Salt-bridge interaction between K26 and E33 is important for maintaining the stability of ALFPm3

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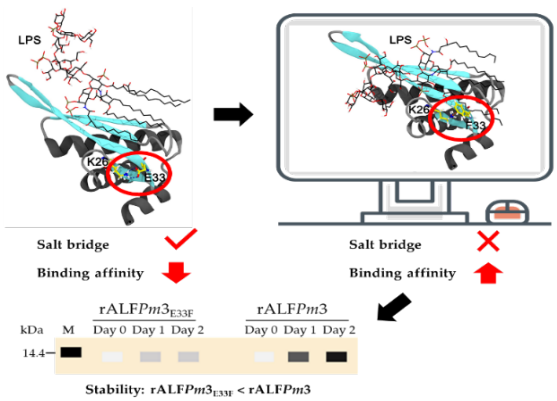
Abstract: The shrimp industry has been persistently affected by production loss from outbreak diseases. Nowadays, using antibiotics in animal feed is illegal. Use of antimicrobial peptide is alternative approach to fight against bacterial infection, and anti-lipopolysaccharide factor isoform 3 (ALF*Pm*3) from *Penaeus monodon* is a promising candidate because it exhibits broad-spectrum antimicrobial activities against various microbes. The lipopolysaccharide-binding domain (LPS-BD) mainly contributes to the antimicrobial activity of ALF*Pm*3. Previous studies reported that the recombinant (r)ALF*Pm*3-supplemented diet can be used to control bacterial and viral infection in shrimp and enhance expression of immune-related genes. However, the possibility of applying rALF*Pm*3 for shrimp disease prevention and control is limited by the high production cost. The more effective rALF*Pm*3 is thus needed. This study aims to produce more effective rALF*Pm*3. ALF*Pm*3 derivatives with better predicted binding affinities to LPS than that wild type were designed using computational techniques. ALF*Pm*3$_{E33F}$ was predicted to have the best binding affinity to LPS (ΔG_{bind} = -14.5 kcal/mol). Site-directed mutagenesis was performed to create the rALF*Pm*3$_{E33F}$ mutant. Following expression and purification, we unexpectedly found that the stability of the rALF*Pm*3$_{E33F}$ protein was lower than that of the wild type. Structural analysis shows that the salt-bridge interaction between K26 and E33, a residue flanking LPS-BD, in the wild type is disrupted when E33 was substituted with F33 in ALF*Pm*3$_{E33F}$. Out results indicate that the salt bridge between K26 and E33 is important to maintain the stability of rALF*Pm*3.

Graphical abstract:



Keywords: Anti-lipopolysaccharide factor isoform 3; Salt-bridge interaction; Protein stability

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