RadA belongs to the RecA/RadA/Rad51 protein superfamily found in all three domains of life, the Bacteria, Archaea, and Eukarya (3, 11, 13). As homologous DNA transferases, these filamenting proteins are responsible for homologous recombination, recombination DNA repair, and other aspects of DNA metabolism (5, 8, 9). Like other members of this family, RadA polymerizes on single-stranded DNA (ssDNA) in the presence of ATP, forming a presynaptic complex (PC) which possesses the ATPase activity. PC interacts with double-stranded DNA (dsDNA) to promote homologous pairing and strand exchange, the two critical steps of recombination (4, 15).

The biochemical and recombination activities of five RadA proteins from different hyperthermophilic crenarchaeans and euryarchaeans have been described (6, 7, 10, 14, 17). One of them, RadA from Pyrobaculum islandicum (RadAPi), has been studied in more detail than the others. The ATPase of its PC exhibits two disparate catalytic modes, and its PC is active within a wide temperature range (from 37 to 90°C) (17).

Earlier, we described a distinguishing property of RadA protein from crenarchaeon Desulfurococcus amylolyticus (RadADa), namely, its ability to promote efficient strand exchange even at 95°C (6). Here, we continued the analysis of biochemical activities of RadADa, and presented its additional characteristics, which include both expected and distinguishing properties of the protein with regard to known activities of the RecA and RadA recombines.

ATP hydrolysis is an intrinsic property of the ternary PC (RecA/RadA/Rad51::ATP::ssDNA) formed by any member of the homologous recombinase family. The hydrolysis rate is strongest for the RecA PC ($k_{\text{cat}} = 30 \text{ min}^{-1}$) and is reduced roughly 10 times for representatives of the RadA/Rad51 subfamily. Though ATP hydrolysis is not necessary for the initiation of recombination by the PC, it is essential for the strand exchange progression and completion (4). Since the accumulation of ADP results in PC inactivation, the ATP hydrolysis is routinely used to monitor an active state of PC.

As ATP hydrolysis catalyzed by RadADa was measured at temperatures around 90°C and above in the absence of ATP-regenerating system, a linear part of ATPase kinetics was observed within the limits of 1 to 4 min of the reaction (data not shown). Unless otherwise specified, the experimental conditions used in all further experiments were as follows. The reaction was carried out at 90°C in the 20-μl mixture containing TMD buffer (25 mM Tris-HCl [pH 7.5], 10 mM MgCl₂, and 1 mM dithiothreitol), 20 mM NaCl, 1 mM ATP, 15 μM RadADa, and 150 μM φX174 ssDNA (in nucleotides [nt]). The amount of [¹⁴C]ATP hydrolyzed was measured by using a thin-layer chromatography method as described previously (6).

The 20 mM NaCl used in this mixture was found to be optimal for ATP hydrolysis catalyzed by RadADa ($k_{\text{cat}} = 3.2 \text{ min}^{-1}$) (Fig. 1). This concentration is fivefold lower than that used for RadAPi (17). Moreover, the addition of 100 mM NaCl resulted in a 3.2-fold decrease of the monomer $k_{\text{cat}}$ value. Similar results were obtained with KCl (data not shown). The data indicate a pronounced sensitivity of the RadADa ATP hydrolysis to monovalent cations.

Besides $k_{\text{cat}}$, two other steady-state kinetic quantitative characteristics of ATP hydrolysis were determined. These included

![FIG. 1. The rate constant for ATP hydrolyzed by the RadADa::ATP::ssDNA PC as a function of NaCl concentration. Optimal experimental conditions (90°C, φX174 ssDNA) were used; see the text for details.](http://jb.asm.org/)

**Characteristic Thermodependence of the RadA Recombinase from the Hyperthermophilic Archæon Desulfurococcus amylolyticus**

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The Desulfurococcus amylolyticus RadA protein (RadADa) promotes recombination at temperatures approaching the DNA melting point. Here, analyzing ATPase of the RadADa presynaptic complex, we described other distinguishing characteristics of RadADa. These include sensitivity to NaCl, preference for lengthy single-stranded DNA as a cofactor, protein activity at temperatures of over 100°C, and bimodal ATPase activity. These characteristics suggest that RadADa is a founding member of a new class of archæal recombinases.
the S05 value, the substrate concentration for the half-maximal observed rate of hydrolysis, and $n_H$, the Hill coefficient that serves as a measure of the degree of cooperativity for binding ATP to RadADa during filament formation. The dependence between the rate at which ATP was hydrolyzed and the ATP concentration (data not shown) gave us an S05 of $75\mu$M. This dependence was converted into a Hill plot, the slope of which gave an $n_H$ of 1.2. Both the low rate constant ($k_{cat}$) and the low cooperativity ($n_H$), measured at optimal conditions (90°C, 20 mM NaCl, and lengthy ssDNA [see below]), characterize RadADa protein as a typical representative of the RadA/Rad51 subfamily (15, 18).

