Nucleotide Cofactor-Dependent Structural Change of *Xenopus laevis* Rad51 Protein Filament Detected by Small-Angle Neutron Scattering Measurements in Solution†

Christine Ellouze,‡ Hye-Kyung Kim,§ Kazuhiro Maeshima,|| Eimer Tuite,§ Katsumi Morimatsu,|| Toshihiro Horii,|| Kell Mortensen,⊥ Bengt Nordén,§ and Masayuki Takahashi*,‡

UMR 216, Institut Curie and CNRS, F-91405 Orsay, France, Department of Physical Chemistry, Chalmers University of Technology, S-412 96 Gothenburg, Sweden, Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, and Physical Department, Risø National Laboratory, Roskilde, Denmark

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ABSTRACT: Rad51 protein, a eukaryotic homologue of RecA protein, forms a filamentous complex with DNA and catalyzes homologous recombination. We have analyzed the structure of *Xenopus* Rad51 protein (XRad51.1) in solution by small-angle neutron scattering (SANS). The measurements showed that XRad51.1 forms a helical filament independently of DNA. The sizes of the cross-sectional and helical pitch of the filament could be determined, respectively, from a Guinier plot and the position of the subsidiary maximum of SANS data. We observed that the helical structure is modified by nucleotide binding as in the case of RecA. Upon ATP binding under high-salt conditions (600 mM NaCl), the helical pitch of XRad51.1 filament was increased from 8 to 10 nm and the cross-sectional diameter decreased from 7 to 6 nm. The pitch sizes of XRad51.1 are similar to, though slightly larger than, those of RecA filament under corresponding conditions. A similar helical pitch size was observed by electron microscopy for budding yeast Rad51 [Ogawa, T., et al. (1993) *Science 259*, 1896−1899]. In contrast to the RecA filament, the structure of XRad51.1 filament with ADP is not significantly different from that with ATP. Thus, the hydrolysis of ATP to ADP does not modify the helical filament of XRad51.1. Together with our recent observation that ADP does not weaken the XRad51.1/DNA interaction, the effect of ATP hydrolysis on XRad51.1 nucleofilament should be very different from that on RecA.

Homologous recombination is a strand exchange between DNA molecules with identical or similar sequences, and is involved in DNA repair and DNA segregation. The molecular mechanism of the homologous recombination reaction has been extensively studied in *Escherichia coli* [for a review, see Kowalczykowski et al. (1994)]. In *E. coli*, RecA[1] protein plays a central role in the reaction by pairing two homologous DNA molecules and catalyzing strand exchange reactions between them. Biochemical and structural analyses have been conducted [for reviews, see Roca and Cox (1990), Radding (1991), West (1992), and Takahashi and Nordén (1994)] and indicate that RecA first binds to ssDNA and forms a nucleofilament, in which RecA subunits are arranged in a helical manner around the DNA. In the presence of ATP, the filament is extended and can bind a second DNA to promote the strand exchange reaction.

Proteins homologous to RecA have been found in eukaryotes as well as in prokaryotes (Shinohara et al., 1992; Bishop et al., 1992). In budding yeast, the Rad51 protein was shown to be involved in genetic recombination in both meiosis and mitosis [for reviews, see Resnick (1987) and Petes et al. (1991)]. Sequence analysis revealed that the sequence of *Saccharomyces cerevisiae* Rad51 protein is somewhat homologous to RecA (Shinohara et al., 1992; Aboussekhra et al., 1992; Basile et al., 1992). Similar proteins have since been found in various eukaryotic organisms, including humans (Bezzubova et al., 1993; Morita et al., 1993; Muris et al., 1993; Shinohara et al., 1993; Maeshima et al., 1995; Terasawa et al., 1995). Structural similarity between Rad51 and RecA was also observed (Ogawa et al., 1993) in addition to sequence homology. Electron microscopy observations have shown that human Rad51 and yeast Rad51 polymerize on DNA and form a helical filament very similar in structure to that formed by RecA (Ogawa et al., 1993; Sung & Robberson, 1995).

