Local Folding of the N-terminal Domain of *Escherichia coli* RecA Controls Protein-Protein Interaction*

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To obtain structural information about the self-association of the protein RecA, we studied urea denaturation of RecA by circular dichroism spectroscopy and gel filtration. Gel filtration analysis showed that urea at low concentrations (1.0–1.2 mol/L) dissociated the RecA oligomer to almost a monomeric state prior to the unfolding of each molecule. Upon treatment with 1.0 mol/L urea, the circular dichroism spectrum showed a decrease in the α-helical content of RecA. A similar decrease was observed in the absence of urea for RecA at an extremely low protein concentration; the RecA oligomer dissociated to an almost completely monomeric state. The properties of RecA at low urea concentrations were similar to those of a truncated RecA lacking the first 33 N-terminal residues (Δ33RecA). Addition of a synthetic peptide corresponding to the 33 N-terminal residues to Δ33RecA increased the α-helical content. These results suggest that local folding of the N-terminal domain is coupled to protein-protein interactions of monomeric RecA, which are involved in the regulation of filament formation. The dissociation constant for interaction between RecA monomers was determined from the ellipticity data to be 0.1 μM.

RecA, a protein from *Escherichia coli*, is a small protein with a relative molecular mass of approximately 38,000 (1, 2), characterized by numerous functional properties in its active form (3–5). These actions include homologous recombination of DNA strands, self-polymerization in the presence or absence of DNA, DNA-dependent hydrolysis of ATP, and stimulation of autocleavage of LexA and certain other repressor proteins. RecA forms various quaternary structures, which are inherent in the activities exhibited by the protein. RecA is active only when it forms a nucleoprotein filament, which consists of a helical array of monomers bound to single-stranded DNA (ssDNA). This highly ordered structure is sustained predominantly by protein-protein interactions between contiguous monomers (6).

RecA is an α/β protein, and its polypeptide chain is folded into three continuous domains, referred to as the N-terminal, central, and C-terminal domains (7). The nucleotide-binding site has been assigned to the central domain by analyzing the structure of the crystal containing ADP (8). Although no crystal of the RecA-DNA complex has been analyzed, two unordered loops in the central domain are presumed to be the DNA-binding sites (9–13). One interesting feature of crystalline RecA is that the RecA molecules form a helical filament even in the absence of DNA (7). Based on the structure in the crystal, two regions involved in protein-protein interaction have been identified; the N-terminal domain interacts with a complementary site in the central domain of the adjacent molecule in the filament. Nevertheless, the filament structure in the crystal resembles that of an inactive filament when observed by electron microscopy (14). Additionally, in the absence of DNA and nucleotides, RecA exists in multiple oligomeric states (15, 16) and protein-protein interactions within these oligomers have not been well studied.

We have recently studied the properties of an N-terminally truncated *E. coli* RecA, designated Δ33RecA, which lacks the first 33 amino acid residues (17). Δ33RecA shows reduced self-association, which results in reduced DNA binding and ATP hydrolysis. By contrast, in the presence of MgCl₂, Δ33RecA does self-associate, although a higher protein concentration and longer time are required for completion of the process than for the wild-type protein. These results suggested that the 33 N-terminal amino acid residues play an important role not only in protein-protein interactions but also in kinetic regulation of the self-association process. Separately, a controversial observation has been reported; a synthetic peptide corresponding to the first 24 amino acids of RecA was able to bind to DNA when the peptide underwent a conformational change from an unordered structure to an α-helix (18). Further study is required to reveal the region involved in the self-association of RecA.

To investigate the self-association process in detail, we studied the urea-induced denaturation of RecA. In the case of an oligomeric protein like RecA, studying denaturation can provide information about protein-protein interactions, because it involves dissociation of the oligomers in addition to unfolding of the monomers. Moreover, for a multifunctional and multidomain protein like RecA, stepwise denaturation may result in the stepwise loss of its functional properties. In this study, we found that partial unfolding was coupled to dissociation of the RecA oligomer. Interestingly, the properties of RecA at low urea concentrations were similar to those of Δ33RecA. We then synthesized a peptide corresponding to the first 33 N-terminal amino acid residues of RecA (Rec33) and investigated its properties. The results obtained suggest that a local folding transition in the N-terminal domain occurs upon protein-protein interaction of monomeric RecA.

