Abstract

Uropathogenic bacteria *Escherichia coli* are the primary cause of urinary tract infections. One of the virulence factors of these bacteria is α-hemolysin (HlyA), a protein belonging to the cytolytic RTX (Repeats in ToXin) toxins secreted by some gram-negative pathogenic bacteria. RTX toxins share several characteristic structural and functional domains and segments: (1) an N-terminal hydrophobic pore-forming domain, (2) an acylated segment where two lysine residues are modified by fatty acid chains, (3) a repetitive (RTX) domain binding calcium ions, and (4) a C-terminal secretion signal recognized by the type 1 secretion system. HlyA is synthesized as an inactive protoxin (proHlyA), which is activated by covalent acylation of the ε-amino groups of two conserved lysine residues, K564 and K690, by the co-expressed acyltransferase HlyC. Acyl-acyl carrier protein (acyl-ACP) serves as the acyl chain donor. However, the molecular mechanism by which the acyltransferase HlyC interacts with acyl-ACP and proHlyA is currently poorly understood.

The aim of this bachelor thesis was to identify amino acid residues involved in the interaction between HlyC and ACP proteins. Based on an *in silico* interaction model of HlyC and ACP, positively charged residues in HlyC and negatively charged residues in ACP were predicted to participate in the electrostatic interactions between the two proteins due to the formation on the following interaction pairs: R49–E61, K105–D57, R108–E48, R120–D36, and K129–D39. First mutant variants of HlyC and ACP, respectively, were prepared with introduced point substitutions of the predicted interacting residues. Subsequently, the effect of mutant HlyC variants on the interaction with ACP was tested using a bacterial two-hybrid system. The residues R108 and R120 in the HlyC structure were found to be essential for the interaction with ACP. The remaining tested substitutions of acyltransferase R49A, K105A, and K129A did not significantly affect the interaction. By testing the influence of mutant variants of ACP on the interaction with HlyC, residues E48 and D36 in ACP were found to be essential for the interaction with HlyC. The substitutions D57A and D39A in ACP reduced the interaction with HlyC by approximately half, and the substitution E41A did not significantly affect the interaction with HlyC. These results indicate that two interaction pairs, R108–E48 and R120–D36, are crucial for the interaction of HlyC with ACP.

Key words

α-hemolysin HlyA, acyltransferase, posttranslational modification, acyl carrier protein, bacterial two-hybrid system