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Effect of Ions and Nucleotides on the Interactions of Yeast Rad51 Protein with Single-Stranded Oligonucleotides[1]

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Rad51 protein is a eukaryotic homologue of RecA protein that is essential for homologous recombination. We developed a simple procedure for purifying yeast Rad51 protein, characterized its interaction with DNA, and compared it with those of RecA from *Escherichia coli* and Rad51 from higher eukaryotes. Fractionation of crude extract with 0.2% polyethylenimine eliminated contaminant proteins and nucleic acids, which can perturb the subsequent purification steps. Binding of Rad51 to single-stranded DNA was detected in solution by measuring the fluorescence anisotropy of a fluorescein probe attached to the 5′ end of the oligonucleotides. The interaction was stabilized by ATP, as is that of RecA, but was neither stabilized by a non-hydrolysable analog of ATP, nor destabilized by ADP, unlike the interaction of RecA. This character was very similar to that of *Xenopus* XRad51.1, although the binding of yeast Rad51 to DNA was more sensitive to Mg^{2+} ion in both the presence and absence of ATP, and was optimal at 5–10 mM Mg^{2+}. The dissociation of Rad51 protein from DNA is not, therefore, favored by the hydrolysis of ATP to ADP, in contrast to that of RecA. On the other hand, the high DNA-binding state of the Rad51-DNA complex promoted by ATP appeared to be short-lived. These features may be linked to the lower activity of Rad51 and the fact that Rad51 activity does not require the hydrolysis of ATP.

Key words: DNA binding, homologous recombination, nucleotide cofactor, Rad51 protein, RecA protein.

Rad51 protein is considered to be a eukaryotic homologue of RecA protein (*1, 2*) and has been found in various organisms from yeast to man (*3–5*). The protein, like RecA, plays an essential role in homologous recombination and can mimic the strand-exchange reaction *in vitro* in the presence of ATP (*6–9*).

The way in which RecA acts is fairly clear (for reviews, see Refs. *10–12*). It first binds to a single-stranded DNA to form a filamentous complex that, in turn, binds a second DNA in the presence of ATP. RecA then promotes the exchange of strands between them. The Rad51 proteins from several organisms can form a filamentous complex with DNA. This complex has a structure very similar to that of the RecA-DNA complex (*13–15*). This similarity suggests that Rad51 and RecA act similarly. However, Rad51 is much less active than RecA (*6–9*) and requires other proteins for the reaction (*16, 17*). Recent biochemical studies on the Rad51/DNA interaction indicate that there are significant differences in the way RecA and Rad51 bind to DNA, and even in the ways Rad51 proteins from different species bind to DNA (*18–21*).

We have therefore studied the interaction of yeast Rad51 with single-stranded DNA *in vitro* and compared it with the way RecA from *Escherichia coli* and Rad51 from *Xenopus* bind DNA. The effect of nucleotides and ions was examined in particular. These factors are required for the strand-exchange reaction of RecA (*22*) and affect the interaction of RecA with DNA (*23–28*). ATP modifies the structure of the RecA-DNA complex and stabilizes it. The association of RecA with DNA in the presence of ATP requires Mg^{2+} ions and is faster at higher Mg^{2+} concentrations. Other ions and pH also affect the interaction of RecA with DNA. The association is faster and the affinity higher at lower pH (*29, 30*). The complex is greatly destabilized by Cl^-, but much less by acetate or glutamate ions (*31*). We have studied their effects on the yeast Rad51/DNA interaction using the method we used to study RecA and *Xenopus* Rad51.

We first developed a simple procedure for purifying the protein. The interaction with DNA was then studied by measuring the change in fluorescence anisotropy of a fluorescein probe attached to the 5′ end of the oligonucleotides. Fluorescence anisotropy depends on the local and global motions of the chromophore in the molecule, and is greater

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Abbreviations: ATPγS, adenosine-5′-*O*-(3-thiotriphosphate); poly(dεA), poly(1,N^6-etheno-deoxyadenosine); ssDNA, single-stranded DNA.

when mobility is restricted (*32*). Complex formation, which increases mass and stiffness, can be detected by a substantial increase in anisotropy (*19*, *30*). The change in signal is proportional to the amount of DNA fragment complexed by the protein, while the total fluorescence is not affected. The measurement thus provides information very similar to that given by a filter binding assay but without the need to separate the complex from free DNA, which can perturb the interaction.