**EXPERIMENTAL PROCEDURES**

*Materials and Reagents—* *E. coli* RecA (19) and Δ33RecA (17) were purified as described previously. The RecA concentration was determined using an ε₇₅₀ value of 2.16 × 10⁴ M⁻¹ cm⁻¹ (19). A peptide, corresponding to 33 N-terminal residues of *E. coli* RecA, was synthesized using a peptide synthesizer (model PSSM-8, Shimadzu, Kyoto, Japan).

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Japan) by the solid-phase method using 9-fluorenylmethoxycarbonyl chemistry. The sequence of the peptide was NH$_2$-AIDENKQKALAAAL-GQIEKQFGKSIMRLGEDR-CONH$_2$. The crude peptide was purified by preparative HPLC on a C$_{18}$ column using a linear gradient of acetonitrile in 0.05% trifluoroacetic acid. The purified peptide was stored by preparative HPLC on a C$_{18}$ column using a linear gradient of ace-

from 30 s after mixing, the turbidity was monitored at 360 nm was increased to 2.0M and beyond, the intensity over the whole wavelength range decreased coincidentally. Addition of 8.0 M urea resulted in substantial loss of the signal.

As shown in Fig. 1B (closed circles), the ellipticity at 222 nm decreased gradually as the concentration of urea increased (in this figure, the CD amplitudes are expressed as the molar ellipticity, $[\theta]_{222}$, to make it easy to compare the amplitudes of wild-type RecA and Δ33RecA). Although cooperative transitions were difficult to see clearly, a small transition was recognized at low urea concentrations. The transition midpoint was estimated to be at approximately 1.0 M urea, although no stable intermediate state was observed and the plateau in the transition curve was difficult to define. The transient intermediate state at a urea concentration of approximately 1.2 M was observed approximately 85% of the native CD spectral amplitude. This reduced intensity indicates that RecA in the presence of 1.2 M urea was in a partially unfolded state. By contrast, such a transition was not observed for Δ33RecA (Fig. 1B, open circles); low concentrations of urea, up to 1.2 M, had little effect on the intensity of its CD signal, which was close to that of wild-type RecA with 1.2 M urea. These results imply that the transition observed for the wild-type protein with approximately 1.0 M urea is associated with the N-terminal region.

Near-UV CD Spectra with Urea—The CD spectra of RecA in the near-UV region, 250–320 nm, are shown in Fig. 2A. The spectra in the presence of 0 and 0.8 M urea were similar to each other. When the concentration of urea was increased to 1.2 M, the shape of the spectrum altered and its sign at around 275 nm changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase. It was of interest that the sign of the signal changed from plus to minus upon the addition of the second phase.
near-UV region and for the transition in the far-UV region were very close to each other.

The spectrum of Δ33RecA in the native state was similar to that of the wild-type protein with 1.2 M urea (17). Its transition curve for urea denaturation was distinct from that of wild-type protein; no transition was recognized in the range of 0–3.0 M urea (Fig. 2B, open circles). Together with the far-UV CD spectra, this result implies that the N-terminal region is associated with the spectral transition of the wild-type protein with approximately 1.0 M urea.

Dependence of CD on Protein Concentration—At 10 μM, RecA exists in multiple oligomeric states (15). To reveal whether the transitions observed above reflected dissociation, unfolding, or both, the dependence of these transitions on the protein concentration was investigated (Fig. 3). In the far-UV region, the transition midpoint for the first transition was shifted to approximately 0.7 M urea at a protein concentration of 1 μM, whereas for 10 μM protein it was approximately 1.0 M urea (Fig. 3A). This dependence suggests that the first transition in the far-UV CD spectrum was associated with an oligomeric state of the protein. At a protein concentration of 40 μM, the transition curve was almost the same as that for 10 μM protein. This implies that, at concentrations above 10 μM, the oligomeric states of RecA were equivalent to changes in the secondary structure caused by the low concentration of urea.

