Interaction of UvrA and UvrB Proteins with a Fluorescent Single-stranded DNA

IMPLICATION FOR SLOW CONFORMATIONAL CHANGE UPON INTERACTION OF UvrB WITH DNA*

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UvrA and UvrB proteins play key roles in the damage recognition step in the nucleotide excision repair. However, the molecular mechanism of damage recognition by these proteins is still not well understood. In this work we analyzed the interaction between single-stranded DNA (ssDNA) labeled with a fluorophore tetramethylrhodamine (TMR) and Thermus thermophilus HB8 UvrA (ttUvrA) and UvrB (ttUvrB) proteins. TMR-labeled ssDNA (TMR-ssDNA) as well as UV-irradiated ssDNA stimulated ATPase activity of ttUvrB more strongly than did normal ssDNA, indicating that this fluorescent ssDNA was recognized as damaged ssDNA. The addition of ttUvrA or ttUvrB enhanced the fluorescence intensity of TMR-ssDNA, and the intensity was much greater in the presence of ATP. Fluorescence titration indicated that ttUvrA has higher specificity for TMR-ssDNA than for normal ssDNA in the absence of ATP. The ttUvrB showed no specificity for TMR-ssDNA, but it took over 200 min for the fluorescence intensity of the ttUvrB-TMR-ssDNA complex to reach saturation in the presence of ATP. This time-dependent change could be separated into two phases. The first phase was rapid, whereas the second phase was slow and dependent on ATP hydrolysis. Time dependence of ATPase activity and fluorescence polarization suggested that changes other than the binding reaction occurred during the second phase. These results strongly suggest that ttUvrB binds ssDNA quickly and that a conformational change in ttUvrB-ssDNA complex occurs slowly. We also found that DNA containing a fluorophore as a lesion is useful for directly investigating the damage recognition by UvrA and UvrB.

UV irradiation and various chemical compounds in the environment cause alterations in the chemistry or sequence of DNA, which lead to mutagenesis or even cell death. To avoid these alterations, living organisms possess DNA repair systems (1). Nucleotide excision repair (NER) is one of the most important repair systems, and it is conserved from prokaryotes to higher eukaryotes (2, 3). The most important feature of NER system is its broad substrate specificity. It excises a wide variety of DNA lesions as follows: pyrimidine dimers, abasic sites, and more bulky adducts, such as benzo[a]pyrene diol epoxide (BPDE) and N-2-acetylaminofluorene.

In prokaryotic NER systems, recognition and excision of DNA lesions is mediated by UvABC excinuclease (2, 3). The properties of the component proteins (UvrA, UvrB, and UvrC) have been studied for Escherichia coli. UvrA recognizes a lesion in DNA and forms UvrA,B complex at the site of the lesion. After UvrA dissociation from damaged DNA, a stable UvrB-DNA preincision complex forms. Then UvrC binds to the UvrB-DNA preincision complex, and UvrBC makes incisions at the fourth or fifth phosphodiester bond from the 3′ side of a lesion and subsequently at the eighth bond from the 5′ side of a lesion. After the incision event, UvrD helicase, DNA polymerase I, and DNA ligase complete the repair process by carrying out excision of the oligonucleotide containing the lesion, repair synthesis, and DNA ligation, respectively.

The molecular mechanism of recognition of the damage site by UvrA and UvrA2B is still not fully understood. The UvrA2 dimer, which can be formed in solution (4), has a higher affinity for damaged double-stranded DNA (dsDNA) than for normal/undamaged dsDNA (5–7), whereas UvrB cannot bind dsDNA even if it contains a lesion (8). However, it has been suggested that UvrB is not merely loaded by UvrA but actively participates in damage recognition (9). UvrB binds single-stranded DNA (ssDNA) and has a higher affinity for damaged ssDNA in the absence of UvrA (8), whereas UvrA shows no preference for ssDNA containing a lesion (5). It has also been shown that dsDNA in UvrB-DNA preincision complex is sharply bent (10) and has one or more DNase I-hypersensitive sites (7, 11–12). The dsDNA in UvrB-DNA preincision complex is supposed to be partly unwound (13), although extensive unwinding may not occur (14). This conformational change is required for binding of UvrC to UvrB-DNA preincision complex (15, 16). As UvrC can bind only to ssDNA (3), it is proposed that a single-

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stranded region may be formed in UvrB-DNA preincision complex. In addition, it has been shown that UvrAB complex binds to bubble or loop regions in dsDNA with an affinity similar to that for damaged DNA (17). These observations suggest the importance of interaction of UvrA and UvrB with ssDNA or ssDNA portion of the dsDNA in the repair process of NER.