We recently cloned and sequenced two *Rad51* gene homologues (*XRAD51.1* and *XRAD51.2*) from *Xenopus laevis*. They share 69% homology with budding yeast Rad51 and 96% with human and mouse Rad51 (Maeshima et al., 1995). We have succeeded in producing the proteins in *E. coli* and purifying them in a large amount (Maeshima et al., 1996). We have verified that the purified protein can promote pairing and strand exchange reactions *in vitro* in the presence of ATP, although reaction is less efficiently

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* Corresponding author: Institut Curie, Bat. 110, Centre Universitaire Paris-Sud, F-91405 Orsay, France. Fax and telephone: 33-1 69 86 30 11. E-mail: Masa.Takahashi@curie.u-psud.fr.

‡ Institut Curie and CNRS.

§ Chalmers University of Technology.

|| Research Institute for Microbial Diseases.

⊥ Risø National Laboratory.

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[1] Abbreviations: XRad51.1, *Xenopus laevis* XRad51.1 protein; RecA, *Escherichia coli* RecA protein; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); SANS, small-angle neutron scattering; R_c, cross-sectional radius of gyration; ^2H_2O, deuterated water.

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catalyzed by *Xenopus* Rad51 than by *E. coli* RecA, as also observed for human Rad51 (Baumann et al., 1996; Gupta et al., 1997). We also characterized the interaction of the protein with DNA. Although largely similar to RecA in binding DNA, XRad51.1 exhibits some differences in the effect of the added nucleotide. In contrast to that of RecA, the binding of XRad51.1 to DNA is not inhibited by ADP (Maraboeuf et al., manuscript in preparation). ATPγS does not stimulate the strand exchange reaction of XRad51.1 (Maeshima et al., 1996).

We previously observed by small-angle neutron scattering (SANS) measurements which indicate that nucleotides can modify the helical structure of RecA and that the structure of the ADP/RecA complex is different from that of the ATP/RecA complex (Ellouze et al., 1995). By taking advantage of the opportunity to prepare a large amount of *Xenopus* Rad51, we here investigate, by SANS measurements, the effect of nucleotides on the helical structure of one of the *Xenopus* Rad51 proteins, XRad51.1, in solution and compare that with the effect on the RecA filament. In contrast to electron microscopy, SANS can provide low-resolution three-dimensional structures of macromolecules without procedures that could affect the structure of the molecules.

The intensity of the SANS signal in the Guinier range (Guinier & Fournet, 1955) is proportional to the mass of the object, while the slope of the intensity *vs* scattering angle is related to its overall mass distribution, *i.e.*, its radius of gyration [for a review, see Jacrot and Zaccai (1981)]. This analysis has been extended to rodlike particles (Luzzati, 1960), when their length is more than 5 times larger than their cross-sectional dimensions. This approach was successfully used to analyze, for example, DNA (Luzzati, 1960), filamentous phages (Torbet et al., 1981), and RecA protein (DiCapua et al., 1990a,b, 1992). SANS data also reflect molecular details within the particle at wider angles. In the case of particles with helical symmetry, the internal regularity of the structure may produce pronounced maxima and minima corresponding to the size of the helical pitch (Nordén et al., 1990, 1992; DiCapua et al., 1992). We could thus examine by SANS the change in the size of the helical pitch and the cross-sectional radius of gyration (R_c) of XRad51.1 upon binding of cofactors and DNA.

Our results show that XRad51.1 molecules are arranged in a helical filament even in the absence of DNA. The structure of the filament depends upon the added nucleotide but is apparently independent of DNA as is that for RecA. However, in contrast to RecA, we did not observe a significant difference in helical structure between the ATP/XRad51.1 and ADP/XRad51.1 complexes, while the structure of the ATPγS/XRad51.1 complex appears to be slightly different.

