDNA substrate, shown in Fig. 4A, was mixed with increasing concentration of the wt NS3h or the mutant protein in the presence or in the absence of ATP. The complex was filtered through the NC-DEAE membranes, and the amount of protein-bound DNA species was quantitated.

As shown in Fig. 7, the amount of protein-bound DNA species was measured at increasing protein concentration both in the absence and in the presence of ATP. The data were fit to a hyperbolic equation, and the fit provided the apparent $K_d$ values for the protein-DNA complexes shown in Table I. The introduced mutation did not affect the equilibrium DNA binding property of the mutant protein. Similar $K_d$ values for the wt and the mutant proteins were obtained in the absence of ATP. Somewhat different $K_d$ values were observed in the presence of ATP.

Effect of the Mutant NS3h Protein on the wt ATPase and Helicase Activities—The ATPase activity of wt NS3h protein was measured in the presence of increasing amounts of either the D1316A or the K1236A mutant NS3h proteins. The experiment was carried out both in the presence and in the absence of DNA. The ATPase activity of the wt protein was essentially unchanged in the presence of the mutant protein (data not shown). This result does not support or contradict oligomerization of NS3h. It merely shows that each helicase subunit hydrolyses ATP independently from the others.

The helicase activity of the wt protein was measured in the presence of increasing mutant protein concentration. Because the concentration of the mixture of proteins was greater than the concentration of the dsDNA substrate, we had to provide equal DNA binding opportunity for both the proteins. To
achieve that, the dDNA substrate was added to the helicase reaction that already contained premixed wt and mutant NS3h proteins. Under these conditions, the chances of each protein binding to the DNA substrate were directly proportional to the protein concentration and inversely proportional to the $K_d$ of the protein-DNA complex.

Fig. 8A shows the unwinding reaction time courses of the reactions that contained a constant amount of the wt NS3h protein and increasing amounts of the D1316A mutant. Each kinetic curve was fit to a single exponential equation. The exponential rates were then plotted against the D1316A mutant concentration (Fig. 8B). A sharp decrease in the unwinding rate was observed as the wt protein was titrated with increasing concentration of the mutant protein. The amplitude of unwinding showed only a mild decrease (Fig. 8C). Similar results were observed also with the K1236A mutant protein (data not shown). The inhibition data were analyzed in a quantitative manner to distinguish between inhibition resulting from interacting subunits versus simple competition for DNA substrate. As shown under “Discussion,” these results provide strong support for interacting subunits in the NS3h oligomer.

**DISCUSSION**

The process of unwinding double-stranded nucleic acid requires helicases to translocate along the nucleic acid substrate in a unidirectional manner. During translocation the helicase has to bind and release the nucleic acid in a cyclical manner. To translocate processively, the helicase must use at least two nucleic acid binding sites in order to maintain contact with the nucleic acid via one of these binding sites (8). This requires that the two nucleic acid binding sites interact and conduct cooperative catalysis. Multiple subunits of an oligomeric helicase can provide the interacting nucleic acid binding sites. Alternatively, domains within a single helicase polypeptide can provide these sites. Thus, in principle, a helicase can act as a monomer or an oligomer as long as interacting DNA binding sites are provided by the structure of the protein.

We have investigated the oligomeric structure of the helicase from hepatitis C virus to understand its mechanism of action. The methods that are usually suitable for studying proteins that form stable oligomers such as x-ray crystallography, analytical sedimentation, and gel filtration chromatography did not detect oligomerization of the helicase domain of the HCV NS3 protein (10, 18, 19, 24, 25). Protein-protein cross-linking studies reported in this paper with a bifunctional cross-linking reagent provide some evidence for oligomerization of the NS3h protein. Upon cross-linking, oligomers ranging in size from dimers to octamers and higher were observed. However, the overall cross-linking efficiency was low, indicating that the oligomers were not stable. The cross-linking efficiency was higher in the presence of the DNA, suggesting that the DNA stimulated some intersubunit contact. However, even in the presence of the DNA, a large proportion of the monomers was not cross-linked.

There are two mechanisms by which DNA can increase the protein-protein cross-linking efficiency. On one hand, binding of the NS3h monomer to the DNA may stimulate binding of another NS3h monomer to the first one, thus increasing the cross-linking efficiency. On the other hand, close positioning of protein monomers on oligo(dT)$_{20}$ may stimulate their cross-linking as well. Our experiments cannot distinguish between the two oligomeric structures. The crystal structure of HCV helicase with a bound oligonucleotide suggested an 8-base binding site for the DNA on the monomer (10). Therefore, two or three NS3h monomers can be bound side by side on a (dT)$_{20}$ DNA used in our experiments.

