Conserved Conformation of RecA Protein after Executing the DNA Strand-Exchange Reaction. A Site-Specific Linear Dichroism Structure Study†

Karolin Frykholm, Katsumi Morimatsu,* and Bengt Nordén

Departments of Chemical and Biological Engineering, and Physical Chemistry, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

Received March 29, 2006; Revised Manuscript Received June 16, 2006

ABSTRACT: RecA protein and its eukaryotic homologue Rad51 protein catalyzes the DNA strand exchange, which is a key reaction of homologous recombination. At the initial step of the reaction, RecA proteins form a helical filament on a single-stranded DNA (ssDNA). Binding of double-stranded DNA (dsDNA) to the filament triggers the homology search; as homology is found, the exchange of strands occurs, and the displaced DNA is released. These are the principal steps of genetic recombination; however, despite many years of extensive study of RecA activities, the details of the mechanism are still obscure. A high-resolution structure of the active nucleoprotein filament could provide information to help understand this process. Using a linear dichroism polarized-light spectroscopy technique, in combination with protein engineering (the site-specific linear dichroism method), we have previously studied the arrangement of RecA in complex with ssDNA. In the present study, we have used this approach to search for structural variations of RecA at the atomic level as the DNA in the complex is changed from ssDNA to dsDNA. The structural data of the RecA−dsDNA filament are found to be very similar to the data previously obtained for the RecA−ssDNA complex, indicating that the overall orientation and also the internal structure of RecA in the active filament are not markedly altered when the bound DNA changes from single- to double-stranded. The implications of the structural similarities as well as the significance of some conformational variations observed for a few amino acid residues that may be involved in interactions with DNA are discussed.

The RecA protein of *Escherichia coli* is a multifunctional enzyme that plays a crucial role in genetic recombination and DNA repair (*1*, *2*). It regulates the synthesis of proteins involved in the SOS pathway of DNA repair and catalyzes the strand−exchange reaction. For these activities, RecA first binds cooperatively to single-stranded DNA (ssDNA[1]) (*1*), forming a filamentous complex in which the protein monomers are arranged in a helical manner around the DNA. In the presence of a nucleotide cofactor, this nucleoprotein filament can bind a second, double-stranded DNA (dsDNA) molecule for the strand−exchange reaction.

The formation of the RecA−ssDNA complex appears as the initial step of the recombination process. Upon incorporation of a dsDNA molecule in the filament, the search for sequence homology takes place, followed by strand exchange. After the leaving of a single DNA strand, the nucleoprotein complex will consist of RecA and dsDNA, representing the final step or the product of the reaction. These are the basic principles of DNA recombination promoted by the RecA protein. RecA-like proteins are found in all organisms, and the bacterial RecA protein has long acted as the model protein for studies of DNA recombination and strand exchange (*1*−*5*). More recently, the activities of the RecA-homologue Rad51 in yeast and human, which are highly homologous in sequence to the bacterial RecA (*6*) and also form helical filaments on DNA, have also been studied (*7*−*9*). Despite many years of research, the detailed mechanism of strand exchange is still not fully understood. Structural information, on a high-resolution level, revealing how the protein monomers interact with each other and with DNA in the filament can give important clues to solve this puzzle.

X-ray crystal structures of the RecA protein itself, in different crystal forms, and its complex with ADP have been determined (*10*−*12*). However, both electron microscopy and small-angle neutron scattering indicate significant differences between the structures of the active RecA−DNA−ATP filament and the compressed, inactive filaments formed by RecA with ADP as a cofactor, with or without DNA (*13*−*15*).

We have previously presented a structural model of the RecA protein in its active filament with ssDNA, using data obtained by linear dichroism (LD) polarized-light spectroscopy (*16*). Our spectroscopic approach, called site-specific linear dichroism (SSLD), uses molecular replacement of amino acid chromophores inside a retained protein structure to determine their angular coordinates and has shown to be very well suited to study the structural organization of the

† We acknowledge the financial support from the European Commission (EU project, SNIPER), defraying a Research Fellowship to K.M., and the Swedish Cancer Foundation (grant from Cancerfonden to B.N.).

* Corresponding author. E-mail: kmorimatsu@ucdavis.edu.

[1] Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; LD, linear dichroism; SSLD, site-specific linear dichroism; poly(dεA), poly(1,N^6-ethenodeoxyadenosine); ATPγS, adenosine 5′-*O*-(3-thiotriphosphate).

10.1021/bi060621q CCC: $33.50 © 2006 American Chemical Society
Published on Web 08/25/2006

filamentous RecA−DNA complex. The SSLD is determined as the differential LD between the wild-type protein complex and a modified protein complex in which one protein residue is replaced by another amino acid. If the LD spectra of wild-type and modified complexes can be normalized with respect to orientation, then one can evaluate the SSLD to determine the angular orientation of the replaced residue. The details of this evaluation have been described elsewhere (*16*).

Having gained information on the structure of the active filament of RecA protein with ssDNA, the starting point of the recombination process, we now use the polarized-light spectroscopy technique developed to study the complex of RecA and a native dsDNA, the resulting product of the strand−exchange reaction.