Spectroscopic analysis using fluorescent DNA is a useful method to examine directly the DNA-protein interaction in solution (18). Additionally, certain bulky fluorophores attached to DNA, such as BPDE, can be recognized as the lesions by UvrABC (19). As the fluorescence spectrum can detect with sensitivity changes in a microenvironment around a fluorophore, such fluorescent adducts may also be used for studying a local conformational change subsequent to binding of UvrA or UvrB to the damaged DNA. These reflections prompted us to employ a bulky fluorophore attached to DNA for studying the interaction of UvrA and UvrB with the damaged DNA.

We have studied several repair systems including NER by using an extremely thermophilic bacterium, *Thermus thermophilus* HB8 (20–24). *T. thermophilus* is a Gram-negative eubacterium that can grow at temperature over 75 °C (25). We have already cloned and sequenced *E. coli* UvrA (ttUvrA) and UvrB (ttUvrB) show homology to genes of *T. thermophilus* [type II] from Sigma; pig heart lactate dehydrogenase (grade II) from Boehringer-Mannheim. The isoforms of ATPase and reverse-phase high pressure liquid chromatography. The isoforms of ATPase were purchased as follows: rabbit muscle pyruvate kinase 

The concentration (nucleotide molar, M) of poly(dT) was determined using an extremely thermophilic bacterium, *T. thermophilus* HB8 (20–24).

Materials—ttUvrA and ttUvrB were purified as described previously (21, 23), and their concentrations were determined using a molar extinction coefficient of $4.82 \times 10^4$ M m$^{-1}$ cm$^{-1}$ at 266 nm, respectively. Enzymes and reagents were purchased as follows: rabbit muscle pyruvate kinase (type II) from Sigma; pig heart lactate dehydrogenase (grade II) from Boehringer-Mannheim; AMP-PNP and AMP-PCP from Roche Molecular Biochemicals; poly(dT) from Amersham Pharmacia Biotech; and tetramethylrhodamine ethyl ester (TMR-TE) from Molecular Probe. The concentration (nucleotide molar, M) of poly(dT) was determined using an molar extinction coefficient of $4.82 \times 10^4$ M m$^{-1}$ cm$^{-1}$ at 266 nm (26). Damage-containing poly(dT) was prepared by irradiating poly(dT) in a quartz cuvette with UV light (mainly 254 nm). All of the other chemicals and reagents were purchased from commercial sources.

An unmodified oligodeoxynucleotide of 30-mer (N-ssDNA, Fig. 1A), was synthesized using an automated DNA synthesizer and purified by reverse-phase high pressure liquid chromatography and gel filtration on Sephadex G-25. For synthesis of fluorescent ssDNA, TMR-ssDNA (Fig. 1B), an oligodeoxynucleotide with a modified thymine residue which contains an amino group (27), was synthesized and purified in the same manner. The resultant modified ssDNA was allowed to react with tetramethylrhodamine-5-isothiocyanate and isolated by gel filtration and reverse-phase high pressure liquid chromatography. The iso-
RESULTS

ssDNA-dependent ATPase Activity—We planned to employ TMR-labeled ssDNA as a fluorescent ssDNA probe to study the interactions between ssDNA and ttUvrA and ttUvrB (Fig. 1). This was because the fluorescence of TMR fluctuates with the polarity of its immediate environment, offering the possibility to study the kinetics of protein-ssDNA interactions by measuring the changes in fluorescence that are expected to occur when protein interacts with the labeled ssDNA. Before making such an analysis, however, we needed to examine whether the complex of ttUvrA or ttUvrB with such fluorescent ssDNA was similar in character to those of lesion-containing ssDNA. It was shown that E. coli UvrB (EcUvrB) binds ssDNA specifically and has a higher affinity to ssDNA with a lesion than to normal ssDNA (8). We previously reported that ttUvrB has ATPase activity even in the absence of ttUvrA and DNA, and this activity is stimulated by the presence of ssDNA (21). However, it was uncertain whether ssDNA with a lesion can activate ATPase activity of UvrB more strongly than do normal ssDNAs.