MATERIALS AND METHODS

Protein Preparations—Rad51 was prepared by cloning the yeast *rad51* gene under the arabinose operon promoter (PBAD) in a plasmid that was expressed in *E. coli*. The cloning was done by M. Campbell (Stanford University, USA) (*33*), who kindly provided the strain. The bacteria were fermented at 37°C in TB medium (10 g/liter Tryptone Broth and 100 mM NaCl) with 25 μg/ml kanamycine. L-Arabinose (Sigma) was added (final concentration: 2%) when the turbidity at 620 nm reached 0.5, and the bacteria were incubated for further 3 h at 37°C for the induction. The bacteria were then harvested by centrifugation, washed with 25% sucrose in 40 mM Tris/HCl, pH 8, and 1 mM EDTA, and suspended in the same buffer (3 ml buffer/1 g bacteria).

The cells were incubated with lysozyme at 4°C and sonicated. All purification steps were carried out at 4°C. Cell debris was removed by centrifugation, and the supernatant was diluted with 25% sucrose buffer to $A_{260} = 60$. Rad51 was precipitated by adding polyethylenimine (Sigma) (0.2% final). The pellet obtained by centrifugation was washed with 0.5 M NaCl, and Rad51 was dissolved in 1 M NaCl in 40 mM Tris/HCl, pH 8.0, 10% glycerol and 5 mM β-mercaptoethanol. Rad51 was then precipitated by adding 0.18 g of ammonium sulfate per ml of solution. The pellet was centrifuged out, dissolved in 10 mM potassium phosphate buffer, pH 6.9, containing 10% glycerol and 5 mM β-mercaptoethanol and loaded onto a hydroxyapatite (BioRad) column (2.5 × 7.5 cm) equilibrated with the same buffer. The proteins were eluted with a linear gradient of potassium phosphate (10–500 mM) with a rise of 2.5 mM/min and a flow rate of 1 ml/min.

The fractions containing Rad51 were collected and dialyzed against 40 mM Tris/HCl, pH 8, 50 mM NaCl, 5 mM β-mercaptoethanol, and 10% glycerol and chromatographed on a POROS HQ (PerSeptive Biosystem) column (1 × 2.5 cm) using a linear gradient of NaCl (50–500 mM) with a rise of 20 mM/min and a flow rate of 1.5 ml/min. The protein was eluted around 250 mM NaCl. The fractions containing Rad51 were collected, dialyzed against 10 mM sodium phosphate buffer with 50% glycerol and stored at −20°C. All liquid chromatography was done at 4°C on an Econo System (BioRad).

Rad51 was detected by Western blotting using an antibody against yeast Rad51 and goat anti-rabbit IgG coupled to alkaline phosphatase (Sigma). The protein was then visualized with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) (Sigma). The concentration of Rad51 was determined from its absorption at 275 nm using the absorption coefficient of $\varepsilon_{275\ nm} = 11{,}200\ M^{-1}\ cm^{-1}$. The absorption coefficient was estimated from the number of tyrosine residues (8 per subunit) (*1*, *2*) and the coefficient of $\varepsilon_{275\ nm} = 1{,}400\ M^{-1}\ cm^{-1}$ for each tyrosine residue.

Oligonucleotides and Other Materials—Fluorescein-labeled oligo(dT) and oligo(dA) (36 bases) were from Genset. ATPγS was from Boehringer Mannheim. Other nucleotides were from Sigma; they were dissolved (neutralized by KOH) in 10 mM potassium phosphate buffer. The concentration of ATP was determined using $\varepsilon_{260\ nm} = 15{,}400\ M^{-1}\ cm^{-1}$. Other chemicals were from Merck. All buffers were prepared with water purified by a MilliQ system (Millipore, USA).

Spectroscopic Measurements—The fluorescence anisotropy of the fluorescein probe attached to the oligonucleotides was monitored in a FP-777 spectrofluorometer (Jasco) equipped with an automatic anisotropy measuring device (ADP301, Jasco). The anisotropy r is defined as:

$$r = (I_{VV} - G \times I_{VH}) / (I_{VV} + 2 \times G \times I_{VH})$$

where I is fluorescence intensity. The first and second indices refer to the orientation of excitation and emission polarizers, respectively. G is the correction factor. The cell holder was thermostated at 20°C. The excitation wavelength was set at 496 nm (bandwidth: 10 nm) and the emission wavelength at 521 nm (bandwidth: 10 nm). Measurements were made in a mini-cell (1 cm × 0.2 cm, Hellema).