MATERIALS AND METHODS

Materials. Wild-type (*17*) and modified RecA proteins (*16*) were prepared as described. Adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS) was purchased from Roche Molecular Biochemicals. Calf thymus DNA was purchased from Sigma-Aldrich.

LD Measurements. LD spectra were measured in a Couette flow device (*18*) as described elsewhere (*19*). The sample containing 4 μM RecA protein, 50 μM ATPγS, and 12 μM calf thymus DNA was preincubated at 22 °C for at least 2 h before loading into the system. The buffer contained 20 mM potassium phosphate (pH 7), 1 mM $MgCl_2$, and 50 mM NaCl. The spectra were averaged over 4 scans (scan speed 50 nm/min; bandwidth 2 nm; shear gradient 90 s^{-1}). As a baseline, spectra were collected without rotation of the cell.

Principles of LD. Linear dichroism (LD) is defined as the differential absorption between orthogonal forms of plane polarized light, where the polarization vector of the incident light beam is oriented parallel and perpendicular to the orientation of the sample as indicated by eq 1.

$$LD = A_{\text{parallel}} - A_{\text{perpendicular}} \quad (1)$$

The total *LD* of the filamentous RecA−DNA complex is a function of wavelength, λ, with contributions from all chromophores (*i*) and transitions (*u*) in it.

$$LD(\lambda) = \Sigma_i \Sigma_u LD_{iu}(\lambda) \quad (2)$$

The reduced linear dichroism is related to the angle α between the light-absorbing transition moment in the chromophore and the orientation axis of the filament as follows.

$$LD^{r}_{iu}(\lambda) = LD_{iu}(\lambda)/A^{iso}_{iu}(\lambda) = S(3/2)(3\cos^2\alpha_{iu} - 1) \quad (3)$$

where A^{iso}_{iu} is the isotropic absorption of the specific transition moment, and *S* is the degree of orientation of the filamentous complex. Note that for the tyrosine chromophore, the overlap in absorption of the L_b and *B* transition moments is negligible and therefore the LD_{iu} and A^{iso}_{iu} can easily be determined by SSLD analysis.

Normalization of Spectra. In our previous study (*16*), the use of ethenoadenine as an internal standard to normalize the LD spectra was crucial for the determination of the SSLD. This time, using a native DNA lacking the probe chromophore present in poly(dϵA), we explored another approach.

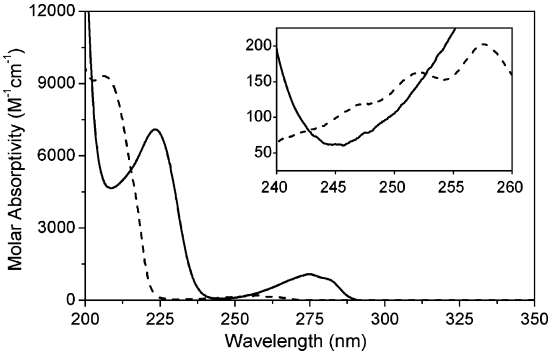

FIGURE 1: Absorption spectra of tyrosine (—) and phenylalanine (---), showing the magic wavelength at 250−255 nm.

The seven tyrosine residues of RecA have been the target for this SSLD analysis. The residues, one at a time, were replaced by phenylalanine, which structurally differs from tyrosine by only one oxygen. Thus, this substitution should not significantly affect the structure of RecA itself or its complex with DNA, which is a prerequisite for the SSLD technique. Assuming the residue change will not cause any significant conformational changes in the protein (an assumption that has to be justified by separate experiments), this molecular replacement is expected to change neither the orientation of the residue inside the protein nor the transition moments. However, the overall fiber orientation (parameter S in eq 3) may change.

Because of the structural similarity of tyrosine and phenylalanine, the replacement of a tyrosine residue in the protein by a phenylalanine will in principle correspond to the replacement of the tyrosine transition moments by the phenylalanine transition moments. The absorption spectra of tyrosine and phenylalanine (Figure 1) show wavelengths where the tyrosine and phenylalanine transitions have coinciding absorption intensities. At these magic wavelengths, we expect the LD intensity, after normalization with respect to the orientation factor *S*, of wild-type and modified nucleoprotein filaments to be the same.

The orientation factor *S* could, however, be a serious source of error because it may vary between different sample preparations and between different complexes. Even very small variations in *S*, if not corrected for, will give rise to large variations in the differentials between large-amplitude LD spectra. Therefore, a prerequisite for useful SSLD spectra is that the differential be formed from LD spectra that are normalized with respect to *S* with high accuracy. In other words, the absolute accuracy of determining *S* in eq 3 does not need to be very high (an error as large as ±20% in *S* will allow many angles to be sufficiently accurately determined for the present purposes). In contrast, the relative accuracy of *S* has to be high (preferably less than 5%) when correcting for variations in the LD spectra between which the differential LD is to be calculated. We have, therefore, normalized the LD spectra to the same LD intensity at a wavelength where the substitute and the substituted chromophores have identical absorption coefficients. This magic wavelength approach is equivalent to a normalization of orientation factors and generally gives the relative accuracy required (Morimatsu, K., and Nordén, B. Manuscript in preparation).