A dependence on the protein concentration was also observed for the spectral transition in the near-UV region (Fig. 3B). At a protein concentration of 1 μM, the transition midpoint was shifted to approximately 0.6 M urea, which appeared to be close to the midpoint, 0.7 M urea, for 1 μM protein in the far-UV region. Contrary to the transition in the far-UV region, however, increasing the protein concentration to 40 μM resulted in a shift of the transition midpoint to approximately 1.2 M urea. These results suggest that this transition in the near-UV region was more directly related to the oligomeric states of the protein than that in the far-UV region. This interpretation may be supported by the fact that the near-UV CD spectra reflect tertiary and quaternary structures of the protein. Therefore, it appears that the spectroscopic transition observed with low concentrations of urea is associated with the dissociation process in the denaturation of RecA.

Gel Filtration in the Presence of Urea—Since complete denaturation of the oligomeric enzyme must involve dissociation to monomers, we wanted to know whether the transient intermediates observed above were oligomeric or monomeric. To evaluate the oligomeric state and the compactness of the molecule, gel filtration of RecA was performed in the presence of urea (Fig. 4A). In the absence of urea, RecA at 10 μM was eluted predominantly in the void volume of the column, and small fractions of the protein were also eluted at larger volumes,
indicating that RecA exists as various forms of oligomers in equilibrium.

As the concentration of urea was increased to 1.0 M, the elution peak broadened and the average size of the RecA molecules decreased, as summarized in the inset in Fig. 4A. This behavior was considered to reflect the process of RecA oligomer dissociation as the urea concentration increased.

When the concentration of urea was increased to 1.2 M, a large peak appeared near the void volume and a small peak was detected at 13.4 ml, which was the largest elution volume among the samples examined in this study. On the basis of the elution volumes of molecular mass standards in the native conformation, an elution volume of 13.4 ml corresponded to a relative molecular mass of approximately 55,000 in a folded conformation. This value is significantly larger than that of the RecA monomer calculated from its amino acid sequence. At a urea concentration of 1.4 M, RecA was eluted almost as a single peak, which was near the void volume of the column. Small peaks near the void volume were also observed for 0.8–1.2 M urea. Since unfolding of the polypeptide chain is expected to cause expansion of the apparent volume of the molecule, it is reasonable to consider that these peaks near the void volume corresponded to an unfolded state of the RecA molecule. The small drifts in the range of elution volume from 8 to 12 ml for urea concentrations above 1.0 M may also correspond to a variety of unfolded molecules. As the concentration of urea was increased further, the elution volumes increased only slightly (Fig. 4A, inset), but the shapes of their peaks became sharper, implying that the apparent size of the molecules had become larger.

As a control experiment, we examined the elution profile of Δ33RecA (Fig. 4B). In the absence of urea, the elution volume of Δ33RecA was a little larger than that of the wild-type monomer, indicating that it exists as a monomer. At a urea concentration of 1.2 M, Δ33RecA was eluted near the void volume of the column (data not shown). As denaturation of Δ33RecA did not involve dissociation, we concluded that this peak corresponded to an unfolded state of the molecule. This result supports the notion that the peak of wild-type RecA that eluted near the void volume corresponds to an unfolded state. Therefore, the results indicate that dissociation of RecA oligomer is followed by unfolding of the monomer.

Aggregation in the Presence of Urea—To further investigate the effect of urea on the oligomeric state of RecA, self-association in the presence of urea was examined by adding MgCl₂ to the solution, since MgCl₂ has been shown to induce association of RecA (15). The turbidity resulting from RecA aggregation is considered to be represented by the optical density of the solution at 360 nm, as shown in Fig. 5A. Without urea, the absorbance at 360 nm increased drastically immediately after the addition of MgCl₂ to a final concentration of 10 mM. At a urea

**Fig. 4.** Gel filtration of RecA in the presence of various concentrations of urea. A, the Superdex 200 HR column was eluted with 25 mM Tris-HCl, 1 mM dithiothreitol, and 100 mM KCl at pH 7.5. RecA (10 μM) was incubated at 25 °C for 24 h in the elution buffer containing the concentrations of urea indicated in the figure and was eluted with buffer containing the same concentration of urea. The inset shows the dependence of the elution volume of the peaks on the urea concentration. Closed and open circles represent the elution volume of the peaks corresponding to the native and unfolded states, respectively (see “Results” for details). B, the elution profile of Δ33RecA on a Superdex 200 HR column. Δ33RecA (10 μM) was eluted with 5 mM Tris-HCl (pH 7.5), 10% glycerol, 10 mM MgCl₂ at 25 °C (17).

