

Analysis of the mutations in the HsdS recognition subunit of the R-M system EcoR124I

Type I restriction-modification (R-M) systems are hetero-oligomeric enzyme complexes, consisted of three subunits: HsdR (restriction), HsdM (modification) and HsdS (specificity). The HsdS subunit recognizes specific target sequences on the DNA and is responsible for specificity of the enzyme complex. HsdS and HsdM together form a methylase (MTase) and are necessary for methyltransferase activity and the third subunit, HsdR, is required for DNA restriction. The HsdS subunit comprises three conserved domains and two target recognition domains (TRD). The conserved domains are probably responsible for binding with other subunits (HsdM, HsdR).

The aim of this work was to analyse the effect of the point mutations in the central and C-terminal conserved regions of HsdS on restriction and modification phenotypes of EcoR124I enzyme *in vivo* and *in vitro*. The mutants were analysed for restriction and modification phenotypes. Classically, mutations within the *hsdS* gene of a Type I R-M enzyme produce an $r^- m^-$ phenotype due to the loss of DNA-binding properties. The substitutions Lys¹⁸⁴Asn and Lys³⁸⁴Asn mutations analysed here were identified as “non-classical” because they expressed an $r^- m^+$ phenotype, which is thought to be due to alterations to protein-protein interactions.

Mutant MTase (K384N) was purified and mixed with wt HsdR to reconstitute the EcoR124I endonuclease *in vitro*. Cleavage assay proved an $r^- m^+$ phenotype in agreement with the *in vivo* test. These results implicate that the point mutations in the central and C-terminal conserved regions of the HsdS subunit can alter ability of HsdS or MTase to bind to HsdR subunit, which shows the importance of the conserved regions for intersubunit interactions. This work is a useful background for further mutagenic analysis in an attempt to identify specific residues that are involved in HsdS-HsdM and MTase-HsdR interactions.

Klíčová slova: Restriktivně-modifikační enzymy, rozpoznávací podjednotka HsdS, bodová mutace, methylasa, interakce protein-protein

Keywords: Restriction-modification enzymes, HsdS – the recognition subunit, point mutation, methylase, protein-protein interactions