Construction of Structural Models. For construction of structural models of RecA−ssDNA−ATPγS and RecA−

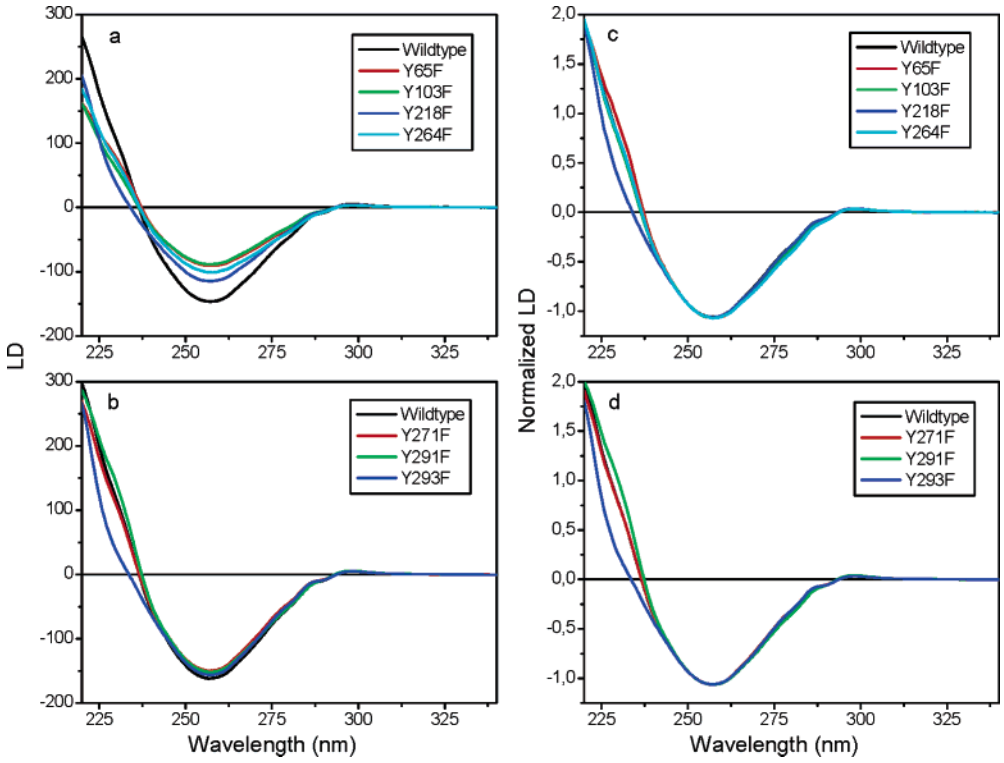

FIGURE 2: LD spectra of wild-type and modified protein complexes with calf thymus DNA. The raw LD spectra (a and b) show some differences in LD intensity due to variations in the degree of orientation. The spectra were normalized (c and d) with respect to LD intensity (set to −1) at 250−255 nm, a magic wavelength.

dsDNA−ATPγS filaments, the L_b and B transition moments of Tyr 218, Tyr 291, and Tyr 293 (six transition moments in total) were used to rotate the RecA monomers in the crystal structure filament (*20*) as described before (*16*). The rotations of RecA that fit the change in orientation for each residue were determined. The rotation of a RecA unit is described by a rotation θ_y of the *z*-axis (the long axis of the filament) around an orthogonal *y*-axis, followed by a rotation θ_z around the *z*-axis. This defines a trial RecA axis vector $\vec{a} = (x,y,z)$ as follows.

$$x = \sin\theta_y \cos\theta_z \quad y = \sin\theta_y \sin\theta_z \quad z = \cos\theta_y$$

Note that because of the cosine-square dependence of LD (eq 3), there is a sign ambiguity in the determination of angles. The trigonometric data of the residues were combined into pairs, and for each pair of angles, the trial angle that best fitted the LD data was iteratively selected; the fit was judged by computing the score $n/\Sigma\,|(\alpha - \alpha')|$, where n is the number of transition moments, α is the effective tilt angle of each transition moment investigated by SSLD, and α' is the tilt angle between the trial axis vector ($\vec{a}$) and the respective transition moment vector in the crystal structure. The trial rotation of the *z*-axis with the highest score, defined as (θ_y, θ_z), was determined as the result of this calculation (Figures S1a and b; see Supporting Information).

To construct a structural model of the RecA−DNA filament, the RecA monomer in the crystal filament was first rotated (θ_y, θ_z) around the origin of space using the following matrix:

$$\begin{bmatrix} \cos\theta_y\cos\theta_z & \cos\theta_y\sin\theta_z & -\sin\theta_y \\ -\sin\theta_z & \cos\theta_z & 0 \\ \sin\theta_y\cos\theta_z & \sin\theta_y\sin\theta_z & \cos\theta_y \end{bmatrix}$$

Then, all RecA monomers were aligned at these angular coordinates on a helical trajectory to make a 9.6 nm pitch filament containing 6 monomers per turn, as exercised before (*16*).

