

TYPE I RESTRICTION-MODIFICATION ENZYMES AS MOLECULAR MOTORS IN BIO-NANOTECHNOLOGY

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ABSTRACT

The ability of type I restriction-modification enzyme to translocate DNA preceding restriction makes them to act as an intelligent molecular motor. We have experience with the complex enzyme *EcoR124I* existing in bacterial cell in equilibrium of $R_1M_2S_1$ and $R_2M_2S_1$ complexes. Complex with only one HsdR subunit is not able to cleave DNA but retains DNA translocation ability. Therefore, there is an opportunity of studying this enzyme as a molecular motor without problems associated with DNA cleavage. However under certain circumstances even the R_1 -complex is able to restrict DNA. To eliminate completely this possibility we will focus on site-directed mutagenesis of three conserved amino acid residues in the endonuclease motif of HsdR subunit. This project is part of EU grant "A Molecular Magnetic Switch that links the Biological and Silicon Worlds."

1. TYPE I RESTRICTION AND MODIFICATION SYSTEMS

Restriction and modification (R-M) systems are divided into three groups; type I, II and III of which the most predominant is the type II enzymes used in genetic engineering. R-M enzymes are generally thought to protect bacteria from invasion by foreign DNA.

The most complex type I R-M systems are coded by three genes, *hsdR*, *hsdM* and *hsdS*. All three genes are required for production of the restriction endonuclease; *hsdR* is absolutely required for restriction and is transcribed from its own promoter (P_{RES}); while *hsdM* and *hsdS* are transcribed from a separate promoter (P_{MOD}) and together are required for modification. The HsdS subunit (product of *hsdS* gene) plays the key role in the recognition of DNA target sequence in both restriction and modification. DNA target sequence consists of two specific components, one of 3 bp and another of 4 or 5 bp, separated by a non-specific spacer; e.g. AAC N6GTGC for *EcoKI* and GAA N6RTCG for *EcoR124I*. The endonuclease requires ATP, S-adenosyl methionine (SAM) and Mg^{2+} as co-factors. ATP and SAM serve as both cofactors and allosteric effectors and determine whether restrictase, or methylase, activity occurs, dependent upon the state of methylation of the DNA substrate. The methylase requires only SAM as a cofactor (for reviews see, [1]).

The type I restriction and modification systems are divided into four families (type IA e.g. *EcoKI*, type IB e.g. *EcoAI* and type IC e.g. *EcoR124I* and type ID e.g. *StySBLI*) based on gene order, amino acid conservation, complementation assays and enzymatic properties [2, 3].

2. THE ROLE OF HsdR SUBUNIT

As mentioned above type I R-M enzymes are composed of three subunits - HsdR is absolutely required for restriction activity, while HsdS is the DNA recognition subunit, which, together with HsdM, produces an independent DNA methyltransferase (MTase). DNA restriction is associated with a powerful ATPase activity during which DNA is translocated by the enzyme until the enzyme stalls and cleavage occurs [4]. Recently, it has been shown that the cleavage complex of the type IC R-M system *EcoR124I* has a subunit stoichiometry of $HsdR_2M_2S_1$ [5].

However, an R_2 -complex exists in equilibrium with the R_1 -complex, which cannot cleave DNA but is capable of translocating the DNA [6, 7]. Therefore, the opportunity exists to study these enzymes as molecular motors without the problems associated with DNA cleavage. The R-M holoenzyme was found to be highly processive, translocating DNA at 400bp/sec in both directions, while the R_1 -complex, although less processive, is unidirectional [7].

Molecular motors move unidirectionally along polymer tracks, producing movement and force in an ATP-dependent fashion [8]. Type I R-M enzymes are similar, but instead of moving along DNA (such as for RNA and DNA polymerases), they move the DNA through the DNA-bound enzyme complex [9].

HsdR is absolutely required for DNA cleavage (restriction activity) and is responsible for ATP-binding and subsequent DNA translocation. Therefore, the HsdR subunit is representing the motor component of the enzyme. HsdR comprises several domains defined by limited proteolysis, sequence analysis and mutational analysis for *EcoKI* [10] and *EcoAI* [11] (Fig.1). Near the N-terminus, there is a proteolytically defined domain of 400 amino acids containing an amino acid motif common to all endonucleases. This region includes a conserved motif X [3], which forms the active site of the nuclease domain. Moreover a further N-terminal domain of unknown function which is not conserved in all HsdR subunit has been revealed upstream the motif X. Part of C-terminal domain is involved in binding to MTase [10].