MATERIALS AND METHODS

X. laevis XRad51.1 protein was produced in *E. coli* and purified as described previously (Maeshima et al., 1996). ATP, ADP, and ATPγS were purchased from Boehringer Mannheim, and freshly dissolved solutions were used without further purification. Poly(dT) was from Pharmacia. The concentrations were determined spectrophotometrically using an ϵ_{260} of 14 500 M^{-1} cm^{-1} for all the nucleotides, an ϵ_{264} of 8520 M^{-1} cm^{-1} for poly(dT), and an ϵ_{278} of 15 823 M^{-1} cm^{-1} for Xrad51.1. The protein concentraiton was estimated after appropriate dilution with the dialysis buffer. The spectrum was corrected for light scattering as described in Eriksson et al. (1992).

For preparation of highly concentrated samples for SANS measurements, the protein was precipitated by addition of ammonium sulfate, redissolved in the ^2H_2O (Sigma, 99% purity) buffer, and dialyzed against a 50-fold volume of the same buffer at 4 °C. The dialysis buffer was changed three times. The final concentration of XRad51.1 is 4 mg/mL. The ^2H_2O buffer contained 20 mM sodium phosphate (pH 6.8), 4 mM $MgCl_2$, and either 60 mM NaCl (low-salt buffer) or 600 mM NaCl (high-salt buffer). A small aliquot of nucleotide cofactor (in ^2H_2O buffer) was subsequently added to XRad51.1. The final cofactor concentration was 4 mM. The complex with DNA was prepared by addition of poly(dT) with a DNA/XRad51.1 ratio of 3 bases per monomer subunit and subsequent incubation for 30 min at room temperature.

The SANS measurements were performed at the Risø National Laboratory (Denmark). A sample-to-detector distance of 2 or 6 m was used, and the neutron wavelength was 0.74 nm. Scattering intensities were measured with a two-dimensional detector of a 128 × 128 array and normalized to the primary beam monitor. The measurements were made in a 2 mm path length quartz cell thermostated at 17 °C (±0.1 °C). Data were accumulated for 2 h. No modification of the SANS pattern was observed during 10 h measurements for any sample, except XRad51.1 alone at low salt concentrations. This sample precipitated during the measurement probably because of low solubility. The SANS data were corrected for buffer background by using the intensity profile obtained with the primary beam blocked with boronate plastic and the profile obtained from the quartz cell filled with dialysis buffer. Detector nonuniformity was eliminated by dividing the signal with the incoherent scattering from water.

The corrected scattering curves of $I(Q)$ *vs* Q at low Q values were interpreted using the Guinier approximation for rodlike particles (Torbet et al., 1981):

$$I(Q)Q = [I(Q)Q]_{Q\to 0} \exp(-R_c^2 Q^2/2) \qquad (1)$$

Hence, a fit of $I(Q)Q$ *vs* Q^2 yields two independent parameters, the cross-sectional radius of gyration, R_c, and the extrapolated zero-angle scattering $[I(Q)Q]_{Q\to 0}$. R_c is related to the radial distribution of scattering density and for a homogeneous distribution would be equal to $^1/_2$ the cross-sectional diameter. $[I(Q)Q]_{Q\to 0}$, when calculated on an absolute scale, reflects the excess scattering mass and is related to the mass per unit length (μ).

$$\frac{[I(Q)Q]_{Q\to 0}}{C} = \pi N_A \mu \left(\frac{\sum b}{M_r} - \rho_S \frac{V}{M_r}\right)^2 \qquad (2)$$

where $[I(Q)Q]_{Q\to 0}$ is as defined above and normalized to the experimental term, which includes geometrical and neutron beam parameters; C is the sample concentration in grams per liter; N_A is Avogadro's number; μ is the mass per unit length; $\sum b/M_r$ is the scattering length per unit mass (2.2453 × 10^{-14} cm/Da); $\sum b$ is the sum of the scattering lengths (scattering power) of the constituent amino acids [calculated from the tables of Jacrot (1976) and Jacrot and Zaccai (1981)