RESULTS

LD Spectra of RecA Protein Complexes and SSLD Spectra of Tyrosine Residues. The formation of a nucleoprotein complex of RecA and ssDNA in the presence of ATP is the initial step of the DNA strand−exchange reaction. As the reaction proceeds, the final complex will be that of the RecA protein, ATP, and dsDNA. As shown previously (*16*), the structure of the RecA−DNA filament can be studied using a polarized-light spectroscopy technique in combination with protein engineering. To compare the initial and final state structures of the RecA−DNA complexes in the DNA strand−exchange reaction, we have applied the SSLD spectroscopic approach on a RecA−ATP−dsDNA filament.

The seven tyrosines of the *E. coli* RecA protein were replaced one at a time by phenylalanine, using site-directed mutagenesis. Flow LD spectra of wild-type and modified RecA proteins in complex with native double-stranded calf thymus DNA in the presence of the nonhydrolyzable ATP analog ATPγS were measured. Assuming that the modified RecA proteins form nucleoprotein filaments of effectively the same structure as that of wild-type RecA protein, the LD differentials can be interpreted in terms of orientations of the substituted residue chromophores.

Figure 2 a and b show the raw LD spectra of wild-type and modified RecA protein−DNA complexes. As expected, there is a negative LD signal around 260 nm, the region of DNA base absorption, in agreement with a more perpendicular than parallel average orientation of the nucleobases

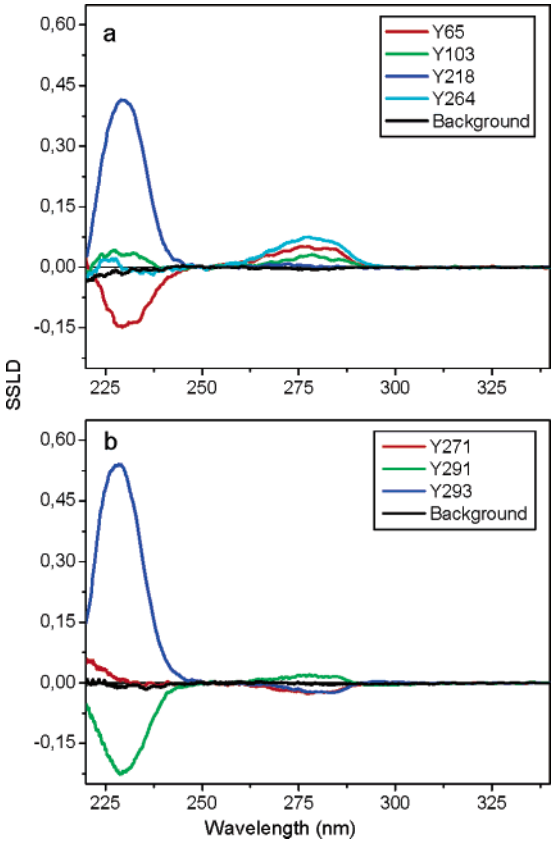

FIGURE 3: (a and b) SSLD spectra of each tyrosine residue computed by subtraction: (LD of wild-type RecA complex) − (LD of modified RecA complex). Background inidcates the differential spectrum of the two wild-type spectra that were recorded first and last in a series of measurements.

relative to the helix axis. The fine structure in the spectra in the 280 to 310 nm region is due to side-group chromophores in the protein, mainly benzene and indole of the tyrosine and tryptophan residues. The positive signal at shorter wavelengths also comes from the protein, with contributions from transitions in the aromatic residues and the peptide bonds. The degree of orientation varies somewhat between the different complexes, as seen from the difference in LD signal intensity. This emphasizes the importance of exact normalization in order to obtain the SSLD spectrum as the differential of wild-type and modified protein LD spectra.

Using the magic wavelength approach, which is equivalent to a normalization of orientation factors, the spectra were normalized with respect to the LD intensity at the magic wavelength 250−255 nm, where tyrosine and phenylalanine have coinciding absorptions (Figure 1). As seen in Figure 2 c and d, there is clearly a difference in the LD spectra of modified protein complexes compared to the LD spectrum of the wild-type protein complex. This difference, computed by subtracting the LD of modified RecA complex from the LD of wild-type RecA complex, is the SSLD of the corresponding substituted residue. Figure 3 a and b show the SSLD spectra of each tyrosine residue. The peaks are centered at 230 and 280 nm, as would be expected from the absorption of the *B* and L_b transition moments, respectively, of tyrosine. This agreement verifies the SSLD approach as a method to achieve structural information on specific residues in the filamentous complex.