Experimental Conditions—Experiments were usually performed at 20°C in a buffer containing 10 mM sodium phosphate, pH 6.9, 0.1 mM EDTA, 1 mM β-mercaptoethanol, and the indicated concentration of $MgCl_2$.

RESULTS

Protein Purification—Expression of the yeast rad51 gene in *E. coli* prevented growth of the cells (data not shown). We therefore expressed the *rad51* gene in an arabinose promoter (P_{BAD}) expression system whose transcription is almost completely repressed in the absence of inducer (arabinose) (*33*). Fractionation with ammonium sulfate alone did not eliminate nucleic acid contaminants, and these perturbed subsequent purification steps by saturating the hydroxyapatite or anion exchange columns. We therefore added fractionation with polyethylenimine to eliminate nucleic acid contaminants. We found that the optimal polyethylenimine concentration was 0.2% (Fig. 1): higher concentrations of polyethylenimine did not increase the yield and decreased the effective elimination of other proteins. This polyethylenimine concentration gave a yield of over 80%, judged from the intensity of the band in a Western blot, and the purity of Rad51 was about 20%.

The subsequent ammonium sulfate fractionation did not increase the purity, but was required to eliminate polyethylenimine. The yield of this step was over 90%. Chromatography of the ammonium sulfate precipitate on hydroxyapatite increased the purity of Rad51 to 65% with a yield of 80%. The final chromatography on POROS HQ (PerSeptive Biosystem) gave a protein over 90% pure with no significant DNase activity when tested by the change in poly(dεA) fluorescence (*34*). The final yield was about 30%. The protein retained its DNA-binding activity after storage for two months at −20°C.

Effect of Mg^{2+} Ion on the Yeast Rad51/DNA Interaction—We determined the optimal Mg^{2+} ion concentration for the binding of yeast Rad51 to DNA in the presence and ab-

sence of ATP. Binding was measured by the increase in fluorescence anisotropy of the fluorescein-labeled oligo(dT) upon adding the protein, as was done for the *Xenopus* Rad51/DNA interaction (*19*). The fluorescence anisotropy *r* reflects the local and global motions of the chromophore, and is close to zero for a freely rotating chromophore. The theoretical upper limit of 0.4 corresponds to a totally non-rotating chromophore (*32*). The fluorescein-labeled oligo-(dT) was small (36 bases long) and flexible, and had a small *r* value (Fig. 2) (0.03–0.05), depending upon the Mg^{2+} concentration, as this affects the condensation state of DNA. The binding of Rad51 molecules increased the mass and hence decreased the motion. Complex formation was thus detected by an increase in fluorescence anisotropy.

In the absence of ATP, the anisotropy of fluorescein-labeled oligo(dT) fluorescence was not significantly modified upon adding yeast Rad51 up to 1.6 μM when the Mg^{2+} concentration was 1 or 5 mM (Fig. 2), indicating little or no binding. *E. coli* RecA and *Xenopus* Rad51 increased the anisotropy about 4-fold under the same conditions (Table I, *19*, *30*). Longer incubation (1 h) did not effect the anisotropy, indicating that there was no slow binding. The anisotropy was increased by adding yeast Rad51 at 10 mM Mg^{2+} (Fig. 2), reflecting complex formation. However, the change did not plateau, even at 1.6 μM Rad51, indicating weak binding (apparent $K_d > 0.5$ μM). There was only a slight increase in the anisotropy upon adding yeast Rad51 at a higher Mg^{2+} concentration (20 mM). The change was much smaller than that at 10 mM Mg^{2+}, indicating weaker binding, probably due to competition between the cation and protein for DNA (*35*). The need for 10 mM Mg^{2+} for the yeast Rad51/single-stranded DNA interaction was checked using other oligonucleotides [fluorescein-labeled oligo(dA) and poly(dεA)].

In the presence of ATP, the fluorescence anisotropy of fluorescein-labeled oligo(dT) was increased greatly by adding

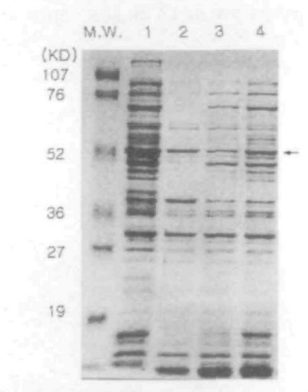

Fig. 1. **Effect of polyethylenimine concentration on the selective precipitation of yeast Rad51 protein.** Polyethylenimine was added to the crude extract (concentration: 0.2, 0.4, and 0.6% for lane 2, 3, and 4, respectively). Each precipitate was subjected to SDS polyacrylamide gel electrophoresis. The Rad51 band was identified by Western blotting. Lane 1 is total crude extract.