**Fig. 5.** Self-association of RecA induced by MgCl₂ in the presence of various concentrations of urea. A, RecA (10 μM) was incubated at 25 °C for 4 h in 3 mM Tris-HCl at pH 7.5 containing the concentrations of urea indicated in the figure, and then MgCl₂ was added to a final concentration of 10 mM. After 30 s, the turbidity was monitored at 360 nm. In B, the protein concentration was 40 μM. Other conditions were the same as those described for A. The dotted line represents the curve for the sample of 10 μM RecA with 1.0 M urea. The numbers in both panels represent the concentrations of urea.
concentration of 0.2 M, an increase in turbidity also occurred immediately after the addition of MgCl₂ and association was almost complete 30 min later. The increase in turbidity became sigmoidal above 0.4 M urea and the time required for aggregation was much longer above 0.8 M urea. For 10 μM RecA, there was little increase in turbidity even after 200 min at a urea concentration above 1.0 M, as shown in Fig. 5B. These results suggest that the structural transition at approximately 1.0 M urea rendered RecA incapable of self-association. By contrast, when the protein concentration was increased to 40 μM, the turbidity increase occurred even in the presence of 1.0 M and 1.1 M urea (Fig. 5B). The increases were sigmoidal and the time required for aggregation was more than 1 h. These results indicate that the deficiency in self-association was compensated for, to some extent, by raising the protein concentration.

ATP Binding in the Presence of Urea—We also studied the ability of RecA to bind to ATP in the presence of urea by monitoring the CD spectra in the near-UV region (Fig. 6A). When ATP bound to RecA in the absence of urea, the CD signal at around 260 nm increased drastically, as shown in Fig. 6A (inset) (21). When ATP was added to RecA with 0.8 M urea, a smaller increase in the signal amplitude was observed only at around 285 nm. With 1.0 M urea, the addition of ATP resulted in a slight increase in ellipticity above 275 nm. These spectral changes indicated binding of ATP to RecA since the spectra of the mixtures were much larger than the sum of the spectra for RecA alone and ATP alone. With 1.2 M urea, little significant increase in the ellipticity was observed, indicating almost no binding of ATP to the protein. Therefore, these results suggest that the presence of urea affects the ability of RecA to bind to ATP. This may rationalize the observation that the addition of adenine nucleotides had no effect on the transition curve in the far-UV CD spectra (Fig. 6B).

Properties at an Extremely Low Protein Concentration—The study of urea denaturation indicated that the oligomeric state of RecA was reflected in the CD spectra. Since equilibrium of a variety of oligomeric forms depends on protein concentration as well as pH, ionic strength, and temperature (15), we speculated that the CD spectrum of RecA varies with the protein concentration decreased. These profiles had no distinguishable peak or shoulder, suggesting a rapid equilibrium among the various forms of the oligomer. Even when RecA was applied at 0.075 μM, the main peak was eluted at a volume of approximately 14.0 ml, corresponding to a globular protein with a relative molecular mass of approximately 46,000. This value was significantly larger than the value of 38,000 calculated from its primary structure, but was considerably smaller than expected for a dimer of RecA. Although this discrepancy might reflect a
A dissociation constant of 0.1 non-globular shape of the RecA molecule, it appears that the equilibrium of oligomeric states of RecA was biased toward the monomeric state at extremely low protein concentrations.

Next, we measured the far-UV CD spectrum of 0.065 μM RecA (Fig. 8A, continuous line). This spectrum was clearly similar to that of Δ33RecA (dotted line). As shown in Fig. 8B, the ellipticity at 222 nm depended on the protein concentration; it increased over the concentration range 0.5–5.0 μM, whereas below 0.5 μM and over 5.0 μM, it remained nearly constant at the respective ellipticities. By contrast, the ellipticity at 208 nm was virtually unaltered in the region between 0.1 and 50 μM protein.

