

Studies on mutated DNA ligase from thermostable bacteria *Thermus scotoductus*

Margrét Helga Ögmundsdóttir, Arnar Pálsson, Jón Már Björnsson, Sigríður Elsa Vilmundardóttir, Arnar Pálsson, Eirný Þöll Þórólfsdóttir, Zophanías Oddur Jónsson, Guðmundur Eggertsson og Sigríður Helga Þorbjarnardóttir, Líffræðistofnun Háskólans. sighelga@hi.is

DNA ligases are essential for all living cells. During DNA replication they ligate Okazaki fragments, they take part in recombination and DNA repair. Ligases are divided into families depending on the cofactor they use in ligation. The cofactors are either NAD or ATP. The ligation takes place in three steps. In the first step the enzyme forms covalent bond with adenylate from either NAD or ATP depending on the cofactor, and NMN or AMP is released. The second step is a transfer of the adenylate to the 5' phosphor end of a nick in double stranded DNA. The third step is the closure of the nick. The 3'OH end of DNA nick attacks the 5' DNA-adenylate and ligates the nick and AMP is released.

DNA ligase gene from the thermophilic bacteria *Thermus scotoductus* (*T. scoto*) was mutated. Seven truncates and two point mutations were made. One of the truncates, $\Delta 568-574$, lacks the BRCT domain of the ligase. The point mutations are in amino acid residues 201 and 219, in both cases Ala was replaced with Arg. The point mutations are in the adenylation domain of the ligase.

NAD ligases are found in bacteria. The first NAD ligase gene was found in *Escherichia coli* (*E. coli*). The gene was found to be essential for the bacteria and only temperature sensitive (ts) mutants were found. We have defined two of those first ts mutants in *E. coli* by sequencing.

In vivo complementation tests were done with *T. scoto* ligase. Wild type ligase, point mutants and the $\Delta 568-674$ truncate were tested on the two *E. coli* ts-ligase mutants. Kinetic studies *in vitro* were also done on three *T. scoto* ligase mutants and wild type. Wild type *T. scoto* ligase gene complements both ts mutants in *E. coli* and the truncate one of the mutants. All other complement tests for the *E. coli* ts mutants were negative. *In vitro* activity of the mutants compared to wild type showed that the K_m value for the truncate was four times higher and k_{cat} value was about four orders of magnitude lower. The point mutant (219) showed 20 times higher K_m and about 2 times lower k_{cat} compared to wild type. The other point mutant (201) was completely ineffective in complementation tests and also showed too low activity *in vitro* to measure kinetic values. We conclude that *in vivo* the truncate is capable of step 1 and 2 in the ligation reaction. The *E. coli* ts mutated enzyme is able to catalyze the third step of the ligation by joining the polynucleotides and release AMP from the 5' end of the nick.