To investigate the effect of DNA lesions on the ATPase activity of ttUvrB, we measured the activity in the presence of UV-irradiated poly(dT). The irradiation of poly(dT) with UV light causes the formation of pyrimidine dimers, including a cyclobutane-type thymine dimer, which is typical of a DNA lesion (1). As shown in Fig. 2A, increasing concentration of UV-irradiated poly(dT) as well as normal poly(dT) stimulated the ATPase activity of ttUvrB. However, the maximum level of ATPase activity in the presence of UV-irradiated poly(dT) was significantly higher than that in the presence of normal poly(dT). In the presence of excess poly(dT), all ttUvrB molecules are considered to be bound by poly(dT) regardless of UV irradiation. This suggests that UV-irradiated poly(dT) activated the ATPase activity of ttUvrB more strongly than did unirradiated poly(dT). This leads to the notion that there is a qualitative difference between the ttUvrB-ssDNA complexes containing unirradiated ssDNA and those containing irradiated ssDNA. This result also suggests that the level of stimulation of the ATPase activity can be used to indicate the presence of DNA lesions on the ssDNA bound to ttUvrB.

Based on this finding, we investigated the effect of the TMR moiety on ttUvrB-ssDNA complex by measuring the ATPase activity of ttUvrB in the presence of TMR-ssDNA and unmodified ssDNA (N-ssDNA, Fig. 1A). Although both ssDNAs could stimulate ATP hydrolysis by ttUvrB, TMR-ssDNA stimulated the ATPase activity more strongly than did N-ssDNA (Fig. 2B). The effect of TMR modification to ATPase stimulation was similar to that of UV irradiation. Therefore, we supposed that the complex of ttUvrB with TMR-ssDNA has a similar property to the complex with UV-irradiated ssDNA. In addition, TMR-ssDNA stimulated ttUvrB ATPase nearly two times higher than N-ssDNA, whereas UV-irradiated poly(dT) stimulated ttUvrB ATPase only 20% higher than unirradiated poly(dT). These results suggest that the activation of ttUvrB ATPase activity depends on the kind of lesion contained on the ssDNA; TMR moiety is a bulky adduct (Fig. 1B), whereas pyrimidine dimer is not. This finding might be consistent with those of previous reports (30), which suggested that the stability of the complex of EcUvrB with damaged DNA depends on the bulkiness of the adduct (see “Discussion”).

To discern the specific binding of ttUvrA and ttUvrB to TMR-ssDNA, fluorescence polarizations were measured. As shown in Fig. 3, the fluorescence polarization of TMR-ssDNA was increased by titrating TMR-ssDNA with ttUvrA or ttUvrB.
These results confirmed the specific interaction of these proteins with TMR-ssDNA. To examine whether ttUvrA and ttUvrB bind TMR moiety itself nonspecifically or not, the fluorescence polarization was measured using tetramethylrhodamine ethyl ester (TMRE, Fig. 1C), as a model compound for TMR moiety in ssDNA. As shown in Fig. 3, no change in the fluorescence polarization of TMRE was observed after addition of ttUvrA or ttUvrB to the assays. These results confirmed that neither ttUvrA nor ttUvrB directly binds TMR moiety. Therefore, we concluded that TMR-ssDNA could be served as a useful model to study the interactions that normally take place between lesions containing ssDNA and the proteins ttUvrA and ttUvrB.