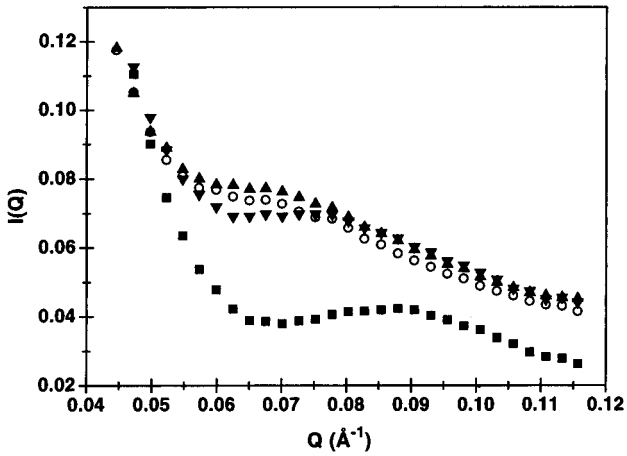

FIGURE 1: Small-angle neutron scattering patterns of various XRad51.1/cofactor complexes. SANS of XRad51.1 without cofactor (■), ATP/XRad51.1 (○), ADP/XRad51.1 (▲), and ATPγS/XRad51.1 (▼) was measured in ^2H_2O containing 20 mM sodium phosphate (pH 6.8), 4 mM $MgCl_2$, and 600 mM NaCl as described in Materials and Methods. For visualization of the subsidiary maxima, large scattering at the center (lower angle) is not shown. The uncertainty in $I(Q)$ is 1%.

using the reported sequence (Maeshima et al., 1996)]; and ρ_S is the solvent scattering density. For water, a ρ_S value of -0.56×10^{-14} cm/Å^3 was used (Jacrot, 1976); V/M_r is the solvent-excluded volume per unit mass (1.2502 Å^3/Da) where the molecular mass (M_r) of XRad51.1 is 37 kDa (336 amino acids) (Maeshima et al., 1996), and V is the total volume of the constituent amino acids [calculated from the tables of Jacrot (1976)]. The helical pitch size of XRad51.1 is obtained from the Q value at the position of the subsidiary maximum in the plots of $I(Q)$ *vs* Q (Nordén et al., 1992).

RESULTS

Effect of Nucleotides. Figure 1 shows SANS patterns for *Xenopus* XRad51.1 in the absence and presence of different nucleotides, ATP, ADP, and ATPγS. Because of the low solubility of XRad51.1 protein under low-salt conditions, the experiments were first performed under high-salt conditions (600 mM NaCl). The patterns for all samples are similar to those of RecA (DiCapua et al., 1990a; Ellouze et al., 1995) and exhibit a small subsidiary maximum. The data clearly indicate that XRad51.1 subunits associate into a helical filament without DNA under these conditions just like RecA.

The SANS patterns obtained in the presence of nucleotide are substantially different from the pattern in the absence of nucleotide (Figure 1). However, the pattern does not depend strongly upon the nature of the nucleotide, although their biochemical effects on XRad51.1 are quite different. The position of the subsidiary maximum, which is quantitatively related to the size of the helical pitch (DiCapua et al., 1990a; Nordèn et al., 1992), is almost the same for the ATP/XRad51.1 and ADP/XRad51.1 complexes (Figure 1). The position is closer to the beam center in the presence of nucleotide than in the absence of nucleotide (Figure 1). This indicates that the helical pitch of the XRad51.1 filament becomes larger upon the binding of any nucleotide. The effect appears to be slightly smaller for ATPγS. We verified experimentally that the hydrolysis of ATP to ADP under the experimental conditions was slow and only about 10% of ATP was hydrolyzed during the course of the measurements (2 h).

Table 1: Helical Pitch and Cross-Sectional Diameter of Various XRad51.1/Nucleotide Complexes, Determined from SANS Measurements at High Salt Concentrations Where [NaCl] = 600 mM

	Rad51	+ATP	+ADP	+ATPγS
cross-sectional diameter (nm)[a] (±0.1)	7.1	6.2	6.3	6.2
helical pitch (nm) (±0.05)	7.80	10.30	10.30	9.00
mass per length (μ)				
subunits per 10 nm (±0.05)	8.75	5.20	5.35	6.00
subunits per turn (±0.05)	6.85	5.35	5.55	5.40

[a] Cross-sectional diameter = $2R_c$ for rodlike particles, where R_c = radius of gyration.