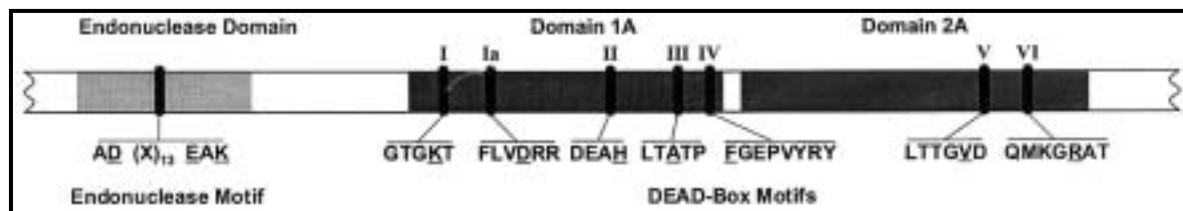


Fig. 1. Domains and motifs of HsdR of *EcoKI*. The two domains that include the DEAD-box motifs correlate with 1A and 2A, as determined for the structures of DNA helicases. Substitutions for the underlined amino acids confer a restriction-deficient phenotype. [1]

In between the endonuclease domain and the C-terminal domain is a region with sequence similarity to domains 1A and 2A of DNA and RNA helicases [12]. This region contains the DEAD-box amino acid motifs implicated in ATP binding, ATP hydrolysis and DNA translocation. Extensive sequence analysis and mutational experimentation have demonstrated the importance of all seven conserved DEAD-box motifs for ATP hydrolysis and DNA translocation. Enzymes with substitutions in DEAD-box motifs fail to hydrolyse ATP and translocate DNA [13, 14]. On the other hand enzymes with conservative substitutions within the endonuclease motif retain their ability to translocate DNA, but these enzymes fail to hydrolyse phosphodiester bonds. Site-directed mutagenesis proved the relevance of motif X to the endonuclease activity of *EcoAI* [11] and *EcoKI* [10].

3. MUTAGENESIS AT MOTIF X OF *hsdR*

It is possible that $R_1M_2S_1$ enzyme would cleave under circumstances where a DNA molecule has two binding sites for the enzyme and a collision might occasionally occur between two bound R_1 -complexes. Therefore, to totally remove such a possibility and provide a fully functional molecular motor, which can never cleave DNA, the motif X amino acids of the HsdR subunit will be mutated using site-directed mutagenesis.

Site-directed mutagenesis proved the relevance of this motif to the endonuclease activity of *EcoAI* [11] and *EcoKI* [10]. In both cases single amino acid substitutions in the conserved residues (D,E,K) of the D-X₁₃₋₁₄-E-X-K sequence motif X (Fig.2) uncoupled the DNA translocation and DNA cleavage activities of the enzymes. Substitution of the conserved Gly in the spacer between the acidic residues of the motif had no effect on restriction function. [11]

<i>EcoKI</i> (IA)	ADYVLFV-- GLKPIAVVEAK 332
<i>EcoAI</i> (IB)	ADIVLYHKPGI-PLAVIEAK 78
<i>EcoR124I</i> (IC)	YDVTILVN- GL -PLVQIELK 167

Fig. 2. Conserved region in the HsdR polypeptide of restriction enzymes *EcoKI*, *EcoAI* and *EcoR124I*

Therefore we will focus on mutagenesis of three conserved amino acid residues, D151, E165 and K167 in the endonuclease motif of HsdR subunit of restriction enzyme *EcoR124I*.

The conserved catalytic residues will be individually substituted by an alanine using site-directed mutagenesis. *Trans*-dominant test will be employed for positive evidence of a restriction-deficient phenotype [15].

4. CONCLUSION

Restriction enzymes Type I are intelligent molecular machines. Intelligence resides in their ability to read the methylation status of the recognition sequence on the substrate DNA and as a consequence only switch to restriction-mode when the recognition sequence is un-methylated. The motor activity reflects their ability to translocate DNA at the expense of ATP.

The availability of the mutated HsdR subunits from the *EcoR124I* R-M will allow us to assemble *in vitro* molecular motors that cannot cut DNA under any circumstances and are also present as HsdR₁-complexes which are unidirectional during translocation of the DNA. The availability of these mutant motors will greatly simplify single-molecule determination of molecular forces and assembly of useful motor-based devices.

