

The Crystal Structure of Archaeal RadA Recombinase in the Presence of Monovalent Ions

X. Qian, K.T. Osman, Y. Luo, University of Saskatchewan

Department of Biochemistry, University of Saskatchewan

49

PRINCIPAL CONTACT:

Yu Luo

Associate Professor
Department of Biochemistry
University of Saskatchewan
Yu.Luo@usask.ca
1(306)9664379

Introduction

Archaeal RadAs are close homologues of eukaryal Rad51s (~40% sequence identities) and remote homologues of bacterial RecAs (~25% sequence identities). These recombinases promote a hallmark strand exchange reaction between homologous single-stranded and double-stranded DNA substrates. This recombinase-facilitated process is essentially identical to the denaturing and annealing steps in a PCR reaction except that no temperature manipulation is

required. Pairing of the 3'-overhangs located at the damaged DNA with a homologous double-stranded DNA enables the re-synthesis of the damaged region using the homologous DNA as template. This DNA-repairing function plays a key role in cancer cells' resistance to chemo- and radiotherapy. Understanding such a process would facilitate the design of future therapeutic strategy against cancer. In our recent studies, occurrence of a highly ordered conformation in the DNA-interacting regions has been correlated with the presence of activity-stimulating potassium [1] or calcium [2] ions. The results presented here further supports the hypothesis that the recurrent conformation seen in the presence of activating cations resembles the active conformation.

Method

The archaeal RadA recombinase is cloned from a *Methanococcus* species and over-expressed in *E. coli* Rosetta cells. The purified RadA were active in promoting DNA strand exchange in the presence of a non-hydrolyzable ATP analogue AMP-PNP, magnesium and a second activating ion (potassium or ammonium). The hexagonal RadA crystals (P61 space group) were grown by the hanging drop method and grew to a maximum dimension of 0.1 mm x 0.1 mm x 0.3 mm. The protein sample contained ~1 mM of concentrated RadA and 2 mM of AMP-PNP. The well solutions contained 6-8% polyethylene glycol 3350, 50 mM MgCl₂, 50 mM Tris-HCl buffer at pH 7.9, and 0.3 M of a monovalent salt (NaCl, KCl, RbCl or NH₄Cl). The crystals were transferred into a stabilizing solution with the well solution supplemented by 30% (w/v) sucrose, looped out of the solution, and frozen on site in a nitrogen cryo-stream at 100 K. The diffraction data sets were collected in August 2007 at CLS beamline 08ID-1 (Canadian Macromolecular Crystallography Facility).

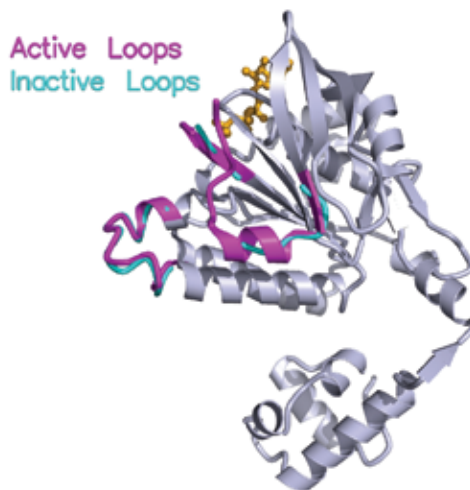


Figure 1: Structure of RadA recombinase with two conformations seen in its DNA-interacting loops highlighted in magenta and cyan.

The resolutions ranges from 2.0 Å to 2.5 Å with the highest resolution data set from a crystal in the presence of the previously unknown activating ammonium ions. Unlike potassium, the nitrogen atom in an ammonium ion does not pose observable anomalous scattering signals to reveal its exact location. In protein crystals, an ammonium ion is indistinguishable from ordered water molecules. Nevertheless, the large-scale conformational change in the DNA-binding regions of RadA can be clearly observed. As expected, structures in the presence of activating potassium (2.4 Å resolution) and ammonium (2.0 Å resolution) showed the recurrent ordered conformation in the DNA-interacting loop 2 (residues 256 to 285). In contrast, structure in the presence of non-activating sodium or rubidium showed another recurrent conformation with largely disordered loop 2 (Figure 1). The electron densities are consistent with either two water molecules or ammonium ions (Figure 2) at essentially identical positions of previously known potassium ions further suggest ammonium mimics the physiologically abundant potassium.

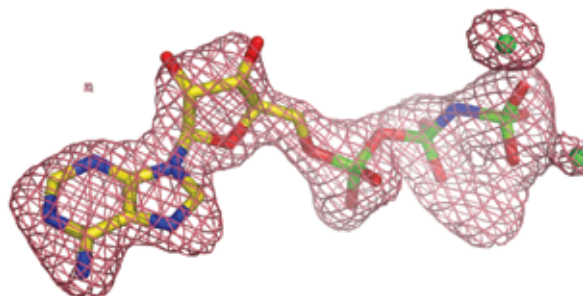


Figure 2: Omit electron difference map around the ATP analogue AMP-PNP and two potential sites for ammonium ions.



Discussion

The structural data acquired at CLS beamline 08ID-1 enabled the correlation between the occurrence of an ordered and possibly active conformation with the newly observed activating effect of ammonium. Though such a correlation is expected, it is worth noting that these RadA crystals reported here showed a different crystallographic packing scheme from all those published before. Yet the crystallized filaments showed a rather smaller range of helical pitches (107-109 Å) compared to previous crystallographic reports (104 -108 Å). This result is in sharp contrast with results from electron microscopy and atomic force microscopy, which have estimated the pitch to be in a wide range between 90 and 130 Å. It is possible that sample-fixing procedures may have distorted the filaments and widened the observed range. Archaeal RadA is the only such recombinase that is crystallized in the presence of all small molecular co-factors (ATP analogue, magnesium, and a second activating ion), further structural evidence is required to verify whether active recombinase filaments in all organisms share a conserved scaffold for assisting DNA strand exchange.

Conclusion

The crystallographic results further support our hypothesis that a second ion is required to activate RadAs and their close eukaryal homologues. The recurrent conformation seen in the presence of a second activating ion appears to resemble an active conformation in the process of DNA strand exchange. Further efforts in co-crystallization are required to reveal interactions between DNA and recombinase.

References

1. Qian, X., He, Y., Wu, Y. & Luo, Y. 2006. Asp302 Determines Potassium Dependence of a RadA Recombinase from *Methanococcus voltae*. *J Mol Biol* 360, 537-47.
2. Qian, X., He, Y., Ma, X., Fodje, M. N., Grochulski, P. & Luo, Y. 2006. Calcium stiffens archaeal Rad51 recombinase from *Methanococcus voltae* for homologous recombination. *J Biol Chem* 281, 39380-7.

Acknowledgements

The authors thank Dr. Michel Fodje at CLS beamline 08ID-1 for his software engineering and technical assistance with the diffraction data collection.