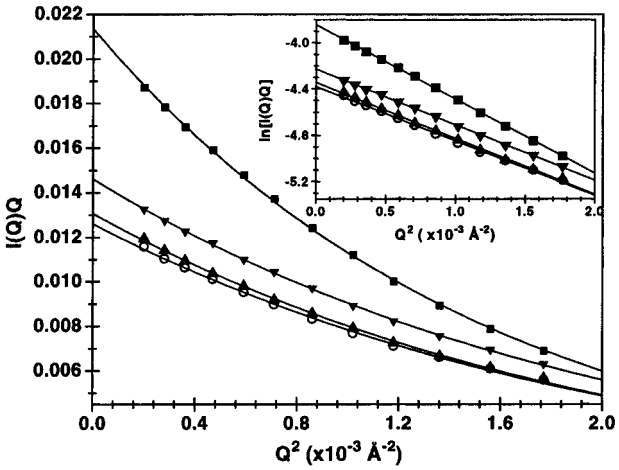

FIGURE 2: Guinier plot of XRad51.1 scattering. The effect of nucleotide cofactors on the cross section of the protein filament. SANS signals of XRad51.1 without cofactor (■), ATP/XRad51.1 (○), ADP/XRad51.1 (▲), and ATPγS/XRad51.1 (▼) are presented as they were collected (main figure) and also as a Guinier plot (inset) for the determination of the cross-sectional diameter of the protein filament. The uncertainty in $I(Q)$ is 1%.

By comparison of our experimental SANS data with those calculated for a helical filament model (DiCapua et al., 1990a; Nordén et al., 1992), we could estimate the helical pitch size of XRad51.1 filament. The results are summarized in Table 1. The results show that ADP or ATP binding enlarges the helical pitch size from 7.8 to 10.3 nm and ATPγS to 9.0 nm.

A second structural parameter can be obtained at smaller scattering angles; the cross-sectional radius of gyration (R_c) can be determined from the modified Guinier plot (Figure 2). The Guinier plot presents the measured scattered intensity as a function of scattering angle in such a way that an exponential fit applying eq 1 gives the cross-sectional radius of gyration (R_c), *i.e.*, the radial distribution of mass around the axis of the filament (Luzzati, 1960; Torbet et al., 1981). The nonlinearity of $\ln[I(Q)Q]$ *vs* Q^2 is diagnostic of the inadequacy of the sample for such a cross-sectional analysis. The inset of Figure 2 shows $\ln[I(Q)Q]$ *vs* Q^2 for XRad51.1 with and without nucleotides. In all cases, the data are approximately linear over a wide range, supporting the validity of the analysis.

In the absence of nucleotide, both the slope of the $\ln[I(Q)Q]$ *vs* Q^2 plot and the scattering intensity at zero angle for the XRad51.1 protein filament are significantly larger than those in the presence of nucleotide. Qualitatively, this implies that nucleotide binding stretches the filament and makes it thinner, and a quantitative analysis leads to the

