6 Summary

Type I restriction-modification enzyme EcoR124I is a multifunctional, hetero-oligomeric enzyme complex that cleaves DNA after extensive ATP hydrolysis coupled to processive DNA translocation. ATP hydrolysis and DNA translocation are conferred by superfamily 2 (SF2) helicase motifs in the central domain of its HsdR subunit. The N-terminal domain carries a conserved region with catalytic residues reminiscent of the PD-(E/D)xK catalytic motif of Type II restriction enzymes.

Single amino acid substitutions in the motifs II and III reduced or removed DNA cleavage activity of the enzyme complex without affecting an assembly of the complex and its DNA-binding properties. Using a combination of bulk solution and single-molecule assays, we investigated the influence of these mutations on the DNA translocation properties of the enzyme, conferred by the helicase domain. Reduced ATPase activity of the mutants was detected by steady-state stopped flow measurements with the use of phosphate-binding protein. These results do not show a clear relationship between the translocation rates and ATPase rates. Probably the broader and bimodal distribution of translocation rates and the stalling events during initiation revealed in single molecule experiments all lead to a lower apparent ATPase rates. We suggest an existence of possible interdomain interactions between the helicase and the nuclease domains of the HsdR subunit, which is indicated by the observed effect of these mutations on the ATPase activity and the DNA translocation.

In addition to the principal PD-(E/D)xK Motifs, I, II and III, bioinformatic analysis of the HsdR subunits of EcoR124I revealed the presence of a QxxxY motif that is characteristic for RecB-family nucleases. The QxxxY motif resides immediately C-terminally to Motif III within a region of the predicted α-helix. We examined the role of the Q and Y residues in DNA binding, translocation and cleavage with the use of site-directed mutagenesis. The mutations in the QxxxY motif did not change the DNA translocation properties; however, all of them altered the DNA binding affinity and reduced both the rate and the efficiency of DNA cleavage. Role(s) of the QxxxY motif in coordination of the catalytic residues and/or in a stabilization of the nuclease domain on the DNA are discussed.