The biochemical evidence for active NS3h oligomers was provided by the observed increase in the $k_{cat}$ for the ATPase activity with increasing concentration of the NS3h protein. As mentioned previously, (dT)$_{20}$ can accommodate the binding of several NS3h subunits side by side. However, the increase of ATPase $k_{cat}$ with the NS3h protein concentration indicates that the NS3h subunits are interacting regardless of their DNA binding mode. Therefore, our results show that NS3h oligomers on the DNA are more ATPase-active than the monomer. The helicase activity of NS3h protein also increased with increasing protein concentration. However, the exact reason for the protein-dependent increase in helicase activity is not clear. The increase in the helicase rate could be due to an increase in DNA binding efficiency, oligomerization, or both.

A strong support for oligomerization was provided by the mutant inhibition experiment. To examine the importance of NS3h subunit interaction, we prepared an ATPase inactive mutant of the NS3h protein. The D1316A mutant of NS3h had normal DNA binding properties, but lacked both the ATPase and helicase activities (Table I). Interestingly, the ATPase activity of the wt protein was not affected to a significant extent when it was mixed with the inactive mutant proteins. These results suggest that the ATPase activity of the NS3h subunits is noninteracting, i.e. the ATP hydrolysis in one subunit does not affect the hydrolysis in other subunits in the helicase oligomer. Thus, although the ATPase activity of the oligomeric NS3h protein is higher than the activity of the monomer, the inactive subunits in the mixture do not affect the ATPase activity of the wt subunits. This property may reflect a more general difference between helicases from superfamily I or II and the hexameric helicases, since the DNA- and RNA-stimulated ATPase activity of the latter class of proteins was inhibited by the addition of a mutant subunit (26, 27).

The presence of a mutant subunit within a mixed oligomer, however, severely inhibited the helicase activity of the NS3h protein. Upon titration of the wt protein with increasing mutant protein, a sharp decrease in the unwinding rate with increasing mutant protein was observed. For instance, addition of only 10 nm (5% of the wt NS3h concentration) of a mutant protein reduced the unwinding rate by a factor of 2. To analyze these results in a more quantitative manner, we consider here the following general models for the helicase activity (Fig. 9).

Model I invokes a monomeric helicase as the active species. According to this model, one would expect no protein-protein cross-linking, and no ATPase $k_{cat}$ dependence on the protein concentration. Neither of these results was observed; nevertheless, we consider two cases to analyze the mutant inhibition data in terms of model I. In case one, the helicase monomer is tightly bound to the DNA. The unwinding, therefore, occurs processively in a single turnover. In this case, the added mutant protein will affect only the amplitude of the unwinding reaction by the wt protein. This was not observed in our experiments, where the addition of the mutant NS3h protein affected mainly the rate of the unwinding (Fig. 8, B and C). In case two, the mutant protein can dissociate from the DNA during the course of the unwinding reaction. Consequently, the effect of the mutant protein will be on the rate of unwinding, which was observed in our reactions. The helicase rate ($H$) will depend on the mutant protein concentration ([E$_{MUT}$]) in the following manner,

$$H = H_{WT} \times \frac{[E_{WT}]}{[E_{WT}] + [E_{MUT}]},$$

(Eq. 1)

where $H_{WT}$ is the helicase rate of the pure wt NS3h protein, [E$_{WT}$] and [E$_{MUT}$] are the concentrations of the wt and mutant NS3h proteins, respectively. The DNA $K_d$ values for wt and
mutant NS3h were found to be somewhat different (Table I). Therefore, the equation was corrected.

\[
H = H_{WT} \times \left( \frac{[E_{WT}]}{K_3^{WT}} \right)^n \quad \text{(Eq. 2)}
\]

The curve simulated for Equation 2 is shown in Fig. 8B with a dotted line. It is clear that the observed inhibition cannot be attributed to simple competition between wt and mutant NS3h proteins for binding the DNA substrate. Therefore, none of the observed results are consistent with model I in which NS3h acts as a monomer.