Table 1: Angles of Tyrosine Transition Moments in the Active RecA Filaments.

transition moment	wavelength (nm)	angle α, (deg), dsDNA	angle α, (deg), ssDNA	angle in crystal (deg)
ssDNA	320−350		70 (80-60)[a]	
dsDNA	250−255	80 (90−70)[a]		
Tyr 65-B	227	67 (68−63)	81 (90[b]−62)	73
Tyr 65-L_b	278	32 (30−39)	47 (44−52)	40
Tyr 103-B	227	51 (51−52)	55 (55−55)	81
Tyr 103-L_b	278	41 (40−45)	46 (42−51)	32
Tyr 218-B	227	19 (13−31)	32 (21−46)	20
Tyr 218-L_b	278	53 (53−53)	58 (60−56)	80
Tyr 264-B	227	53 (53−53)	63 (67−58)	82
Tyr 264-L_b	278	18 (12−31)	37 (29−48)	12
Tyr 271-B	227	53 (53−54)	59 (61−56)	71
Tyr 271-L_b	278	68 (70−64)	77 (90[b]−62)	70
Tyr 291-B	227	79 (86−70)	77 (90[b]−62)	78
Tyr 291-L_b	278	46 (45−48)	50 (48−53)	19
Tyr 293-B	227	0[b] (0[b]−19)	10 (0[b]−40)	26
Tyr 293-L_b	278	66 (67−62)	66 (72−59)	65

[a] Angles of transition moments determined assuming ssDNA and dsDNA orientations in the filament are 70 ± 10° and 80 ± 10°, respectively. [b] LD^r/S value is out of range.

Orientation Angles of Tyrosine Residues. The angular orientation α of the transition moments of each tyrosine residue can be determined from the reduced LD (LD^r) if the degree of orientation, *S*, of the sample is known; there is a simple correlation between the angle and the LD^r/S ratio, as seen in eq 3. Assuming that the effective orientation angle of the DNA bases is 80 ± 10° (*19*), an approximate absolute value of the *S* factor can be determined from the normalized LD intensity at the magic wavelength 250−255 nm.

The LD^r/S spectra of all tyrosine residues can then be determined from the SSLD spectra, and hence, the α values can be calculated. Table 1 shows the calculated orientations relative to the helix axis of the tyrosine transition moments in RecA in complex with both ssDNA (*16*) and dsDNA. The corresponding angles of these transition moments calculated from the crystal structure of RecA in the presence of ADP (*20*) are also included. In general, the tyrosine orientation angles determined for the different RecA−DNA complexes are very similar. The most significant differences are found for the L_b and *B* transition moments of Tyr 65 and Tyr 264, while other differences in orientation angle are negligibly small or within the confidence interval. These results suggest that the global conformation of the RecA protein is conserved between the complexes with single- and double-stranded DNA, with only local conformational changes around Tyr 65 and Tyr 264.

The confidence intervals indicated for the calculated angles in Table 1 are determined from the limits given for the DNA base orientation. As discussed by Nordén et al. (*19*), the determination of the angle α for the DNA bases in the RecA−dsDNA complex is associated with rather large uncertainties. However, several different observations indicate a roughly orthogonal base orientation. Thus, a large α value is expected to be more accurate, the lower angle limit given possibly resulting from an overestimation of errors.

Orientation of RecA in the Filament with dsDNA. As discussed before (*16*), the changes in orientation of the tyrosine residues in the RecA−DNA complexes, compared to the protein structure in the crystal in the absence of DNA,