Fluorescence Titration of TMR-ssDNA—In an aqueous solution, TMR-ssDNA shows the fluorescence emission spectrum with an emission maximum at 571 nm upon excitation at 543 nm, as can be seen in Fig. 4A (dotted line). In the presence of ttUvrA, its fluorescence emission was considerably enhanced (broken line). This enhancement occurred in the instant of addition of ttUvrA and did not change after that. The presence of both ttUvrA and ATP resulted in even further enhancement of the fluorescence emission (solid line). In the presence of ATP, as that seen without ATP, the fluorescence intensity changed instantly and did not change after that.

Also, enhancement of the fluorescence intensity was observed in the presence of ttUvrB (Fig. 4B), and this fluorescence was further enhanced by the addition of ATP, like in the case of ttUvrA. However, it should be mentioned that the fluorescence intensity took a long time to reach saturation in the presence of ATP (Fig. 6A). For this reason, the spectral data in the presence of ttUvrB and ATP were collected after incubation for more than 200 min. It should be noted that the measurement in the presence of ATP was performed associated with an ATP regeneration system, as ttUvrA and ttUvrB has an ATPase activity. A slight blue wavelength shift of the maximum fluorescence was observed for ttUvrB in the presence of ATP, whereas little shift was observed for ttUvrA. This might represent a difference in protein-DNA interaction between ttUvrA-TMR-ssDNA and ttUvrB-TMR-ssDNA complexes.

The fluorescence titration of TMR-ssDNA with ttUvrA and ttUvrB is shown in Fig. 5. The titration curves at saturation for each protein were hyperbolic both in the absence and presence of ATP. From these data, we determined the dissociation constants ($K_d$) of ttUvrA and ttUvrB for TMR-ssDNA (Table I), assuming bimolecular reaction between these proteins and TMR-ssDNA (see under “Experimental Procedures” for details). The $K_d$ values showed a slightly higher affinity of ttUvrA for TMR-ssDNA in the presence of ATP than in the absence of ATP.

**Fig. 3.** Fluorescence polarization titration of TMR-ssDNA and TMRE with ttUvrA or ttUvrB. Reaction mixtures contained 0.1 μM TMR-ssDNA and the indicated concentrations of ttUvrA (A) or ttUvrB (B) in 50 mM Tris-HCl, 100 mM KCl, and 10 mM MgCl$_2$, pH 7.5. Fluorescence emission at 571 nm was measured using an excitation wavelength of 543 nm at 25 °C. Fluorescence polarization was measured by a spectrofluorometer equipped with polarizers. The symbols used are as follows: closed circles, fluorescence polarization of TMR-ssDNA; closed squares, fluorescence polarization of TMRE; open circles, fluorescence intensity of TMR-ssDNA.

**Fig. 4.** Emission spectra of TMR-ssDNA with ttUvrA (A) or ttUvrB (B). Reaction mixtures contained 2 μM ttUvrA or 8 μM ttUvrB in 50 mM Tris-HCl, 100 mM KCl, and 10 mM MgCl$_2$, pH 7.5. In the presence of ATP, 10 mM ATP, 25 mM creatine phosphate, and 25 units/ml creatine kinase were further added. The emission spectra were measured at 25 °C using an excitation wavelength of 543 nm. The symbols used are as follows: dotted lines, TMR-ssDNA alone; broken lines, in the presence of ttUvrA or ttUvrB; solid lines, in the presence of each protein and ATP.
The dissociation constants of ttUvrA and ttUvrB to TMR-ssDNA or N-ssDNA

A 0.1 μM sample of TMR-ssDNA was incubated with ttUvrA or ttUvrB in the absence and presence of ATP, as described under "Experimental Procedures." The dissociation constants to N-ssDNA were determined as the inhibition constants from competition experiments.

| ssDNA     | ttUvrA  | ttUvrB |
|-----------|---------|--------|
|           | -ATP    | +ATP   | -ATP    | +ATP   |
| TMR-ssDNA | 0.43 ± 0.10 | 0.10 ± 0.01 | 0.85 ± 0.14 | 1.32 ± 0.17 |
| N-ssDNA   | >30     | 0.12 ± 0.01 | 0.56 ± 0.14 | 0.92 ± 0.12 |

ATP. In addition, it should be noted that the addition of ATP caused further enhancement of the fluorescence intensity, suggesting that ATP elicits changes in the interaction of ttUvrA with TMR-ssDNA.

