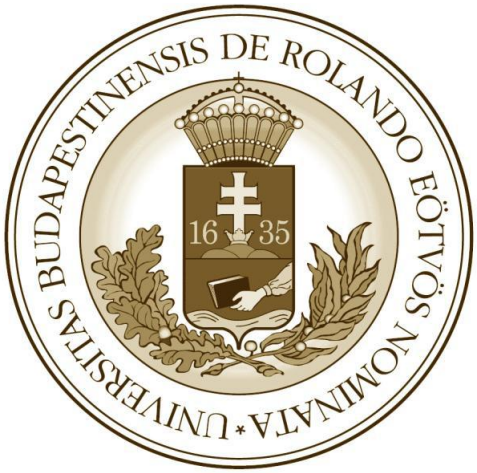
Structural changes and domain movements in RecQ helicases

Zsuzsa Kocsis

Eötvös Loránd University,
Doctoral School of Biology
Director: Prof. Anna Erdei

Structural Biochemistry Doctoral Program
Director: Prof. László Nyitray

Supervisor:
Dr. Mihály Kovács,
PhD, DSc, Research Associate Professor



Eötvös Loránd University,
Faculty of Science, Institute of Biology, Department of Biochemistry

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Introduction

Helicases are nucleotide binding proteins that unwind nucleic acid strands. RecQ helicases play roles in double-stranded DNA break repair by homologous recombination, thus promoting the genome maintenance. RecQ helicases suppress illegitimate recombination, dissolve D-loops, and promote the production of non-crossover recombination products. Furthermore, they participate in the processing of stalled replication forks, and maintaining telomeres. The importance of their role can be demonstrated by the fact that the functional deficiency of RecQ proteins lead to severe genetic diseases including Bloom's, Werner's and Rothmund-Thomson syndromes, which are associated with high cancer predisposition and, in some cases, premature aging. *Escherichia coli* (Ec) possesses only one (the prototypic) RecQ helicase homolog. In humans there exist five family members: Recq1, Bloom (BLM), Werner (WRN), Recq4 and Recq5 helicases. There are numerous RecQ helicase activities including ATP hydrolysis-driven single stranded (ss) DNA translocation and double stranded DNA unwinding, all of which require DNA binding.

RecQ helicases contain two RecA domains, which are responsible of ATP hydrolysis, a Zn^{2+}-binding (ZB) domain, a winged-helix (WH) domain, and mostly a HRDC domain. All of the conserved RecQ domains are proposed to bind DNA, but the information about the DNA-bound crystal structures, DNA-binding sites, and the mechanism of DNA binding is scarce. The crystal structure of HRDC-truncated RecQ is described, as well as the structure of Recq1 (also lacking HRDC) in the presence of DNA. In the Recq1 structure, the WH domain appears in a rotated position compared to the RecA domains. It is unknown whether this difference is caused by the difference between the RecQ homologs, or there is a DNA-induced conformational change occurring.

Problems and aims

1. Although RecQ helicases share a conserved structure, the structures of Ec RecQ and DNA-bound human Recq1 differ significantly. The difference can be caused either by variation between the homologs or by a DNA-driven isomerisation.
   We aimed to explore if DNA causes a conformational change in RecQ helicase.

2. The effect of the enzymatic cycle (nucleotide state) on the communication between binding sites, the strength of the binding and the proposed structural changes is unknown.
   We wished to explore how the proposed structural change (and the extent of the DNA interaction) is affected by the change of the enzymatic (nucleotide) state cycling through the ATPase cycle.

3. In the structure of RecQ helicases there are numerous conserved principles, but the contributions of the RecQ helicase domains to DNA binding are unexplored.
   We aimed to assess the contribution of the RecQ helicase domains to DNA binding.

4. The features of RecQ helicase steady-state translocation along ssDNA have been described, but the contribution of the nucleotide states to the process of translocation is unknown, which is the basis of DNA structure processing.
   We aimed to study which nucleotide states (and DNA-bound structures) are most abundant in the RecQ translocation mechanism.