According to this relationship, ellipticity at 222 nm can be considered to be an indication of an oligomeric state of RecA. The dependence of the oligomeric states, estimated from the ellipticity, on protein concentration seems similar to that obtained by electron microscopy (15). Furthermore, an equilibrium constant for the interaction between RecA monomers can be estimated from the data shown in Fig. 8B, although there are several possible descriptions of this oligomerization process, which depend on the relative stabilities of distinct oligomers (15). If the monomers (A) and hexamers (A₆) are significantly populated states in the transition zone, the overall reaction will be described as a concerted reaction in the following manner.

$$K_d = \frac{[A_6]}{[A]}$$  

The dissociation constant ($K_d$) is defined as shown in Equation 1.

$$K_d = [A]^6[A_6].$$  

(Eq. 1)

The brackets denote concentration. The total concentration ($[A]$) of monomers and hexamers is related to the respective concentrations, as shown in Equation 2.

$$[A] = [A] + 6[A_6]$$  

(Eq. 2)

The observed ellipticity ($\theta_{obs}$) is expressed as shown in Equation 3.

$$\theta_{obs} = \theta_A \cdot [A] + \theta_{A6} \cdot [A_6]$$  

(Eq. 3)

$\theta_A$ and $\theta_{A6}$ are the molar ellipticities of the monomers and hexamers, respectively. The values of $\theta_A$ and $\theta_{A6}$ were determined from the data in Fig. 8B. The parameter $K_d$ was determined by fitting Equation 3 to the observed ellipticities at various concentrations of $A_0$. The determined value of $K_d$ was approximately 0.1 μM.

The CD amplitude was restored by concentrating the diluted RecA solution (data not shown). In addition, the CD spectra of the 5 μM RecA solution containing 1.0 M urea recovered after dialysis to approximately 90% of those seen in the absence of urea (data not shown). These results suggest that alterations to the secondary structure of RecA caused by lowering the protein concentration or by adding low concentrations of urea are reversible, and they also appear to support the notion that this transition is associated with the dissociation process.

Conformational Change of Rec33—The properties of RecA at low concentrations of urea were similar to those of Δ33RecA, which lacks the first 33 amino acid residues (see “Discussion”). This similarity suggests that the partial unfolding upon dissociation of RecA oligomers occurs in the N-terminal region. To verify the involvement of the N-terminal region in the association/dissociation process, we synthesized a peptide corresponding to residues 1–33 of the wild-type protein, designated Rec33, and characterized its structural properties.

The far-UV CD spectrum of Rec33 did not show any feature characteristic of an ordered structure, as depicted in Fig. 9A (thin line). When Δ33RecA was added to this peptide, the ellipticity of the mixture showed an apparent increase in the regions centered at around 220 nm and 210 nm compared with the sum of the spectra of Rec33 and Δ33RecA. The difference between the spectrum of the mixture and that of Δ33RecA alone is shown in Fig. 9A (shaded line). The difference spectrum appears to be characteristic of an α-helix, since it shows double maxima at around 210 and 220 nm. Furthermore, the spectrum of the mixture of Δ33RecA and Rec33 was similar to that of the wild-type protein, although the ellipticity of the former was slightly smaller than that of the latter (data not shown). These results indicate that some secondary structures including an α-helix were induced upon interaction between Δ33RecA and Rec33.

Addition of Rec33 to RecA, even up to 10 times the amount of the wild-type protein (1–10 μM), resulted in little induction of
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**DISCUSSION**

In the absence of DNA and nucleotides, RecA exists in multiple oligomeric states in reversible equilibrium with the distribution depending on solution conditions. Equilibrium ultracentrifugation has indicated that monomers, trimers, hexamers, and dodecamers are the predominant oligomeric species (16). Electron microscopic analyses also revealed four discrete aggregation states: monomers, rings, rods, and large bundles of rods (15). Among them, the rings are hexameric and most likely planar and the protein-protein interactions within the rings must be significantly different from those within the filaments (22). In the filament structure observed in the crystal form, the N-terminal domain directly contributes to the contacts between the proteins (7). We had previously studied Δ33RecA, a truncated RecA protein lacking the first 33 amino acid residues, and our results indicated that the N-terminal region of RecA plays an important role in protein-protein interaction not only in the filaments but also in smaller oligomeric forms (17). Yu and Egelman (23) recently proposed a structure for the hexameric rings, in which the N-terminal domain is involved in protein-protein interactions but in a different manner from that within the filaments.