To compare the affinity of ttUvrA and ttUvrB for TMR-ssDNA with that for normal ssDNA, we performed competition experiments using N-ssDNA. We performed measurements in two different concentrations of N-ssDNA. For ttUvrA, no inhibition by N-ssDNA was observed in the absence of ATP, whereas considerable inhibition was observed in the presence of ATP. These results indicate that ATP drastically affects the affinity of ttUvrA to N-ssDNA. In contrast, significant inhibition by N-ssDNA was observed for ttUvrB, irrespective of the presence of ATP. Assuming that N-ssDNA is competitive with TMR-ssDNA for binding to the proteins, the inhibition constants (Ki) were determined in each case, except for ttUvrA without ATP (Table I). Based on the obtained values, we deduced that ATP exerted no significant effect on the interaction of ttUvrB with N-ssDNA.

Kinetics of Interaction between TMR-ssDNA and ttUvrB—As shown in Fig. 6A, it took over 200 min before the fluorescence intensity reached saturation after the addition of ttUvrB and ATP. We used UvrB from the extreme thermophile T. thermophilus and measured its binding activity at 25 °C. This raised the possibility that the observed slow change was due to the measurement at a lower temperature than the optimal growth temperature. Thus, we performed the same experiments at higher temperatures, 37 and 50 °C, and observed the similar slow change of fluorescence (Fig. 6C). The ordinate of Fig. 6C was represented by (F – F0)/F0 to correct the differences of fluorescence intensities at different temperatures. At 50 °C the fluorescence intensity reached saturation within an hour. This relatively rapid saturation was considered to be due to the lack of ATP because ATP regeneration system became inactivated during incubation at high temperature.

Two phases could be recognized during the increase in fluorescence. The first phase, which took place within a minute, was characterized by a rapid increase in fluorescence intensity above that of TMR-ssDNA alone, and the second phase was characterized by a slower increase following again by a rapid increase. In this second slow phase, the rate constant was obtained 0.023 min⁻¹ at 25 °C by assuming the single molecular reaction. Change of fluorescence intensity can be considered to reflect alteration of the environment around the fluorophore. The fluorescence polarization also showed two phases for the interaction of ttUvrB with TMR-ssDNA (Fig. 6B). The extent of the enhancement of fluorescence polarization in the first rapid phase was greater than that in the second slow phase, whereas in the case of fluorescence intensity the extent of the increase in the first phase was smaller than that in the second phase. These results suggest that the first and second phases reflect different events during the interaction of ttUvrB with TMR-ssDNA. Fluorescence polarization reflects mobility or dynamics of the molecule containing a fluorophore. Generally, binding of a small fluorescent molecule to a large molecule, like a protein, leads to an increase in the fluorescence polarization (18), so the binding reaction itself of TMR-ssDNA to ttUvrB should cause an increase in the fluorescence intensity and polarization. The saturated value of the fluorescence intensity in the absence of ATP was almost equal to the value after the first phase in the presence of ATP (Fig. 6A). As ttUvrB can bind TMR-ssDNA in the absence of ATP, it is more likely that the first phase corresponds to the binding reaction itself.

A clue to the nature of the second phase is that this phase was not observed in the absence of ATP (Fig. 6A). As ttUvrB has ATPase activity, ATP hydrolysis should occur under our experimental condition. This suggests an involvement of ATP hydrolysis in an unidentified event during the second phase. It should be mentioned here that the saturation of the fluorescence in the presence of ATP was not due to a decrease of ATP concentration, because further addition of ATP near the saturation point had no effect on the fluorescence (Fig. 6A). To investigate further the involvement of ATP hydrolysis in the second phase, we examined the effect of several adenine nucleo-
otides on the interaction of ttUvrB with TMR-ssDNA. In the presence of ADP, AMP-PNP, or AMP-PCP, no time-dependent change in the fluorescence was observed, and the fluorescence emission spectra were almost the same as those in the absence of any nucleotide (data not shown). These results suggested that ATP hydrolysis is involved in the second phase. The $K_d$ values for TMR-ssDNA determined from the fluorescence titration (Fig. 7) were 1.06, 1.08, and 1.32 $\mu$M in the presence of ADP, AMP-PNP, and AMP-PCP, respectively. These values were similar to those in the absence and presence of ATP (Table I).

