Abstract

DNA as the primary carrier of genetic information guarantees organisms to live, grow, develop and reproduce. However, this most vital molecule in the cell is subject to various damages every moment. If it is not repaired, the cell and the organism will eventually succumb to inevitable destruction. One of the most serious damages is abasic site interstrand crosslink (Ap-ICL). Ap-ICL is formed spontaneously when an abasic site covalently pairs with an adenine on the opposite strand. The lack of information on repair mechanisms, the influence of the local sequence and its stability leads to questions about the fate, toxicity and occurrence of these lesions in cells.

During evolution, several mechanisms have evolved to repair these and other damages to ensure the organism's survival. A recently discovered pathway known to repair Ap-ICL is named after the DNA glycosylase responsible for removing Ap-ICL. NEIL3 DNA glycosylase is recruited to Ap-ICL by ubiquitylation of DNA helicase, which is part of the DNA replication complex. NEIL3 glycosylase contains several zinc-finger domains, that bind to the damaged DNA and ensure its catalytic function. The molecular mechanism of the NEIL3 glycosylase repair process is currently not known.

In order to answer the aforementioned unknowns, the rate of formation, yield and stability of Ap-ICL depending on the local sequence were investigated *in vitro*. These experiments revealed the impact of different bases in the vicinity of the abasic site on Ap-ICL formation. AT-rich sequences were found to undergo Ap-ICL more rapidly than GC-rich sequences. Surprisingly, Ap-ICL is formed from the abasic site virtually independently of the surrounding sequence, albeit with different rates and yields.

In the second part of the work, the mechanism of recognition of native DNA substrate by NEIL3 glycosylase was investigated. The structure of two zinc-finger GRF domains and catalytic Nei domain from NEIL3 is clarified. Via structural and experimental data, the presented work reveals the molecular details and preference interaction of GRF domains to DNA replication fork. The obtained crystal structure of the Nei catalytic NEIL3 domain with DNA-replication intermediate allowed the proposal of a mechanism for the recognition of two stalled replication forks upon Ap-ICL by NEIL3 glycosylase.

Keywords: DNA damage, DNA interstrand crosslink, abasic site, non-enzyme kinetics, DNA repair, NEIL3, GRF