In this study, we investigated further the self-association process using denaturation experiments, and our results indicate that partial unfolding and folding accompany the dissociation of RecA oligomers and the association of RecA monomers, respectively. Partial unfolding of the RecA molecule at low concentrations of urea or at an extremely low protein concentration can explain our inability to detect a compact monomer by gel filtration. Another possible explanation is that the RecA monomer exists in a molten globule state; however, the near-UV CD spectra obtained with 1.2 M urea showed that RecA retained an ordered tertiary structure, although it was different from that of the native state. In addition, no increase in the fluorescence intensity of 1-anilino-naphthalene-8-sulfonic acid was observed in the course of urea-induced denaturation of RecA (data not shown). These results exclude the possibility that the intermediate state corresponds to a molten globule state. Therefore, it is probable that unfolding upon dissociation occurs locally in a specific region of the molecule.

The results also suggest the region at which the local unfolding occurs. RecA with low concentrations of urea showed some properties distinct from the intact protein: 1) compared with that of the intact protein, the CD amplitude centered at around 220 nm was reduced, as was that of the protein at an extremely low concentration without urea; 2) the near-UV CD spectrum was changed drastically and the CD signal centered at around 275 nm was altered from plus to minus; 3) the CD spectral change upon binding to ATP was much smaller than that for the native protein and a signal increase was observed only at around 285 nm; 4) RecA showed reduced self-association induced by MgCl₂, but a higher protein concentration and longer incubation time than that used without urea enabled the protein to self-associate and aggregate in the presence of urea. All these biochemical properties closely resemble those of Δ33RecA (17). In addition, no transition was recognized in the denaturation curves of CD spectra for Δ33RecA in the region of low urea concentrations. Therefore, we conclude that a folding transition of the N-terminal domain occurs upon protein-protein interaction.

The results of the present study, together with the crystal structure (7, 23), yield a picture of the protein-protein interaction of RecA (Fig. 10). In the absence of urea, the N-terminal domain is unfolded at low protein concentrations and it folds into an α-helix upon self-association when the protein concentration is increased. In the presence of low concentrations of

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**Fig. 9. CD spectra of Rec33.** A, the spectra of 10 μM Rec33 alone (thick line) and in the presence of 10 μM Δ33RecA (thin line) and 10 μM RecA (dotted line) were measured with a 1-mm path length cell in 5 mM Tris-HCl (pH 7.5). The spectra of Δ33RecA and RecA were subtracted to give the thin and dotted lines, respectively. B, the spectra of 4 μM Rec33 alone (thick line) and in the presence of 20 μM poly(dT) (thin line) and 90% trifluoroethanol (broken line) were measured with a 1-mm path length cell in 5 mM Tris-HCl (pH 7.5). The spectra of poly(dT) and trifluoroethanol alone were subtracted to give the thin and broken lines, respectively.

α-helical content, as shown in Fig. 9A (dotted line), indicating very weak interaction between them (Kd > 1 mM). Therefore, the possibility that a nonspecific interaction between Rec33 and RecA had induced the conformational change was ruled out. As a control experiment, we replaced Δ33RecA with carbonic anhydrase, which is similar to RecA in size (Mₐ, approximately 30,000) and isoelectric point (5.4), and observed no additional increase in the spectrum of the mixture compared with the sum of the individual spectra (data not shown). This result excludes the possibility that Rec33 nonspecifically interacted with Δ33RecA.

To analyze the functional interaction of these fragments, the effect of Rec33 binding to Δ33RecA was studied using an ssDNA-dependent ATPase assay. Δ33RecA showed no ssDNA-dependent ATPase activity, as reported previously (17). Addition of Rec33, even up to 10 times the amount of Δ33RecA, induced no increase in the ATPase activity (data not shown). As RecA-mediated reactions require an active nucleoprotein filament, this result indicates that the Rec33-Δ33RecA complex failed to form a helical filament, which is directly related to the self-association process. This result also suggests the mode of interaction between Rec33 and Δ33RecA (see “Discussion”).
the peptide could not adopt an arrangement to the N-terminal domain of the wild-type protein; however, we speculate that Rec33 occupied the position on the RecA molecule (23), respectively. See "Discussion" for details.