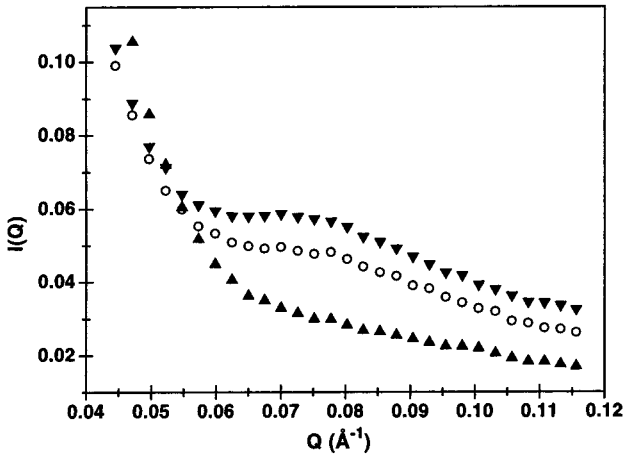

FIGURE 3: Effect of DNA and nucleotide on SANS patterns of XRad51.1 filaments. Modification of the subsidiary maxima position. SANS patterns of ATP/XRad51.1 (○), XRad51.1/poly(dT) (▲), and ATP/XRad51.1/poly(dT) (▼) are measured in a low-salt buffer (60 mM NaCl). Differences in the position of the subsidiary maxima indicate differences in the size of the helical pitch. The uncertainty in $I(Q)$ is 1%.

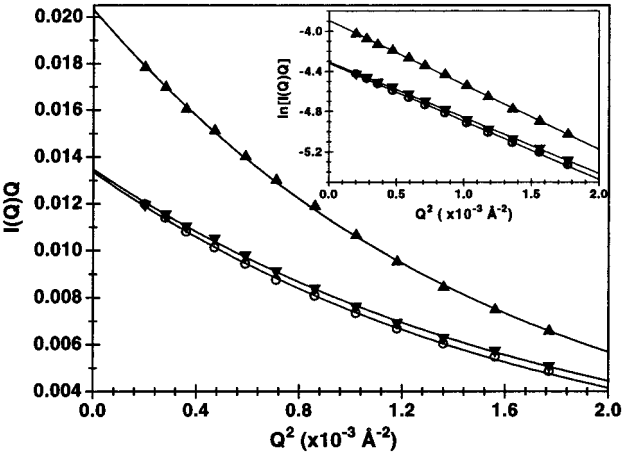

FIGURE 4: Effect of ATP and DNA binding on the cross section of XRad51.1 filament. SANS patterns of ATP/XRad51.1 (●), XRad51.1/poly(dT) (▲), and ATP/XRad51.1/poly(dT) (▼) are presented as they were collected (main figure) and also as a Guinier plot (inset) for the determination of the cross-sectional diameter of the protein filament. The uncertainty in $I(Q)$ is 1%.

values collected in Table 1 for cross-sectional diameter and mass per length. From the values of mass per length and helical pitch size, we can compute the number of protein subunits per helical turn. Approximately six subunits per turn was obtained for all the filaments with nucleotide cofactor (Table 1), as observed also for RecA (Egelman, 1993), although the number of subunits per helical turn is, however, somewhat larger for the protein filament in the absence of nucleotide than in its presence.

Effect of DNA. We have also examined the effect of DNA binding on the XRad51.1 protein filament. Since DNA binding occurs only under low-salt conditions, these experiments were performed under low-salt conditions (60 mM NaCl instead of 600 mM). Under such conditions, XRad51.1 has low solubility and precipitated during the experiments in the absence of DNA and nucleotide which excluded analysis of data for XRad51.1 alone. However, the other complexes with single-stranded polynucleotide or nucleotide were all soluble under these conditions and could be analyzed. The data are shown in Figures 3 and 4.

In Figure 3, a distinct subsidiary maximum can be seen for the ATP/XRad51.1 and ATP/XRad51.1/poly(dT) complexes, while the maximum is not so clear for the XRad51.1/poly(dT) complex formed in the absence of nucleotide. The absence of a clear subsidiary maximum may be an indication of a reorganization of protein subunits in the Rad51/poly(dT) complex. However, this may be due to the fact that the complex has a less well-ordered structure perhaps because of an insufficient amount of added poly(dT) or due to the instability of the complex. The positions of the subsidiary maxima of the ATP/XRad51.1 and ATP/XRad51.1/poly(dT) complexes are very similar, indicating that DNA binding does not affect the helical pitch size of the XRad51.1/nucleotide filament (Table 2). We also note that the size of the helical pitch under low-salt conditions (9.6 nm) is slightly smaller than that under high-salt conditions (10.3 nm) for the ATP/XRad51.1 complex. The effect of nucleotide may be larger under higher salt concentrations as reported by DiCapua et al. (1990a) for the RecA filament. Here again, we experimentally verified that the ATP hydrolysis was slow and negligible.