If the increase of the fluorescence intensity in the second phase corresponds to an increase in the number of the complexes between ttUvrB and TMR-ssDNA, the ATPase activity of ttUvrB should also have increased during the second phase. Thus, we measured the time-dependent change in ATP hydrolysis by ttUvrB. In the absence of DNA, the rate of ATP hydrolysis by ttUvrB was 0.80 $\mu$M$\cdot$sec$^{-1}$ and remained unaltered during the period of measurement (200 min). In the presence of TMR-ssDNA, the initial rate (during the first phase) was 2.41 $\mu$M$\cdot$sec$^{-1}$, which was three times higher than that in the absence of TMR-ssDNA, whereas the rate after 100 min (during the second phase) was 2.84 $\mu$M$\cdot$sec$^{-1}$. The ratio of the rate after 100 min to the initial rate was 1.18, whereas the ratio of the enhancement in the fluorescence intensity at the same time point was approximately 4.5 using the fluorescence intensity of TMR-ssDNA alone as a reference. These results disagree with the notion that the increase in the fluorescence intensity during the second phase represents the increase in the number of complexes of ttUvrB with TMR-ssDNA over time. Some other event must be occurring during the second phase (see "Discussion").

**DISCUSSION**

Based on the fluorescence measurements using TMR-ssDNA, we analyzed the interaction of Uvr proteins with TMR-ssDNA and normal ssDNA. In the absence of ATP, ttUvrA could bind to TMR-ssDNA with at least 100 times higher affinity than N-ssDNA. In the presence of ATP, however, ttUvrA
showed little difference in its affinity for TMR-ssDNA and N-ssDNA. This result is consistent with previous work using filter binding assays, which indicated that in the presence of ATP *E. coli* UvrA (ecUvrA) binds to ssDNA regardless of UV irradiation (5). Comparison of *Kd* values (Table I) indicates that the loss in damage specificity of ttUvrA by the addition of ATP results from enhancement of the ability of ttUvrA to bind normal ssDNA. The fluorescence intensity in the presence of ATP was much higher compared with that in the absence of ATP. This change in fluorescence suggests that ATP alters the interaction between ttUvrA and TMR-ssDNA and that this also occurs with N-ssDNA. It has been suggested that hydrophobic interactions are an important driving force for ecUvrA binding to BPDE-damaged DNA (19). As the fluorescence emission of the DNA backbone (31). Therefore, the increase of the fluorescence intensity was increased by a factor of about 4.5, whereas the fluorescence intensity was increased by a factor of about 1.2. This was unexpected as the increase in ATPase activity should have paralleled the increase in the number of ttUvrB molecules bound to TMR-ssDNA. Thus, it is unlikely that the increase in the fluorescence intensity during the second phase represents an increase in the ttUvrB-TMR-ssDNA complex.

An alternative explanation for the second phase is that a slow conformational change occurred in the complex between ttUvrB and TMR-ssDNA. According to this hypothesis, the first phase may be considered to correspond to the initial binding of ttUvrB to TMR-ssDNA and the second phase to a slow change of a microenvironment around the TMR moiety in the complex. Presumably, this conformational change would make the environment more polar, resulting in an increase in the fluorescence intensity of TMR. TMR-ssDNA contains a C-6 alkyl linker between the TMR moiety and the thymine base of the DNA (Fig. 1B). Such a linker is supposed to cause the mobility of a fluorophore, which is independent on the DNA backbone (31). Therefore, the increase of the fluorescence polarization during the second phase might be explained by a conformational change that restrains the mobility of dynamics of the TMR moiety. The slight increase of ATPase activity can be also explained by the assumption that the ATPase activities of the initial and final ttUvrB-ssDNA complexes are different.