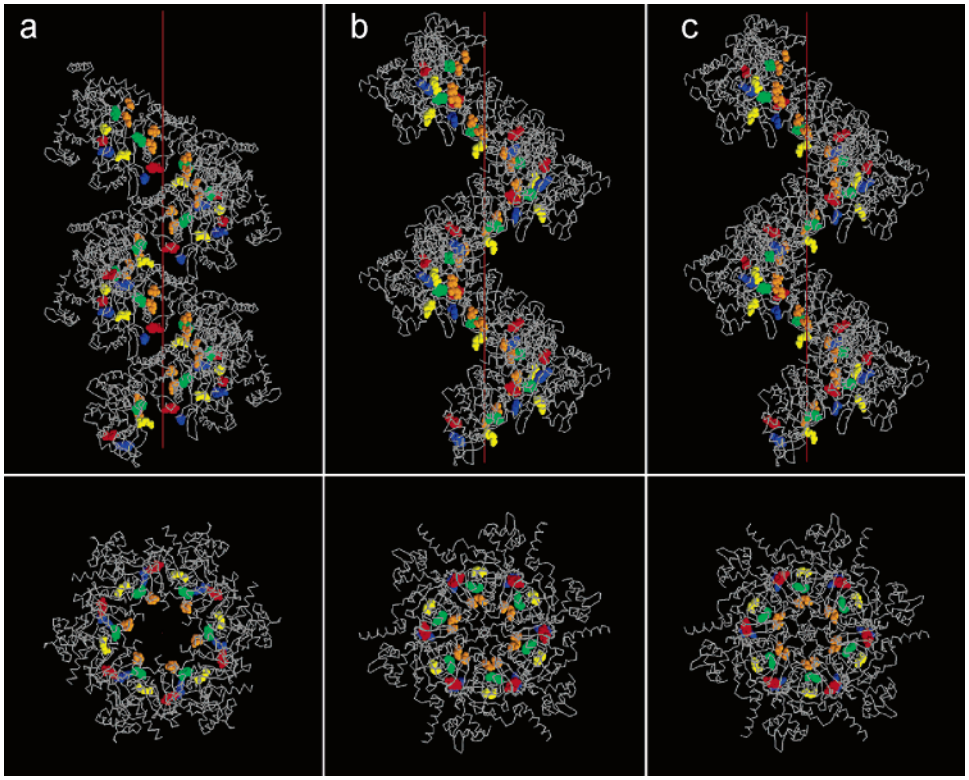

FIGURE 4: Comparison of the different RecA filament structures. The structural models of the RecA filaments with ssDNA (b) and dsDNA (c) were constructed by modification of the crystal filament structure (a) (*20*). The proposed DNA binding regions, Tyr 65 (green), Tyr 103 (red), residues 194 and 210 of loop 2 (orange), Arg 243 (yellow), and Tyr 264 (blue), were space-filled and colored. The helix axis (*z*-axis) of the filament is indicated by a red line. The RasMol program (version 2.7) was used for viewing.

could be due to local conformational changes and/or a change in orientation of the whole RecA unit in the filament. In the latter case, a concerted degree of rotation of all protein residues, relative to their orientation in the crystal structure, can be calculated, and a structural model of the RecA−DNA filament can be constructed. By considering the difference in orientation angles for the tyrosine transition moments between the RecA−dsDNA filament studied by SSLD and the RecA−ADP complex in the crystal, the degree of rotation of the whole protein unit was calculated, and a structural model was constructed (Figure 4) using the same method as that used previously (*16*). The L_b and B transition moments of Tyr 218, Tyr 291, and Tyr 293 (six transition moments in total) were used to rotate the RecA monomers in the crystal structure filament. Note that Trp 290 and Trp 308 were also used for the model construction in the previous study but were omitted in this article because of the lack of data due to the magic wavelength approach used for the normalization of LD spectra of RecA−dsDNA complexes. The rotation, (θ_y, θ_z), of the RecA monomer in the RecA−dsDNA complex was determined to be (36°, 349°), that is, 36° rotation around an axis orthogonal to the long axis of the filament followed by 349° rotation around the long axis, which is within the experimental uncertainty identical to the (36°, 351°) rotation previously determined for the RecA−ssDNA complex (*16*). Recalculating the rotation degree for the RecA−ssDNA complex, using the same six transition moments of Tyr 218, Tyr 291, and Tyr 293 used for the RecA−dsDNA complex, gives (41°, 348°), which only slightly differs from the previous value. This result again supports the suggestion that the global conformation of the RecA protein is the same in both RecA−ssDNA and RecA−dsDNA complexes. However, there are some variations in residue orientations, the significance of which will be discussed below.

DISCUSSION

The DNA strand exchange promoted by the RecA protein has been in focus for many years. However, despite numerous studies, the details of the mechanism of the strand−exchange reaction still remain obscure. Structural information is of great importance when it comes to understanding the function of the RecA protein and explaining the events of the recombination process. Gaining this information has been complicated by the fact that the active form of the RecA filament, in its complex with DNA and ATP, differs from the filament structure in the crystal form. Creating a model of the strand−exchange reaction also requires knowledge regarding the specific interactions between the protein and DNA.

Using a combined protein engineering and spectroscopic approach, we have previously presented a structural model of RecA in its active nucleoprotein filament with ssDNA (*16*). In that model, the proposed regions for DNA binding, Tyr 65 (*21*), Tyr 103, and Tyr 264 (*17*), Arg 243 (*22*, *23*), and loop 2 (*20*, *24*−*26*), are clustered near the center of the helical filament, in agreement with findings showing the DNA to be located close to the filament axis (*14*, *27*). Although the results of our previous study was concerned with the structure of RecA in complex with ssDNA, the present work extends the structural study of the RecA−DNA complex to also include the nucleoprotein filament with dsDNA.

Not surprisingly, the LD data of the RecA−dsDNA filament are very similar overall to the data previously obtained for the RecA−ssDNA complex. Calculating the orientation angles of the tyrosine transition moments, relative to the helix axis, also indicates that the changes in orientation of these residues are generally small. It is, thus, likely that the overall orientation and internal structure of RecA in the active filament is not significantly altered as the bound DNA is changed from single- to double-stranded. Interestingly, the most notable differences in orientation are seen for the L_b and *B* transitions of Tyr 65 and Tyr 264, and some minor changes are also observed for the transitions of Tyr 103 and Tyr 271. Tyrosines 65, 103, and 264 have been suggested to interact with DNA, and thus, a small conformational change at these residues as the DNA incorporated in the filament changes from one to two strands does not seem to be unreasonable. Tyr 271 has so far not been reported to be involved in any specific function of RecA, but the small change in orientation at this residue might be related to a conformational change at Tyr 264 and a role for this residue in the interaction with nucleic acid or reflecting a concerted motion within a protein machinery, which we think is less likely.

The RecA protein has long acted as the model protein for studying DNA recombination and strand exchange. In recent years, the interest for these processes has been increased to also include the activities of Rad51, the eukaryotic homologue of RecA. The Rad51 genes are highly conserved in eukaryotes, from yeast to human (*6*). All of these proteins show homology with the RecA protein of *E. coli*, but only in a 221-residue core region including residues 33−240 of RecA.