In model II (Fig. 9, II), the helicase functions as a stable oligomer, i.e. the subunit interactions are strong and the subunits do not exchange during the course of the reaction. Once again, two cases should be considered. In the case of stable protein-DNA interactions, only the amplitude of the unwinding reaction should be affected upon addition of an ATPase-deficient mutant. This result was not observed, as shown in this paper. If the protein-DNA interactions are not stable, and the protein dissociates from the DNA during the time scale of the unwinding reaction, then the rate of unwinding rather than the amplitude will be affected. The unwinding rate will be proportional to the fraction of the unwinding active complexes in the mixture of wt and mutant NS3h proteins. Assuming that only homo-wt complexes are capable of unwinding at a maximal rate,

\[
H = C + H_{WT} \times \left( \frac{[E_{WT}]}{[E_{WT}]+[E_{MUT}]} \right)^n, \quad \text{(Eq. 3)}
\]

where \( n \) is the number of subunits in the complex, and \( C \) is the unwinding rate by heterooligomers. Equation 3 was used to fit the data in Fig. 8B (solid line). The value of \( n \) obtained from the fit was 13.5, indicating the formation of tri- or tetradecamers. This subunit composition is inconsistent with the observed results. In addition, although an increase in ATPase rate with increasing NS3h concentration was observed, stable oligomer formation was not detected by cross-linking (Fig. 2), size exclusion chromatography (25), and sedimentation centrifugation (24). Therefore, we conclude that NS3h protein is oligomeric, but it does not function as a stable oligomer.

In model III (Fig. 9, III), the active species is an oligomer, but the protein-protein interactions are transient. Thus, the subunits exchange during the course of translocation and unwinding. Here we consider a dimer as the simplest case. In this model, the unwinding reaction can proceed only when both subunits are wt. Therefore, the reaction will pause every time a mutant subunit forms a tertiary complex with the wt subunit and the DNA substrate. The reaction will continue when the mutant subunit dissociates with a wt subunit rebinds. When the concentration of the mutant protein increases in the reactions, the chances of homo-wt complex formation reduces. Therefore, the unstable oligomer model predicts a decrease in the rate of unwinding rather than a decrease in amplitude of unwinding.

The lack of quantitative protein-DNA and protein-protein interactions of NS3h protein precludes quantitative analysis of this model in the present study. However, by process of elimination, we arrive at model III, which is the most consistent with the reported results on NS3h protein.

The mutant inhibition of the helicase activity can be the ultimate test for interacting subunits of an oligomer (13, 27). However, it provides useful information only when the results are analyzed in a quantitative manner. Two kinds of inhibition are to be looked for. The first kind, a reduction in the unwinding amplitude, occurs when a monomeric helicase or a stable oligomeric helicase has tight interactions with the DNA during unwinding. The second kind, a decrease in the unwinding rate, occurs in case of unstable oligomers or when the interactions with the DNA are reversible during the course of DNA unwinding. To make a distinction and to determine if the helicase functions as a monomer or oligomer, one must measure the time course of DNA unwinding. Similar mutant inhibition of the helicase activity were reported with the E. coli UvrD helicase. In a recent report, mutant inhibition was not observed (13), and it contradicts a previous report where inhibition of unwinding and a dominant phenotype of the UvrD mutant gene was observed (28). Unfortunately, the single time point helicase assays do not allow one to distinguish between the effects on the rate or the amplitude of unwinding and to examine the results in terms of the various models described above.

Several helicases have been shown to be oligomeric, and models for the oligomeric helicase activity have been proposed (7–9). Recently suggestions have been made that some helicases may act as monomers (10, 11, 13). If these suggestions are correct, then it appears that the dimeric and monomeric helicases have similar structures, but use different mechanisms for DNA unwinding. Alternatively, the individual subunits in the oligomeric helicase and the monomeric helicase use similar unwinding mechanisms, however, the monomers are not as efficient as the oligomers. An analogy can be drawn from the motor protein field. Although it was shown previously that monomeric myosin was capable of translocation (29), a recent study shows that two-headed myosin can generate force and movement about twice as much as one-headed myosin (30). The same may be true for helicases as well. The oligomerization may be required for increasing the processivity or the rate of unwinding. Oligomerization, for instance, may allow the protein to translocate with larger step-sizes, which may not be possible in monomeric helicases due to structural constraints. Fairly small conformational changes were detected in the structure of PcrA helicase proposed to act as a monomer (11).

More detailed studies are necessary in which the efficiency of unwinding, in terms of processivity and rate, are measured for a monomeric subunit or a monomeric helicase and compared with the activity of a helicase that acts as an oligomer.