TABLE I. **Effect of nucleotides on the interaction of Rad51 and RecA (1 μM) with fluorescein-labeled oligo(dT) (3 μM) at pH 6.9, 20°C and 5 mM $MgCl_2$.**

Nucleotide	Fluorescence anisotropy (*r*)		
	Yeat Rad51	*Xenopus* Rad51*	*E. coli* RecA
—	0.10	0.30	0.20
ATP	0.18	0.34	0.30
ADP	0.09	0.31	0.06
ATPγS	0.09	0.31	0.32
GTP	0.09	n.d.	0.08
CTP	0.08	n.d.	0.07
dTTP	0.09	n.d.	0.07

*The data from Maeshima *et al.* (*19*) and Mg^{2+} concentration 2 mM.

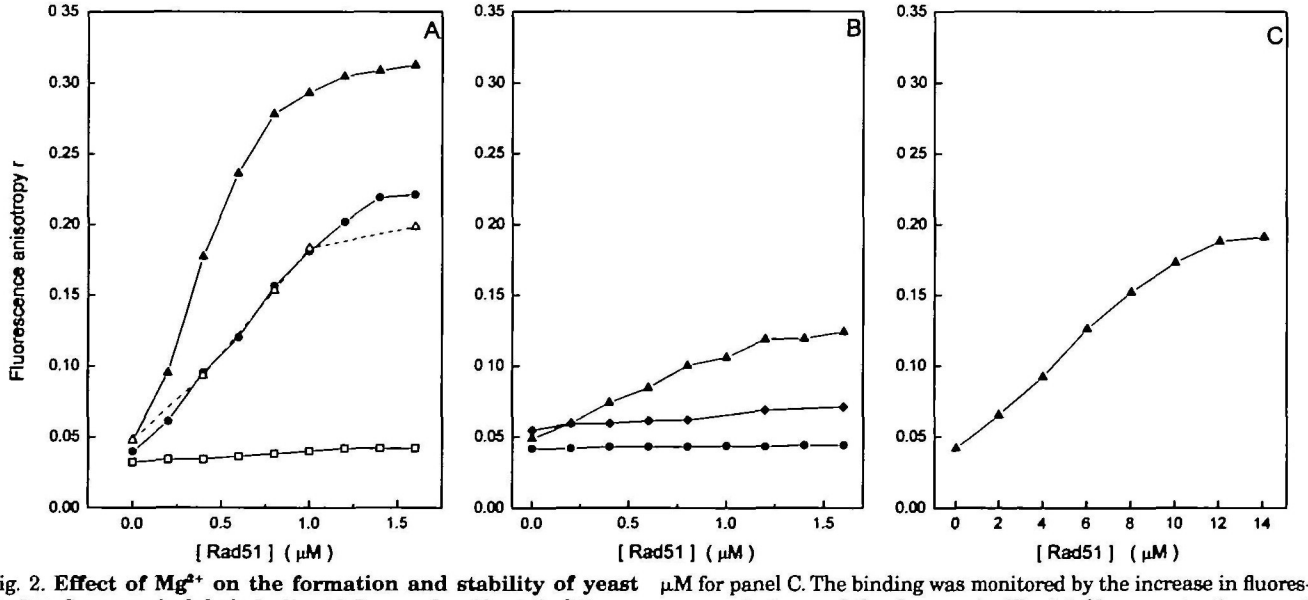

Fig. 2. **Effect of Mg^{2+} on the formation and stability of yeast Rad51–fluorescein-labeled oligo(dT) complex.** Yeast Rad51 was added step-wise to fluorescein-labeled oligo(dT) with (Panel A) or without (Panels B and C) 0.3 mM ATP. The concentrations of fluorescein-labeled oligo(dT) were 3 μM (in bases) for panels A and B, and 60 μM for panel C. The binding was monitored by the increase in fluorescence anisotropy of the fluorescein. The $MgCl_2$ concentration was 1 (□), 5 (●),10 (▲), or 20 mM (◆). Since the anisotropy decreased with time in the presence of ATP and 10 mM $MgCl_2$, the anisotropy values at equilibrium after incubation for 1 h are also shown (△).

yeast Rad51 when the Mg^{2+} concentration was 5 or 10 mM (Fig. 2). But there was no change at 1 mM Mg^{2+}, showing that 1 mM Mg^{2+} is not sufficient for efficient binding. This Mg^{2+} concentration is sufficient for the binding of RecA or *Xenopus* Rad51 to DNA (*19*, *30*). The lack of binding of yeast Rad51 to fluorescein-labeled oligo(dT) at 1 mM Mg^{2+} was probably not due to slow association, because there was no significant increase in anisotropy even after 1 h.

