“Molecular mechanism of thermal stability of the endolysin Ts2631 from bacteriophage vB_Tsc2631 infecting *Thermus scotoductus*”

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People have been fighting pathogens for centuries. The discovery of antibiotics and bacteriophages has contributed to improving the quality of live, but every organism, including bacteria, develops survival mechanisms. Humanity is increasingly experiencing a crisis related to bacterial drug resistance. That is why it is so necessary to develop new strategies to combat it. One of the desirable characteristics of a suitable drug is its stability. Therefore, bacteriolytic enzymes should be sought in the thermostable organisms which are highly resistant to chemical denaturation and proteolysis. In the current study, I have investigated the mechanism of thermal stability of endolysin Ts2631 based on its amino acid sequence. The melting temperature (T_m) initially determined for this protein was 99.82°C, but in a buffer consisting of 20 mM HEPES, pH 7.4, 25 mM NaCl and 10% glycerol, it was determined to be 104.73°C. As a result of my analyses, I proved that residues W102, W109, W145 and P140 are important for the thermostability of endolysin Ts2631. In addition, disruption of Zn^{2+} coordination resulted in a significant decrease in T_m. During my research, I also demonstrated that other proteins derived from thermophiles similar to the Ts2631 have the aforementioned tryptophan and proline in the same position, which suggest that the mechanism of thermal stability may be similar in these proteins. I set a goal of checking whether the thermostability of mesophilic proteins with structural homology to endolysin Ts2631 can be increased without affecting their lytic activity, after replacing the amino acids in the mesophilic proteins with those responsible for thermostability of Ts2631 endolysin. I examined K11gp3.5 endolysin derived from bacteriophage K11, which infects *Klebsiella pneumoniae* and the amidase domain of the LytO protein, which derived from *Staphylococcus aureus* subsp. *aureus* NCTC 8325. In the case of the Q115W_F179W substitution variant of the protein LytO amidase domain, the T_m was increased by 9.7°C compared to the wild-type protein. In the case of N91W_Q137W variant, T_m decreased by approximately 6°C. The results obtained show that the substitution of amino acid residues alone is not sufficient to improve the thermostability of a protein, and that it is also necessary to analyse the environment of the residues to be replaced. There is therefore no universal research procedure, and each case requires individual analysis.