Interestingly, the limited helicase activity of UvrB has been shown to display such time-dependent behavior (32, 33). It requires about 90 min to release the short strand with a lesion from the duplex, and this event is dependent on ATP hydrolysis. The time-dependent change observed in our study may reflect a process occurring during limited helicase activity because of the necessity of ATP hydrolysis and similar time-dependent behavior of both reactions. It has been proposed that UvrAB complex translocates on DNA by its limited helicase activity, and it is stalled at the damaged site and allows the following steps (32, 33). However, another research group has proposed that this release of an oligonucleotide from a duplex does not involve the translocation of UvrAB on DNA but rather involves a local conformational change around the damage site in DNA (34). As the DNA used in our study was not a duplex but an oligonucleotide of 30-mer, it seems unlikely that ttUvrB translocated on this short ssDNA with helicase-like activity. Our results support the latter model, which proposes a local conformational change around the damage site. We also consider that a local conformational change can take place even in damaged ssDNA.

The UvrB-DNA preincision complex is stable with a half-life about 2 h (35). This complex causes some characteristic conformational changes, and the dsDNA in the ecUvrB-DNA complex is thought to be partly unwound (7, 10–13). The formation of UvrB-DNA preincision complex requires the limited helicase activity (15, 16). In addition, it was recently shown that ecUvrB specifically binds to ssDNA containing a lesion but not normal ssDNA or dsDNA (8). The conformational changes observed in UvrB-DNA preincision complex are necessary for the following UvrC binding (15, 16), and UvrC also can bind only ssDNA (3). These observations raise the possibility that UvrB binds to the ssDNA portion in the damaged dsDNA, and this ssDNA portion is necessary for the UvrC binding. The tertiary structure of the core domains of ttUvrB was quite similar to those of some helicases (36–38). Based on the similarity to the structures of helicases, it is considered that UvrB interacts with ssDNA portion in its complex with dsDNA (37). Therefore, the conformational change in TMR-ssDNA observed in our study might partly reflect that in UvrB-DNA preincision complex.

The cryptic ATPase activity of ecUvrB was detected only in the presence of ecUvrA and DNA, and the ATPase activity of ecUvrAB-DNA complex is more highly stimulated by a damaged dsDNA than by a normal dsDNA (39). Although this stimulation has been supposed to represent an increase of the ATPase activity of ecUvrB (40), it has been difficult to verify this clearly because ecUvrB alone exhibits no ATPase activity. Unlike ecUvrB, ttUvrB can hydrolyze ATP by itself, which enabled us to investigate the dependence of the UvrB ATPase activity on different kinds of DNA. In this work, we showed that an UV-irradiated poly(dT) stimulated the ATPase activity of ttUvrB more effectively than did an unirradiated poly(dT). This result indicates that a damaged ssDNA is capable of stimulating the ATPase activity of ttUvrB. It is interesting to note that TMR-ssDNA also stimulated ttUvrB ATPase activity much more effectively than did the corresponding normal ssDNA. These results suggest that TMR-ssDNA can be recognized by ttUvrB as a damaged ssDNA.

The above results also suggest that the ssDNA-dependent ATPase activity of ttUvrB has an important role to play in the recognition of a lesion on DNA. In this regard, it is interesting that TMR-ssDNA, which contains a bulky TMR moiety, stimulated the ATPase activity of ttUvrB more strongly than UV-irradiated poly(dT), which contains a non-bulky pyrimidine dimer. It was shown that ecUvrB forms a more stable complex with DNA having bulky adducts than with DNA having non-bulky adducts (30). The efficiency of NER is considered to depend on the stability of UvrB-DNA complex (41, 42). Thus, it is probable that the ATPase activity of UvrB-DNA complex relates to its stability and that the strength of ATPase activity directly influences the efficiency of NER. Although UvrB has been suggested to take a key role in NER, the activity of UvrB itself has been little studied. Spectroscopic analysis of ttUvrB,
described in this study, is expected to be a useful way to investigate further the reaction mechanism of UvrB.

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