5. Earlier experiments suggested that human BLM helicase can carry out different activities in different oligomerisation states, but this is still a contentious issue.
   We aimed to determine the oligomerisation state of BLM helicase, in part by measuring the occupied binding site size along DNA.

Experimental approach

1. The tryptophan fluorescence signal of RecQ helicase was used to follow the proposed structural changes of RecQ helicase, as this signal allowed the detection of the change of the structure of the whole protein.

We labelled the RecQ protein with a double pyrene label, because the stacking interaction of the pyrene molecules leads to a detectable excimer emission. Therefore we prepared a new RecQ construct by placing one of the pyrenes into the WH domain, and the other one into the N-terminal RecA domain in order to monitor domain movement. For double pyrene labelling, a mutant was created (4CRecQ), which was prepared with QuikChange kit, having two amino acids changed to cysteines at the appropriate sites, and two intact cysteines removed from the molecular surface (C94A, T187C, C351S, A487C). We made the one-in and two-out cysteine constructs too: 187C-RecQ (C94A, T187C, C351S) and 487C-RecQ (C94A, C351S, A487C).

We used stopped-flow transient kinetic experiments to explore DNA-binding and determine experimentally the kinetic and equilibrium constants.

2. We examined the DNA binding of RecQ helicase in different nucleotide states using stopped-flow system.

3. We studied the contribution of the conserved RecQ domains to DNA binding. We employed wild-type RecQ, RecQ-d523 and RecQ-d414 constructs, which end at the referred amino acid; RecQ-Y555A mutant, which possesses a tyrosine-to-alanine change. The RecQ-d523 construct lacks the HRDC, while RecQ-d414 lacks both the HRDC and WH domains. The Y555A mutation was shown to abolish the ssDNA-binding capability of the HRDC domain.

4. We compared the mean DNA-bound lifetimes and dissociation constants during ssDNA translocation with those in different nucleotide states, in order to identify the most abundant enzymatic state(s) during translocation.

5. To study the oligomerisation of BLM helicase, we employed steady-state fluorescence measurements with fluorescently labelled (Cy3) DNA molecules of different length.

Theses and conclusions

1/A. thesis: We detected biphasic RecQ DNA binding stopped-flow curves. The first phase had linear length dependence, whereas the second one showed hyperbolic dependence on DNA concentration.

Conclusion: After the fast DNA-binding step, there occurs a slower, isomerisation step.

$$\underbrace{\mathrm{RecQ.(N) + DNA} \overset{K_1}{\rightleftharpoons} \mathrm{RecQ^{\dagger}.(N).DNA}}_{K_{eq}} \overset{K_2}{\rightleftharpoons} \mathrm{RecQ^{\dagger\dagger}.(N).DNA}$$

Proposed mechanistic scheme of DNA binding by RecQ and RecQ-nucleotide complexes (RecQ.N), involving an initial binding step (K_1) followed by an isomerisation (K_2) representing a structural change occurring in the RecQ.DNA complex. Both steps are associated with a quench in Trp fluorescence (indicated by † and †† symbols). K_1 and the overall equilibrium constant of DNA binding (K_{eq}) are defined as dissociation constants, whereas K_2 is defined in the rightward direction.

1/B. thesis: The rate constants of DNA binding are not length dependent. However, the amplitude of the slow phase increases with the DNA length.

Conclusion: There are no detectable DNA end effects, or cooperative DNA binding effects. The increase in the amplitude shows that there may be affected ssDNA regions outside the primary occupied DNA binding-site size.

1/C. thesis: The rate constants of the first step, k_{-1}, k_1, both decreased with temperature, but the extent of the decrease was the same. The overall dissociation constant of the reaction (K_{eq}) decreased as the temperature increased.