The structures of wild-type RecA and the ssDNA-dependent ATPase activity of RecA are according to the crystal structure (7) and the intrinsic conformation of the N-terminal domain in a monomeric state is in an unordered structure. Furthermore, based on the tertiary structure, it seems unlikely that Rec33 was located at the position corresponding to the N-terminal domain of the wild-type protein because the N-terminal domain protrudes from the RecA molecule (7).

The finding of a local folding transition of the N-terminal domain enabled us to investigate a primary reaction of the self-association process. By fitting the equation for an equilibrium between the monomers and hexamers to the observed ellipticities, the dissociation constant for the interaction between RecA monomers was determined to be approximately 0.1 μM. Such spectroscopic analyses will be useful for further investigations of the self-association processes of RecA.

Another important aspect of the proposed model is that the N-terminal domain is involved in kinetic regulation of the self-association process. We suggested previously that the N-terminal region of RecA plays an important role not only in protein-protein interactions but also in kinetic regulation of the self-association process (17). Since treatment with a low concentration of urea resulted in a slow rate of association/dissociation, it is probable that an energy barrier between oligomeric states of RecA is reduced by the unfolding of the N-terminal domain. It has been shown that the association/dissociation process of RecA is entropically driven and that a significant number of water molecules is required to facilitate association/dissociation (16). There are several well ordered water molecules largely buried in the interface between neighboring monomers (7). Therefore, the absence or unfolding of the N-terminal domain, which constitutes one complementary face in the interface, should result in a decrease in the number of bound water molecules, leading to slow kinetics for interactions between RecA molecules. Although the intermediates in the self-association pathways, including nucleoprotein filament formation, have not been defined, it has been indicated that the oligomeric structure must disassemble into smaller oligomeric structures, probably monomers, prior to filament formation (16). As these processes necessarily include the dissociation/association processes, the folding transition of the N-terminal domain may have relevance to the oligomeric equilibrium of RecA.

It has been reported that a peptide corresponding to the first 24 amino acid residues of RecA stoichiometrically binds to ssDNA, which is associated with a folding transition from an unordered structure to an α-helix (18). In this study, we showed that induction of an α-helical conformation by the Rec33 occurs upon interaction not only with Δ33RecA but also with poly(dT) (Fig. 9B). It is uncertain whether or not the N-terminal region is involved in binding to ssDNA, since the structure of the RecA-DNA complex has not been determined.

The central and C-terminal regions, in addition to the N-terminal region, may be involved in protein-protein interactions and the accompanying structural changes. Near-UV CD spectra may help us to obtain information about these parts of the protein; these spectra are affected by alterations of the microenvironments of the aromatic residues and most of the aromatic residues are contained in those regions. The midpoint of the first phase on [θ]_275 in urea denaturation of RecA was dependent on the protein concentration over the range 1–40 μM (Fig. 3B). When 40 μM RecA was treated with 1.0–1.1 μM urea, the ability of the protein to self-associate was retained to some extent (Fig. 5B), even though the N-terminal domain was unfolded (Fig. 3A). Therefore, the spectral change in the near-UV region is thought to be closely associated with the protein-protein interaction itself, whereas the far-UV region is related to the folding state of the N-terminal region. The E. coli RecA protein contains seven tyrosine and two tryptophan residues (7). Tyr<sup>103</sup> is situated in the center of the central domain, Tyr<sup>218</sup> and Tyr<sup>218</sup> are located on the surface of the interface between contiguous RecA molecules in the crystal structure and it seems likely that these residues contribute to the CD spectrum in the near-UV region. The other three tyrosine and both tryptophan residues are located in the C-terminal domain.
environment of the side chains of these residues may be altered upon association/dissociation. If this is the case, a dynamic movement or conformational change would be expected to occur in the C-terminal domain.

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