We further investigated the optimal Mg^{2+} concentration by measuring the change in anisotropy of the yeast Rad51/DNA/ATP mixture with increasing Mg^{2+} (Fig. 3). There was significant change in anisotropy at 2 mM Mg^{2+}, with the largest value at about 5 mM Mg^{2+}. Surprisingly, the anisotropy was lower at 10 mM, and smaller (r = 0.2) than that produced by adding the protein to DNA at the same concentration of Mg^{2+} (r = 0.35). The anisotropy of the complex formed by adding the protein to DNA in 10 mM Mg^{2+} decreased slowly with time to a level after 1 h that was similar to that obtained by adding Mg^{2+} step-wise to the yeast Rad51/DNA mixture (Fig. 4). The titration of fluorescein-labeled oligo(dT) by Rad51 at 10 mM Mg^{2+} was therefore revised by measuring the anisotropy at equilibrium after 1 h of incubation. The anisotropy plateaued at 1 μM yeast Rad51 and thus the binding stoichiometry was similar to that estimated from the anisotropy measured just after adding the protein (Fig. 2). Aggregates may be formed when the protein is added at 10 mM Mg^{2+}.

Effect of Nucleotide on the DNA Binding of Yeast Rad51—The above results also show that the binding of yeast Rad51 to single-stranded DNA depended upon ATP, as do those of *E. coli* RecA and *Xenopus* Rad51 (*19*, *26*). The anisotropy increased rather linearly and plateaued with about 1 μM yeast Rad51 in the presence of ATP (Fig. 2) when the Mg^{2+} concentration was 5 or 10 mM, indicating a binding stoichiometry of 3–4 bases per Rad51 subunit for the yeast Rad51-ssDNA complex with ATP. The (reverse) titration of Rad51 by oligonucleotide at 5 mM Mg^{2+} showed a similar DNA/protein ratio (3 bases, data not shown) for

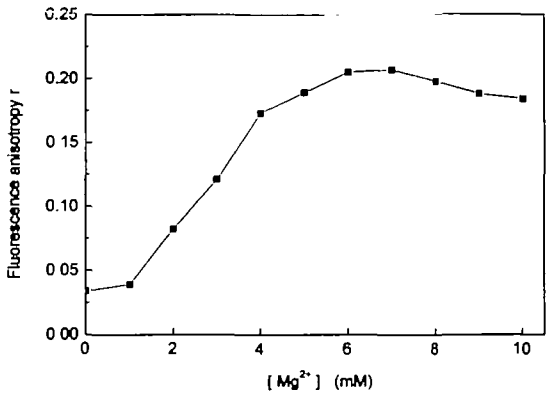

Fig. 3. **Effect of $MgCl_2$ on the interaction of yeast Rad51 with fluorescein-labeled oligo(dT).** $MgCl_2$ was added step-wise to a mixture of 1.5 μM Rad51, 3 μM fluorescein-labeled oligo(dT), and 300 μM ATP, and incubated for 5 min before measuring the fluorescence anisotropy.

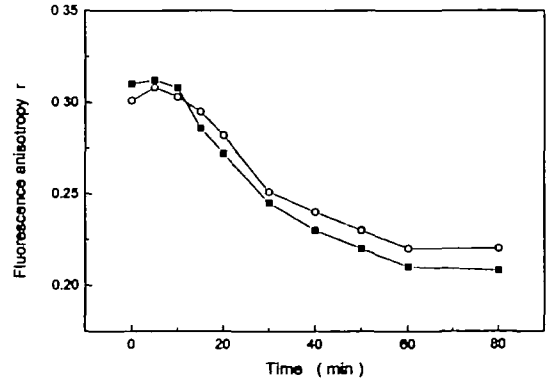

Fig. 4. **Slow change in fluorescence anisotropy of yeast Rad51–fluorescein-labeled oligo(dT) complex formed in the presence of 10 mM $MgCl_2$.** The change with time of anisotropy of fluorescein-labeled oligo(dT) (3 μM in bases) complexed by yeast Rad51 (1.6 μM) in the presence of ATP and 10 mM $MgCl_2$ was measured (■). The data in the presence of an ATP-regeneration system are also shown (○).