Conclusion: In the first step of DNA binding, the equilibrium constant unchanged. The overall affinity is smaller at 5^{o}C, suggesting an endothermic reaction.

1/D. thesis: In the single pyrene-labelled constructs, the monomer pyrene signal is apparent, as the excimer signal is absent. In the double pyrene 4CRecQ mutant, both the monomer pyrene and excimer emission signals are detectable.

Conclusion: The excimer signal in the emission suggests that the pyrenes are attached to the appropriate sites on the RecA and WH domains, and are stacked.

1/E. thesis: The excimer emission peak of double pyrene-labelled RecQ4C protein decreases upon ssDNA binding, and the monomer pyrene signal increases slightly. The single pyrene-labelled RecQ constructs did not show any significant DNA-induced change in the emission spectra.

Conclusion: The reduced excimer emission indicates that the pyrenes are displaced, suggesting a rotation of the WH domain.

1/F. thesis: The DNA binding stopped-flow curves of the double pyrene-labelled RecQ are biphasic. The rate constants of the first phase are the same as the ones calculated from Trp fluorescence measurements, but those of the second phase differ.

Conclusion: We succeeded in observing the same DNA-binding step with the pyrene excimer signal as with the Trp signal. We propose that we found an other isomerisation step, which is caused by the rotation of the WH domain.

2/A. thesis: The DNA binding properties of the RecQ protein depend on its nucleotide state.

Conclusion: The nucleotide-state dependence of the isomerisation step suggests an ATP hydrolysis-driven conformational change.

2/B. thesis: In the case of the ADP.AlF_4 state, the first binding step is much slower without any difference in the equilibrium constant. We found a marked the decrease of the rate constant of the backward reaction of the second step (k_{-2}), which resulted in a massive increase in ssDNA affinity.

Conclusion: Together with the DNA-binding stoichiometry, this kinetic change suggests a larger binding-site size of RecQ.ADP.AlF_4 complex occupied on ssDNA than in other nucleotide states. Previously, the ADP.AlF_4 complex was proposed to represent different ATPase cycle states in different proteins. As the features of the RecQ.ADP.AlF_4 complex differ greatly from the RecQ.AMPPNP prehydrolysis state, we propose that it represents a transient state during ATP hydrolysis, or a posthydrolytic, ADP.P_i-bound state. Our data show

that ATP hydrolysis by RecQ helicase is coupled to a conformational change closing the DNA binding site.

3. thesis: The DNA binding transients of RecQ mutants are monophasic. The unchanged ATPase activities of the mutants suggest that the RecA domains are intact and correctly folded.

Conclusion: The DNA-bound isomerisation (K_2) step is not detectable in the absence of a fully functional HRDC domain. The RecQ-d523 and RecQ-Y555A constructs contain all native tryptophans. Thus the results suggest that, during the isomerisation, there is a change in the the HRDC-DNA interaction. These two mutants preserved the strong ssDNA affinity, which suggests that the HRDC domain has other roles than the stabilisation of DNA binding.

4. thesis: In the steady-state RecQ helicase appears mostly in the prehydrolytic (mimicked by AMPPNP) and ADP.AlF_4 (proposed to be posthydrolytic, or ADP and phosphate-bound) states.

Conclusion: This suggests that ATP-hydrolysis, phosphate release, or coupled structural changes is/are the rate limiting step(s) of the ATPase cycle of RecQ.

5. thesis: BLM helicase concentration dependence of the labelled DNA fluorescence spectra revealed a 40-nt binding site size.

Conclusion: Together with other complex experiments, we showed that BLM is a monomer in the DNA bound state, but slightly oligomerises (mostly dimerises) when bound to more complex DNA structures. BLM occupies a 40-nt stretch along DNA, shows no cooperative tendency, and translocates as a monomer.