Tyr 65 of RecA, in the homologous region, is conserved among the recombination proteins corresponding to Phe 184 in *Saccharomyces cerevisiae*. Investigation of the crystal structure of the *S. cerevisiae* Rad51 filament (*28*) shows that the orientation angles of the Phe 184 transition moments, relative to the filament axis, are similar to the angles determined for Tyr 65 of RecA. The Rad51 filament has a pseudo 6-fold symmetry, but alternating protein−protein interfaces are slightly different, indicating a dimeric functional unit. This is also seen in the calculated orientation of Phe 184 in two adjacent Rad51 monomers, where the angles are slightly different. The crystals were grown in the presence of ssDNA, and the orientation of the Phe 184 B-transitions is in good agreement with the angle obtained for the *B* transition of Tyr 65 in the RecA−ssDNA filament. The orientation angles of the L_b transitions in the Rad51−Phe184 are in the same range as the angles calculated for RecA Tyr 65 in both the ssDNA and dsDNA filaments. However, comparison of exact angles should be made with some caution because the structural model of the Rad51 filament is based on crystallographic data of only 3.25 Å resolution, and experimental errors of angles from the LD measurements are relatively large for angles that are small (0−30°) as well as large (70−90°). Nevertheless, the overall similarity of the orientation angles is encouraging and indicates that this residue is conserved not only sequentially but also structurally.

The results presented in this study contain information that gives rise to several important questions regarding the mechanism of DNA recombination and strand exchange. The overall orientation and internal structure of RecA in the active filament is not significantly altered because the bound DNA changes from single stranded, representing the initial state of the strand−exchange reaction, to double-stranded DNA, which is the final state of the filament after the DNA strand−exchange reaction has been performed. This could indicate that the protein is on the whole a static structure during the recombination process, which in turn suggests that the recombination protein might not have the most advanced function in the recombination machinery but may rather act in a more assisting way, like a static scaffold. It is important to note the possibility that during the recombination process there is a transient state (or even transition state) in which the filament structure is totally different from the initial and terminal structures. This transient/transition state could be related to the hydrolysis of ATP to ADP, known to extensively change the structure of the RecA−DNA filament. ATP hydrolysis is required for efficient branch migration after the formation of heteroduplex joints, whereas homologous pairing can occur in the presence of the nonhydrolyzable ATP analogue ATPγS (*29*). The static model for RecA during the initial phases of the strand−exchange reaction is interesting; as the protein searches for homology between two DNA molecules, the static scaffolding would provide a proficient environment for the DNA molecules to react. Even if there is a transient state during the process, the scaffolding that is found in the RecA−ssDNA complex must play an important role for the homology-search stage of the recombination reaction. By its formation of a helical structure onto DNA and its interactions with DNA, the protein helps DNA to perform the strand−exchange reaction, providing the conditions needed for the recombination reaction to occur. The importance of the unusual DNA structure induced by the RecA protein in the nucleoprotein filament is also suggested by NMR analysis for the structure of DNA bound to RecA or Rad51 proteins (*30*, *31*). Thus, the details of the specific interactions between the recombination protein and the DNA and the consequences these interactions may have on the DNA structure could be a key to a better understanding of the mechanism of genetic recombination and DNA strand exchange.

SUPPORTING INFORMATION AVAILABLE

Figures showing the calculated scores for the trial rotation used to construct the structural models of the RecA−DNA filaments. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Prévost, C., and Takahashi, M. (2003) Geometry of the DNA strands within the RecA nucleofilament: role in homologous recombination, *Q. Rev. Biophys. 36*, 429−453.
2. Roca, A. I., and Cox, M. M. (1997) RecA protein: structure, function, and role in recombinational DNA repair, *Prog. Nucleic Acid Res. Mol. Biol. 56*, 129−223.
3. Cox, M. M. (1999) Recombinational DNA repair in bacteria and the RecA protein, *Prog. Nucleic Acid Res. Mol. Biol. 63*, 311.
4. Radding, C. M. (1991) Helical interactions in homologous pairing and strand exchange driven by recA protein, *J. Biol. Chem. 266*, 5355.
5. Takahashi, M., and Nordén, B. (1994) Structure of RecA-DNA complex and mechanism of DNA strand exchange reaction in homologous recombination, *Adv. Biophys. 30*, 1.

6. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K., and Ogawa, T. (1993) Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA, *Nat. Genet. 4*, 239−243.
7. Baumann, P., and West, S. C. (1998) Role of the human RAD51 protein in homologous recombination and double-stranded-break repair, *Trends Biochem. Sci. 23*, 247−251.
8. Bianco, P. R., Tracy, R. B., and Kowalczykowski, S. C. (1998) DNA strand exchange proteins: a biochemical and physical comparison, *Front. Biosci. 3*.
9. Sung, P., Krejci, L., Van Komen, S., and Sehorn, M. G. (2003) Rad51 recombinase and recombination mediators, *J. Biol. Chem. 278*, 42729.
10. Story, R. M., and Steitz, T. A. (1992) Structure of the recA protein-ADP complex, *Nature 355*, 374−376.
11. Xing, X., and Bell, C. E. (2004) Crystal structures of *Escherichia coli* RecA in complex with MgADP and MnAMP-PNP, *Biochemistry 43*, 16142−16152.
12. Xing, X., and Bell, C. E. (2004) Crystal structures of *Escherichia coli* RecA in a compressed helical filament, *J. Mol. Biol. 342*, 1471−1485.
13. Ellouze, C., Takahashi, M., Wittung, P., Mortensen, K., Schnarr, M., and Nordén, B. (1995) Evidence for elongation of the helical pitch of the RecA filament upon ATP and ADP binding using small-angle neutron scattering, *Eur. J. Biochem. 233*, 579−583.
14. Timmins, P. A., Ruigrok, R. W., and DiCapua, E. (1991) The solution structure of recA filaments by small angle neutron scattering, *Biochimie 73*, 227−230.
15. Yu, X., VanLoock, M. S., Yang, S., Reese, J. T., and Egelman, E. H. (2004) What is the structure of the RecA-DNA filament? *Curr. Protein Pept. Sci. 5*, 73−79.
16. Morimatsu, K., Takahashi, M., and Nordén, B. (2002) Arrangement of RecA protein in its active filament determined by polarized-light spectroscopy, *Proc. Natl. Acad. Sci. U.S.A. 99*, 11688−11693.
17. Morimatsu, K., Horii, T., and Takahashi, M. (1995) Interaction of Tyr103 and Tyr264 of the RecA protein with DNA and nucleotide cofactors. Fluorescence study of engineered proteins, *Eur. J. Biochem. 228*, 779−785.
18. Wada, A., and Kozawa, S. (1964) Instrument for Studies of Differential Flow Dichroism of Polymer Solutions, *J. Polym. Sci., Part A: Gen. Pap.* 2, 853−864.
19. Nordén, B., Elvingson, C., Kubista, M., Sjoberg, B., Ryberg, H., Ryberg, M., Mortensen, K., and Takahashi, M. (1992) Structure of RecA-DNA complexes studied by combination of linear dichroism and small-angle neutron scattering measurements on flow-oriented samples, *J. Mol. Biol. 226*, 1175−1191.
20. Story, R. M., Weber, I. T., and Steitz, T. A. (1992) The structure of the *E. col*i recA protein monomer and polymer, *Nature 355*, 318−325.
21. Morimatsu, K., Funakoshi, T., Horii, T., and Takahashi, M. (2001) Interaction of tyrosine 65 of RecA protein with the first and second DNA strands, *J. Mol. Biol. 306*, 189−199.
22. Morimatsu, K., and Horii, T. (1995) Analysis of the DNA binding site of *Escherichia coli* RecA protein, *Adv Biophys. 31*, 23−48.
23. Rehrauer, W. M., and Kowalczykowski, S. C. (1996) The DNA binding site of the *Escherichia coli* RecA protein, *J. Biol. Chem. 271*, 11996−12002.
24. Malkov, V. V., and Cameriniotero, R. D. (1995) Photocross-links between single-stranded-DNA and *Escherichia-coli* reca protein map to loops L1 (amino-acid-residues 157-164) and L2 (amino-acid-residues 195-209), *J. Biol. Chem. 270*, 30230−30233.
25. Morimatsu, K., and Horii, T. (1995) DNA-binding surface of RecA protein photochemical cross-linking of the first DNA binding site on RecA filament, *Eur. J. Biochem. 234*, 695−705.
26. Wang, Y., and Adzuma, K. (1996) Differential proximity probing of two DNA binding sites in the *Escherichia coli* recA protein using photo-cross-linking methods, *Biochemistry 35*, 3563−3571.
27. Yu, X., and Egelman, E. H. (1992) Structural data suggest that the active and inactive forms of the RecA filament are not simply interconvertible, *J. Mol. Biol. 227*, 334−346.
28. Conway, A. B., Lynch, T. W., Zhang, Y., Fortin, G. S., Fung, C. W., Symington, L. S., and Rice, P. A. (2004) Crystal structure of a Rad51 filament, *Nat. Struct. Mol. Biol. 11*, 791−796.
29. Cox, M. M., and Lehman, I. R. (1981) recA protein of *Escherichia coli* promotes branch migration, a kinetically distinct phase of DNA strand exchange, *Proc. Natl. Acad. Sci. U.S.A. 78*, 3433−3437.
30. Nishinaka, T., Shinohara, A., Ito, Y., Yokoyama, S., and Shibata, T. (1998) Base pair switching by interconversion of sugar puckers in DNA extended by proteins of RecA-family: a model for homology search in homologous genetic recombination, *Proc. Natl. Acad. Sci. U.S.A. 95*, 11071−11076.
31. Shibata, T., Nishinaka, T., Mikawa, T., Aihara, H., Kurumizaka, H., Yokoyama, S., and Ito, Y. (2001) Homologous genetic recombination as an intrinsic dynamic property of a DNA structure induced by RecA/Rad51-family proteins: a possible advantage of DNA over RNA as genomic material, *Proc. Natl. Acad. Sci. U.S.A. 98*, 8425−8432.

BI060621Q