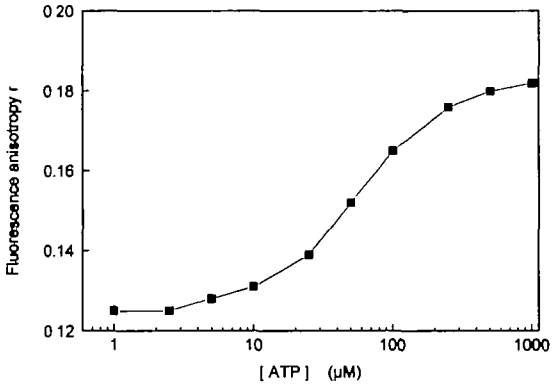

Fig. 5. **Effect of ATP on the binding of yeast Rad51 to fluorescein-labeled oligo(dT).** ATP was added step-wise to a mixture of 1.5 μM Rad51, 3 μM fluorescein-labeled oligo(dT), and 10 mM $MgCl_2$. The binding was monitored as in Fig. 2.

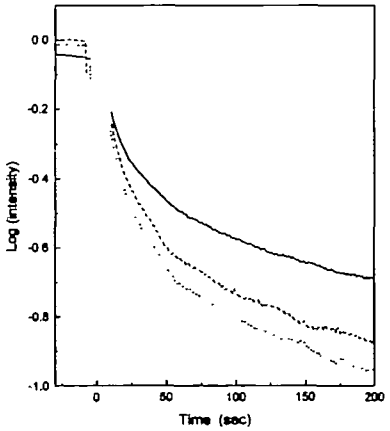

Fig. 6. **Effect of ATP and ADP on the dissociation of yeast Rad51–fluorescein-labeled oligo(dT) complex.** The dissociation kinetics of yeast Rad51–fluorescein-labeled oligo(dT) complex [0.3 μM Rad51 and 0.6 μM fluorescein-labeled oligo(dT)] was measured in the absence (continuous line) and presence of 1 mM ATP (dots) or ADP (broken line) by monitoring the change in the polarized fluorescence of oligo(dT) upon adding excess of non-labeled oligo(dT) (60 μM).

saturation. The anisotropy did not plateau even at 1.6 μM yeast Rad51 in the absence of ATP, indicating weaker binding (Fig. 2B). The stoichiometry was estimated from the experiments with a 20-fold higher DNA concentration, giving a stoichiometry of about 5 bases per yeast Rad51 (Fig. 2C). Similar differences in the stoichiometry with and without ATP have been observed for RecA (*11, 24*) and *Xenopus* Rad51 (*19*). They reflect the difference in binding.

The concentration of ATP required for DNA binding was assayed by measuring the change in fluorescence anisotropy with step-wise increases in ATP concentration (Fig. 5). The fluorescence anisotropy increased with the addition of ATP to a maximum value at about 300 μM ATP, with a half-effect at around 60 μM ATP. Thus the apparent dissociation constant (K_d) of ATP/yeast Rad51 interaction is about 60 μM, which is close to the K_m of the ATPase activity of RecA (*38*). The reported K_d of ATP/RecA interaction in the absence of DNA is 18 μM (*37*). The maximum fluorescence anisotropy obtained by adding ATP to the yeast Rad51/DNA mixture was less than 0.2, and smaller than the fluorescence anisotropy obtained by adding Rad51 to fluorescein-labeled oligo(dT) in the presence of ATP. Here again, the formation of an ATP-Rad51-DNA complex depended upon the order in which the constituents were added. As noted above, the fluorescence anisotropy (0.35) of the complex formed by adding Rad51 to the DNA-ATP mixture slowly decreased with time to reach a value (0.2) close to that obtained by adding ATP to the Rad51-DNA complex.

We also examined the effect of other nucleotides (ADP, ATPγS, GTP, CTP, TTP) (Table I). None of the other nucleotides, including ADP and ATPγS, significantly altered the fluorescence anisotropy of the yeast Rad51/fluorescein-labeled oligo(dT) mixture. This feature differs from that of the RecA-DNA complex, which was destabilized by ADP and strongly stabilized by ATPγS (Table I and *40*), but is similar to that of *Xenopus* Rad51-ssDNA complex (Table I), except for that of ATPγS. ATPγS stabilizes the complex with *Xenopus* Rad51 as did ATP, although to a lesser extent than ATP (*19*).