Summary

The results in my dissertation show that DNA-bound RecQ helicase undergoes a nucleotide-state dependent conformational change. This is the first report of such a change found in any RecQ helicase. Furthermore, we observed a strongly DNA-bound state, which likely plays a role in mechanochemical coupling between ATP hydrolysis and ssDNA translocation. The described isomerisation includes the DNA binding of the HRDC domain. Taken together with earlier works studying ssDNA translocation, unwinding and ATPase activity of RecQ helicase, our research contributes to elucidating genome maintenance activities by describing the DNA binding mechanism of RecQ helicases.

Publications in the topic of the dissertation

Journal articles:

International peer-reviewed journal publications:

- Gyimesi M., Pires R. H., Billington N., Sarlós K., **Kocsis Z. S**., Módos K., Sellers R. J., Kellermayer M. S., Kovács M.
  Visualization of Human Bloom's Syndrome Helicase Molecules Bound to Homologous Recombination Intermediates. FASEB Journal 2013; 27(12):4954-64.
- **Kocsis Z. S**., Sarlós K., Harami G. M., Martina M., Kovács M.
  A nucleotide- and HRDC-domain-dependent structural transition in DNA-bound RecQ helicase. Journal of Biological Chemistry, 2014, e-pub 2014 Jan 8, in press (MS ID: JBC/2013/530741)

Conference presentations:

- 2013, Hungarian Molecular Life Sciences 2013 Conference, Siófok, Hungary:
  Kocsis Z. S., Sarlós K., Kovács M., Harami G. M., Gyimesi M., Martina M., Seol Y., Ferencziová V., Neuman K. C.
  Domain Movements and Sequence-specific Pausing of RecQ Helicases.
- 2013, The Student Scientific Conference on Biotechnology and Biomedicine, Brno, Czech Republic:
  Kocsis Z. S., Sarlós K., Kovács M., Harami G. M., Gyimesi M., Martina M., Seol Y., Ferencziová V., Neuman K. C.
  Domain Movements and Sequence-specific Pausing of RecQ Helicases.

Other publications

Journal articles:

International journal publications:

- **Kocsis Z. S**., Molnár C. S., Watanabe M., Daneels G., Moechars D., Liposits Z., Hrabovszky E. Demonstration of vesicular glutamate transporter-1 in corticotroph cells in the anterior pituitary of the rat. Neurochem Int 2009; 56:479-486.

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- **Kocsis Z. S**.
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Conference abstracts:

- 2008, Meeting of Hungarian Neuroscience Society, Debrecen, Hungary:
  Kocsis Z. S.; Deli L.; Kalló I.; Horváth C. M.; Keller É.; Liposits Z.; Hrabovszky E.
  Localisation of synaptic vesicle protein-2 in rat and human hypophysis.
- 2009, Meeting of Hungarian Neuroscience Society, Budapest, Hungary:
  Kocsis Z. S.; Kalló I.; Wittmann G.; Fekete C.; Liposits Z.; Hrabovszky E.
  Occurrence and phenotype of glutamatergic elements in the adenohypophysis of the rat.
- 2011, FASEB Summer Research Conference, Steamboat Springs, USA:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Mechanistic basis of the DNA-restructuring activity of the Rad5 double-stranded DNA translocase.
- 2011, Annual Meeting of Hungarian Biochemical Society, Pécs, Hungary:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Mechanistic basis of the DNA-restructuring activity of the Rad5 double-stranded DNA translocase.
- 2012, Biophysical Society 56th Annual Meeting, San Diego, USA:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Mechanochemistry of the Rad5 double-stranded DNA translocase.
- 2012, FEBS3+ Meeting, Opatija, Croatija:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Fork reversal by Rad5: Molecular basis.
- 2012, IUBMP&FEBS YSP, Cadiz, Spain:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Molecular mechanism of the fork reversal by Rad5.
- 2012, IUBMP&FEBS, Sevilla, Spain:
  Kocsis Z. S., Pintér L., Haracska L., Kovács M.
  Molecular mechanism of the fork reversal by Rad5.