The effect of ssDNA chain length on $k_{cat}$ is presented in Table 1. Two oligonucleotides (21 and 53 nt), four PCR fragments (from 250 to 940 bp) of human genomic DNA, dsDNA, and ssDNA of two phages were used as DNA cofactors in PC-dependent ATP hydrolysis. Different forms of M13 phage DNA were used to control the complete melting of dsDNA molecules under experimental conditions. Circular and linearized M13 ssDNA demonstrated the same ability to stimulate ATP hydrolysis as a linearized dsDNA of this phage, whereas the supercoiled dsDNA (replicative form I) was a weak cofactor due to the only partial denaturation at 90°C (Table 1). At optimal assay conditions, the RadADa PC was able to hydrolyze ATP efficiently with ssDNA cofactors of 940 nt and greater. This cofactor size is about 30-fold longer than that for RecA protein from Escherichia coli (RecAEc) (2). Thus, RadADa prefers lengthy ssDNA as cofactors for ATPase activity, and the same preference could probably be applied to the formation of the PC.

As was shown before (1), a single amino acid replacement (of Cys129 with Met) alters the basic characteristics of wild-type RecAEc resulting in the reduced kinetics of strand transfer, lower $k_{cat}$ of DNA-dependent ATP hydrolysis, increased sensitivity to monovalent cations, and the requirement for a longer ssDNA for the PC ATPase activity. Interestingly, the features of RadADa addressed here repeat the alterations in properties of this RecAEc mutant. Therefore, the suggestion that a limited number of amino acid replacements (possibly just one) are capable of standardizing the RadADa characteristics listed above to make them closer to those of wild RecAEc cannot be excluded.

The ability of RadADa PC to promote recombination at 95°C encouraged us to look for the highest temperatures at which the PC can hydrolyze ATP and to determine factors which provide such thermostability. In Fig. 2A, the rate constants of PCs formed at the temperatures indicated (80 to 100°C) are compared with those of PCs formed at 80°C. Results suggest that the preformation of PCs at 80°C guarantees the conservation of at least a part of the activity up to 102°C.

Another important factor turned out to be the stable oligomer formation at high protein concentration. We used 180 $\mu$M RadADa as the concentration which corresponds to roughly 10,000 protein molecules per bacterium, the number reported for RecA in E. coli (12). Figure 2B shows that after boiling for 30 min (180 $\mu$M RadADa in TMD buffer with 20 mM NaCl), RadADa protein retained one-third of its original activity (this residual activity was measured at 90°C with 15 $\mu$M

![FIG. 2. Factors of the RadADa PC thermostabilization. (a) ATPase activity as a function of temperature. The PC was formed (circles) or preformed at 80°C (triangles), and its ATPase was measured at the temperatures indicated. (b) The residual ATPase activity as a function of RecADa preliminary temperature preincubation in TMD buffer with 20 mM NaCl, RadADa protein retained one-third of its original activity (this residual activity was measured at 90°C with 15 $\mu$M](http://jb.asm.org/)

### TABLE 1. The rate constant of ATP hydrolysis as a function of ssDNA chain length in composition of the RadADa presynaptic complex

<table>
<thead>
<tr>
<th>DNA cofactor</th>
<th>Length (nt)</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligonucleotide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>PCR fragment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>404</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>569</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>940</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>4X174 (viral ssDNA)</td>
<td>5,386</td>
<td>3.1</td>
</tr>
<tr>
<td>M13mp18</td>
<td>7,250</td>
<td></td>
</tr>
<tr>
<td>Closed circular ssDNA (viral)</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Linearized ssDNA</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Supercoiled dsDNA (RFI)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Linearized dsDNA</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

$a$ All measurements of ATPase activity were carried out at optimal experimental conditions (90°C, 20 mM NaCl, and a 10-fold excess in the nucleotide concentrations of each DNA cofactor used in the reaction). ND, not detected. RFI, replicative form I.
RadADa, under other standard conditions). For comparison, a preincubation of 15 μM RadADa at 90°C for 6 min resulted in a complete inactivation of the protein. The data indicate that two factors, preliminary PC formation and high protein concentration, save, at least partially, the activity of RadADa protein, even at 100°C. This conclusion gives the basis of a possible thermodependence of the crenarchaeal recombinases RadADa and RadAPi, respectively. \( E_\text{act} \) (activation energy) was calculated from the formula \( E_\text{act} = RT \ln(k_\text{cat}) \) for each linear part of the plot. The transition temperatures between two catalytic modes (70 and 75°C) are shown. The data for RadAPi were published earlier (17).

Figure 3 shows the thermodependence of the RadADa protein. The graph reveals two catalytic modes of ATPase activity with the transition point at 70°C and may reflect the transition between two conformation states of the protein with different modes of functional activation. The first mode has a relatively high energy of activation (46 kcal/mol), whereas the second one has a much lower activation energy (13.9 kcal/mol). In addition, the protein is characterized by a negligible activity below 65°C. For comparison, the thermodependence of RadAPi is presented in the same coordinates (see the data from Fig. 6 in reference 17). The latter also exhibits a biphasic Arrhenius plot with two characteristic energies of activation and a transition point at 75°C. The difference between thermodependence of the crenarchaeal recombinases RadADa and RadAPi appears obvious. RadAPi belongs to a class of thermotrophic proteins which maintain activity in a wide range of temperatures (37 to 90°C), whereas the activity of RadADa is strongly temperature dependent, being close to zero at temperatures below 65°C. Summarizing all data presented in the paper, we suggest that RadADa represents a new class of thermophilic recombinases.

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