The effect of nucleotide on the dissociation rate of yeast Rad51 was examined. The dissociation kinetics was followed by monitoring the fluorescence change of fluorescein-labeled oligo(dT) upon adding 100-fold excess of non-labeled oligo(dT) to the yeast Rad51- fluorescein-labeled oligo(dT) complex as described in (*39*). The dissociation was faster in the presence of ATP or ADP (Fig. 6). The effect of ADP was similar to that of ATP. The hydrolysis of ATP to ADP thus does not accelerate the dissociation of yeast Rad51 from DNA.

Effect of Anions on DNA Binding—Like other protein-DNA interactions, the yeast Rad51-DNA complex was dissociated by adding salt, probably due to competition between the cation and protein for DNA (*35*). The interaction with RecA also depends upon the nature of the anion. The RecA-DNA complex is not easily dissociated by sodium glutamate, but is readily dissociated by NaCl (*31*). We examined the effect of NaCl, sodium acetate, and sodium glutamate on the destabilization of the yeast Rad51-DNA complex. The dissociation pattern of the yeast Rad51-fluorescein-labeled oligo(dT) complex monitored by the change in fluorescence anisotropy revealed no significant difference between the salts (Fig. 7). Anions do not affect the binding of yeast Rad51 to DNA.

Effect of pH—The binding of RecA depends upon the pH, both with and without nucleotide (*28, 36*). Binding is greater at lower pH and association is faster. The binding of yeast Rad51 was examined at pH 6.4, 6.9, and 7.4. A higher Rad51 protein concentration was required for saturation of

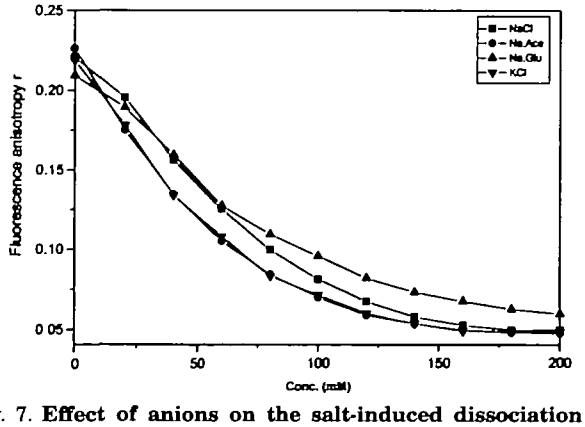

Fig. 7. **Effect of anions on the salt-induced dissociation of yeast Rad51–fluorescein-labeled oligo(dT) complex.** The ATP–Rad51–fluorescein-labeled oligo(dT) complex was dissociated by adding NaCl (■), Na glutamate (▲), Na acetate (●), or KCl (▼) at pH 6.9 and 20°C. [fluorescein-labeled oligo(dT)] = 3 μM, [ATP] = 300 μM and [Mg^{2+}] = 10 mM.

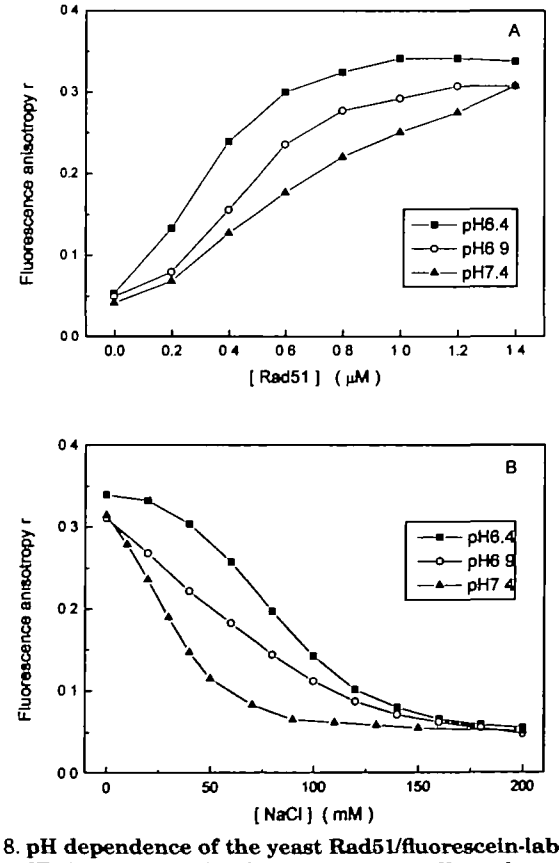

Fig. 8. **pH dependence of the yeast Rad51/fluorescein-labeled oligo(dT) interaction.** Panel A: association profile, and panel B: NaCl-induced dissociation profile of Rad51/fluorescein-labeled oligo(dT) at pH 6.4 (■), 6.9 (○), and 7.4 (▲). The experimental conditions are the same as those in Fig. 6.