Table 2: Helical Pitch and Cross-Sectional Diameter of Various XRad51.1/Nucleotide/ssDNA Complexes, Determined from SANS Measurements at Low Salt Concentrations Where [NaCl] = 60 mM

	+ATP	+poly(dT)	+ATP and poly(dT)
cross-sectional diameter (nm)[a] (±0.1)	6.8	7.1	6.7
helical pitch (nm) (±0.15)	9.60	−[b]	9.60
mass per length (μ)			
subunits per 10 nm (±0.05)	5.50	8.35	5.55
subunits per turn (±0.1)	5.3	−[b]	5.3

[a] Cross-sectional diameter = $2R_c$ for rodlike particles, where R_c = radius of gyration. [b] No clear subsidiary maximum was observed.

The modified Gunier plot is almost linear for all the complexes also under low-salt conditions (Figure 4 inset). The slope shows that the cross section of the ATP/XRad51.1 complex is slightly larger at low salt concentrations. The binding of poly(dT) to the ATP/XRad51.1 complex did not change the cross section or the mass per length (Figure 4 and Table 2). From the binding stoichiometry (3 DNA bases per XRad51.1 subunit) (Maeshima et al., 1996) and the number of subunits per turn of the ATP/XRad51.1/poly(dT) complex, one can estimate the axial distance between two DNA bases. The value is about 0.6 nm, indicating more than 50% elongation of DNA compared to a normal stacked configuration as in the case of RecA/DNA complexes formed in the presence of ATP or ATPγS (Williams & Spengler, 1986). Unfortunately, no direct comparison with the XRad51.1/poly(dT) complex could be made since the absence of a clear subsidiary maximum precluded an estimation of the helical pitch.

Nonetheless, the cross section of the XRad51.1/poly(dT) complex without nucleotide could also be determined and is very similar to that of XRad51.1 alone under high-salt conditions. Hence, poly(dT) binding does not appear to modify the filament structure either in the presence or in the absence of nucleotide. By contrast, nucleotide binding affects the structure of the XRad51.1 protein and the XRad51.1/DNA complex filaments. Thus, the determining factor for the structure of the protein filament is nucleotide cofactor but not DNA. The same conclusion has been reached previously for RecA protein (Ellouze et al., 1995).

DISCUSSION

To gain information about the formation and the structure of the Rad51 protein filament, we have carried out SANS measurements of *Xenopus* Rad51 protein and examined the effect of DNA and nucleotide binding. Structural parameters determined for the filament can be compared with those of RecA.

Our results show that XRad51.1 can form helical filaments, even in the absence of DNA, by autoassociation as observed for RecA (Williams & Spengler, 1986; Brenner et al., 1988; DiCapua et al., 1990a,b; Ellouze et al., 1995). The general origin of filament formation on DNA is, therefore, ascribed to the subunit/subunit interaction of the protein. This conclusion is further supported by the observation that DNA does not affect the filamentous structure of XRad51.1. By contrast, the nucleotides significantly modify the structure. The factor which primarily determines the nucleofilament structure is the protein/nucleotide complex, and it appears that DNA may adjust its structure to fit the protein filament and be stretched more than 50% in the complex with ATP. The high-salt condition increases the solubility of the protein and enhances the elongation effect of nucleotides as observed for the RecA filament (DiCapua et al., 1990a,b, 1992).