REFERENCES

- [1] N. E. Murray, Type I restriction systems: sophisticated molecular machines (a legacy of Bertani and Weigle), *Microbiol Mol Biol Rev* 64 (2000) 412-434.
- [2] N. Redaschi, and T. A. Bickle, Posttranscriptional regulation of *EcoP1I* and *EcoP15I* restriction activity, *J. Mol. Biol.* 257 (1996) 790-803.
- [3] A. J. Titheradge, D. Ternent, and N. E. Murray, A third family of allelic *hsd* genes in *Salmonella enterica*: sequence comparisons with related proteins identify conserved regions implicated in restriction of DNA [published erratum appears in *Mol Microbiol* 1997 Feb;23(4):851], *Mol. Microbiol.* 22 (1996) 437-447.
- [4] P. Janscak, M. P. MacWilliams, U. Sandmeier, V. Nagaraja, and T. A. Bickle, DNA translocation blockage, a general mechanism of cleavage site selection by type I restriction enzymes, *Embo Journal* 18 (1999) 2638-2647.
- [5] P. Janscak, A. Abadjieva, and K. Firman, The type I restriction endonuclease R.*EcoR124I*: over-production and biochemical properties, *J. Mol. Biol.* 257 (1996) 977-991.

- [6] P. Janscak, D. T. Dryden, and K. Firman, Analysis of the subunit assembly of the type IC restriction-modification enzyme EcoR124I, *Nucleic Acids Res.* 26 (1998) 4439-4445.
- [7] K. Firman, and M. D. Szczelkun, Measuring motion on DNA by the type I restriction endonuclease EcoR124I using triplex displacement, *Embo J* 19 (2000) 2094-2102.
- [8] W. Kliche, S. Fujita-Becker, M. Kollmar, D. J. Manstein, and F. J. Kull, Structure of a genetically engineered molecular motor, *Embo J* 20 (2001) 40-46.
- [9] M. D. Szczelkun, M. S. Dillingham, P. Janscak, K. Firman, and S. E. Halford, Repercussions of DNA tracking by the type IC restriction endonuclease EcoR124I on linear, circular and catenated substrates, *Embo Journal* 15 (1996) 6335-6347.
- [10] G. P. Davies, I. Martin, S. S. Sturrock, A. Cronshaw, N. E. Murray, and D. T. Dryden, On the structure and operation of type I DNA restriction enzymes, *J. Mol. Biol.* 290 (1999) 565-579.
- [11] P. Janscak, U. Sandmeier, and T. A. Bickle, Single amino acid substitutions in the HsdR subunit of the type IB restriction enzyme EcoAI uncouple the DNA translocation and DNA cleavage activities of the enzyme, *Nucleic Acids Res.* 27 (1999) 2638-2643.
- [12] A. Gorbalenya, and E. Koonin, Endonuclease (R) subunits of type-I and type-III restriction-modification enzymes contain a helicase-like domain., *FEBS Lett* 291 (1991) 277-281.
- [13] G. P. Davies, L. M. Powell, J. L. Webb, L. P. Cooper, and N. E. Murray, EcoKI with an amino acid substitution in any one of seven DEAD-box motifs has impaired ATPase and endonuclease activities, *Nucleic Acids Res.* 26 (1998) 4828-4836.
- [14] G. P. Davies, P. Kemp, I. J. Molineux, and N. E. Murray, The DNA translocation and ATPase activities of restriction-deficient mutants of Eco KI, *J Mol Biol* 292 (1999) 787-796.
- [15] J. Hubáček, V. E. Zinkevich, and M. Weiserová, The location of a temperature-sensitive trans-dominant mutation and its effect on restriction and modification in *Escherichia coli* K12, *Journal Of General Microbiology* 135 (Pt 11) (1989) 3057-3065.

RESTRIKČNĚ-MODIFIKAČNÍ ENZYMY TYPU I JAKO MOLEKULÁRNÍ MOTORY V BIO-NANOTECHNOLOGII

Schopnost restrikčně-modifikačních enzymů translokovat DNA před vlastním štěpením činí z těchto enzymů inteligentní molekulární motor. Máme zkušenosti s komplexním enzymem *EcoR124I*, který se vyskytuje v bakteriální buňce ve formě dvou komplexů $R_1M_2S_1$ a $R_2M_2S_1$, jež jsou ve vzájemné rovnováze. Komplex obsahující jen jednu podjednotku HsdR není schopen štěpit DNA, ale zachovává si schopnost DNA translokovat. Tím se nabízí možnost studovat tento enzym jako molekulární motor bez problémů spojených s restrikcí. Je však známo, že za určitých okolností i R_1 -komplex může štěpit DNA. Abychom zcela odstranili tuto možnost chceme se zaměřit na řízenou mutagenézi tří konzervovaných aminokyselinových zbytků endonukleasového motivu podjednotky HsdR. Tento projekt je součástí EU grantu " A Molecular Magnetic Switch that links the Biological and Silicon Worlds.