fluorescein-labeled oligo(dT) at pH 7.4 than at pH 6.4 (Fig. 8A), indicating weaker binding. The stability of the complex, analyzed by salt-induced dissociation, showed that half dissociation occurred at 50 mM NaCl at pH 7.4 and at 80 mM at pH 6.9 (Fig. 8B). The complex was more stable at the lower pH, as is the RecA complex.

DISCUSSION

We have attempted to understand why the strand-exchange activity of Rad51 is lower than that of RecA by analyzing the binding of yeast Rad51 to DNA using the method used to study *E. coli* RecA and *Xenopus* Rad51 and compared the findings with the data for the latter proteins. We have developed a method for purifying yeast Rad51, which can be also used to purify human Rad51 (H-K. Kim and M. Takahashi, unpublished results).

There is some difference between RecA and Rad51 in their interactions with DNA. The most important one is the effect of nucleotides. ADP destabilizes the interaction of neither yeast nor *Xenopus* Rad51, while it dissociates the RecA-ssDNA complex. Zaitseva *et al.* also reported that adding ADP to the yeast Rad51-etheno DNA complex did not alter the fluorescence of etheno DNA (*20*), indicating no structural change due to ADP. We recently observed that ADP does not dissociate the human Rad51- ssDNA complex (H.-K. Kim, K. Morimatsu, B. Norden, and M. Takahashi, unpublished result). This feature is probably general for Rad51 from all species. By contrast, RecA exists in two states: a high DNA-binding state which can be promoted by ATP and a low DNA-binding state which can be promoted by ADP (*41*). The hydrolysis of ATP to ADP thus promotes the change from the high to the low state and allows the recycling of RecA for the strand-exchange reaction. This may not occur with Rad51, because Rad51, hydrolyses ATP very slowly (*6–8*) and ADP cannot promote the dissociation from the DNA.

On the other hand, the high DNA-binding state of Rad51 promoted by ATP appears to be less stable and to have a shorter life span than that of RecA. Adding ATP to the *Xenopus* Rad51-ssDNA complex accelerates the dissociation from DNA without hydrolysis of ATP (*19*). The *Xenopus* Rad51-ssDNA complex formed with ATPγS, a non-hydrolysable analogue of ATP, can be dissociated by adding salt, while that of RecA is not dissociated. The fluorescence of *Xenopus* Rad51-poly(dεA) complex is increased in intensity by adding ATP, but decreased with time, indicating a change from a high to a lower state without hydrolysis of ATP (*8*). A similar result has been observed for human Rad51 (H.-K. Kim, K. Morimatsu, B. Norden, and M. Takahashi, unpublished result). ATP decreases the intrinsic binding affinity of human Rad51 (*21*). Adding yeast Rad51 to ssDNA at 10 mM $MgCl_2$ strongly increased the anisotropy at first, but it then decreased. This change was probably not due to the dissociation of yeast Rad51 from DNA, because the binding stoichiometry was not modified by the incubation (Fig. 2A). Since the anisotropy is related to the motion of the chromophore, the result suggests that the complex changes its structure and becomes more flexible with time, or that aggregates form at first and slowly dissociate.

In contrast to this ephemeral nature of the complex with Rad51, the ATPγS-RecA-DNA complex is extremely stable and cannot be dissociated by adding salt or changing the pH. This difference may be linked to a less efficient strand-exchange reaction by Rad51 compared to that by RecA. Probably only a part of Rad51-DNA complex filaments is "active" to bind the homologous DNA. The association rate of the human Rad51-DNA filament to the homologous DNA is one order less than that of the RecA-DNA filament (*42*). On the other hand, these features may also be linked to the fact that ATP hydrolysis is not required for the activity of yeast Rad51, even *in vivo* (*43*). Rad51 can dissociate from DNA and can be recycled for the strand-exchange reaction without ATP hydrolysis.

The English text was checked by Dr. Owen Parkes.

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