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REFERENCES

1. Matson, S. W., Bean, D. W., and George, J. W. (1994) BioEssays 16, 13–22
2. Lu¨ king, A., Stahl, U., and Schmidt, U. (1998) Crit. Rev. Biochem. Mol. Biol. 33, 259–296
3. Gorbalenya, A. E., and Koonin, E. V. (1993) Curr. Opin. Struct. Biol. 3, 419–429
4. Tai, C. L., Chi, W. K., Chen, D. S., and Hwang, L. H. (1996) J. Virol. 70, 8477–8484
5. Gwack, Y., Kim, D. W., Han, J. H., and Cho, J. (1997) Eur. J. Biochem. 256, 47–54
6. Korolev, S., Yao, N., Lohman, T. M., Weber, P. C., and Waksman, G. (1998) Protein Sci. 7, 605–610
7. Lohman, T. M., and Bjornson, K. P. (1996) Annu. Rev. Biochem. 65, 169–214
8. Lohman, T. M., Thorn, K., and Vale, R. D. (1998) Cell 93, 9–12
9. Ahnert, P., and Patel, S. S. (1997) J. Biol. Chem. 272, 32267–32273
10. Kim, J. J., Morgenstern, K. A., Griffith, J. P., Dyer, M. D., Thomson, J. A., Murchko, M. A., Lin, C., and Caven, P. R. (1986) Structure 6, 89–100
11. Velankar, S. S., So◄lantsan, P., Dillingham, M. S., Subramanayya, H. S., and Wigley, D. B. (1999) Cell 97, 75–84
12. Bird, L. E., Subramanayya, H. S., and Wigley, D. B. (1998) Curr. Opin. Struct. Biol. 8, 14–18
13. Mechanic, L. E., Hall, M. C., and Matson, S. W. (1999) J. Biol. Chem. 274, 12489–12496
14. Di Biscaglia, A. M. (1988) Lancet 331, 351–355
15. Moussali, J., Opolon, P., and Poynard, T. (1998) J. Viral Hepat. 5, 73–82
16. Kim, D. W., Gwack, Y., Han, J. H., and Cho, J. (1995) Biochem. Biophys. Res. Commun. 215, 160–166
17. Vishnyavtsian, D., Kakiuchi, N., Urvi, P. T., Shimotohno, K., Kamar, P. K., and Nishikawa, S. (1997) FEBS Lett. 402, 209–212
18. Yao, N. H., Hesson, T., Cable, M., Hong, Z., Kwong, A. D., Le, H. V., and Weber,
P. C. (1997) Nat. Struct. Biol. 4, 463–467
19. Cho, H. S., Ha, N. C., Kang, L. W., Chung, K. M., Back, S. H., Jang, S. K., and Oh, B. H. (1998) J. Biol. Chem. 273, 15045–15052
20. Hingorani, M. M., and Patel, S. S. (1993) Biochemistry 32, 12478–12487
21. Heilek, G. M., and Peterson, M. G. (1997) J. Virol. 71, 6264–6266
22. Kim, D. W., Kim, J., Gwack, Y., Han, J. H., and Choe, J. (1997) J. Virol. 71, 9400–9409
23. Weng, I., Chao, K. L., Bujalowski, W., and Lohman, T. M. (1992) J. Biol. Chem. 267, 7596–7610
24. Porter, D. J. T., Short, S. A., Hanlon, M. H., Preugschat, F., Wilson, J. E., Willard, D. H., Jr., and Conslor, T. G. (1998) J. Biol. Chem. 273, 18906–18914
25. Preugschat, F., Averett, D. R., Clarke, B. E., and Porter, D. J. T. (1996) J. Biol. Chem. 271, 24449–24457
26. Richardson, J. P., and Ruteshouser, E. C. (1986) J. Mol. Biol. 189, 413–419
27. Patel, S. S., Hingorani, M. M., and Ng, W. M. (1994) Biochemistry 33, 7857–7868
28. George, J. W., Brush, R. M. J., and Matson, S. W. (1994) J. Mol. Biol. 235, 424–435
29. Molloy, J. E., Burns, J. E., Kendrick-Jones, J., Tregear, R. T., and White, D. C. S. (1995) Nature 378, 209–212
30. Tyska, M. J., Dupuis, D. E., Gaulford, W. H., Patlak, J. B., Waller, G. S., Trybus, K. M., Warshaw, D. M., and Lowey, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4402–4407
31. Choo, Q. L., Richman, K. H., Han, J. H., Berger, K., Lee, C., Dong, C., Gallegos, C., Coit, Medina-Selby, R., and Barr, P. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2451–2455