This feature, together with the structural parameters of the corresponding filaments, is very similar to that of the RecA filament. As for RecA, XRad51.1 filaments consist of about six protein subunits per turn and the DNA in the complex with ATP is stretched in length. The difference in the number of subunits per turn between the filaments with and without nucleotide could be in the range of error due to uncertainty in the estimation of hydration and thus of *V*, the hydrated volume. The RecA filament has a helical pitch of 7 nm and a cross-sectional diameter of 8 nm in the absence of cofactor (inactive form) and 9 and 7 nm in the presence of ATP and ATPγS, respectively (DiCapua et al., 1990a; Ellouze et al., 1995). The corresponding parameters of *Xenopus* Rad51 are 8 and 7 nm in the absence of nucleotide and 10 and 6.5 nm in the presence of ATP. The filaments of XRad51.1 are slightly more elongated and thinner than those of RecA. Moreover, there are some differences in the effect of nucleotide on the filament structure. In contrast to the case of RecA, the effect of ADP on Rad51.1 is very similar to that of ATP, although ADP cannot stimulate XRad51.1 for the strand exchange reaction (unpublished result). This means that the hydrolysis of ATP to ADP does not affect the structure of XRad51.1 filament, whereas a significant change occurs in the case of RecA and could play a role in the strand exchange reaction. We recently observed that the XRad51.1/DNA interaction is not weakened by ADP while the RecA/DNA complex can be completely dissociated by ADP (unpublished results). Sung and Stratton (1996) recently reported that yeast Rad51 can catalyze the strand exchange reaction without ATP hydrolysis under certain conditions. Together with the fact that ATP hydrolysis by XRad51.1 is the much slower than that by RecA (Maeshima et al., 1996), the difference may be an indication of some difference in the reaction mechanism and be related to the much less efficient catalytic activity of XRad51.1 for strand exchange reaction compared to that of RecA.

We also observe that the structure of the ATPγS/XRad51.1 complex is different from that of the ATP/XRad51.1 complex in terms of helical pitch (although the cross section and number of subunits per turn are the same). This contrasts with the case of RecA. ATPγS does not activate Rad51 for the strand exchange reaction except under particular conditions (Maeshima et al., 1996; Sung & Stratton, 1996; Gupta et al., 1997), while ATPγS stimulates the RecA/DNA interaction even more strongly than ATP and promotes the strand exchange reaction up to certain step (Menetski et al., 1990). The filamentous structure of the ATPγS/RecA complex is very similar to that of the ATP/RecA complex (Ellouze et al., 1995). Probably the binding mode of nucleotides to RecA and to Rad51 is not the same, and thus, nucleotides have different effects on the structures of RecA and Rad51.

Filamentous structures of yeast and human Rad51 proteins in complexes with DNA have previously been observed by electron microscopy (Ogawa et al., 1993; Benson et al., 1994; Sung & Robberson, 1995). Ogawa et al. (1993) reported that the ATP/yeast Rad51/dsDNA complex has a larger helical pitch than the ATP/RecA/dsDNA complex. The pitch (9.6 nm) estimated from electron micrographs is very close to the helical size of *Xenopus* Rad51 in the presence of ATP. However, Sung and Robberson (1995) have reported a smaller pitch (8.0 nm) for the dsDNA complex but a similar value for the ATP/yeast Rad51/ssDNA complex (9.6 nm). Ogawa et al. (1993) could not observe any well-ordered ATP/yeast Rad51/ssDNA complex. Benson et al. (1994) observed an ordered complex of human Rad51 with ssDNA but did not observe the extended form. This may be because they replaced ATP with ATPγS by adding the latter cofactor at the end of the reaction to prepare an electron microscopy sample. ATPγS does not stabilize the complex as much as ATP (Gupta et al., 1997), and the structure of the complex may be modified during sample preparation for electron microscopy. The results for the Rad51 filaments are thus controversial with regard to values of the helical pitch. Further careful analysis by SANS and electron microscopy under various conditions may be required.

In summary, our SANS experiments with *Xenopus* Rad51 protein have shown that, like RecA, XRad51.1 forms a helical filament independently of cofactor or DNA. The presence of cofactor modifies the structure of the filament, making it longer and thinner with fewer subunits per helical turn. In a notable contrast to RecA, the filaments with ADP and ATP are almost identical while the filament with ATPγS is somewhat different (smaller helical pitch). Addition of DNA has an insignificant effect on the filament dimensions, indicating that the DNA must adapt to the structure of the protein filament, as with RecA.

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