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Temperature and Concentration-dependences of Tolaasin-induced Hemolysis

Kwang-Hyun Cho*, Sung-Soo Bhan, and Young-Kee Kim

Dept. of Agricul. Chem., Chungbuk Nat'l Univ

Tolaasin, a pore-forming 1.9 kDa peptide toxin released by *Pseudomonas tolaasii*, produces brown blotch disease on cultivated oyster mushrooms. To investigate the mechanism of tolaasin-induced cell disruption, we studied the effect of temperature on the hemolytic process. In the kinetic analyses, single exponential function was fitted to the data obtained from temperature-dependent velocity of hemolysis($1/t_{50}$), implying that there is a major time-limiting factor on the temperature-dependent hemolysis. No hemolysis occurred at 4℃ in the presence of toxins. Subsequently, the cells were harvested and resuspended with toxin-free fresh HBS, no hemolysis was observed at 37℃. However, after erythrocytes were incubated with tolaasin at 37℃ in the presence of PEG 2000, an osmotic protectant, and cells were recovered and resuspended with fresh HBS which is free of toxin and PEG, hemolysis was observed at 4℃. These results indicate that tolaasin molecules do not bind to cell membrane at 4℃. The results also suggest that once tolaasin binds to membrane, there is negligible temperature dependence on the step of pore formation. Meanwhile, The effect of erythrocyte concentration on the hemolysis at constant amount of tolaasin was measured to understand the binding properties of tolaasin to the erythrocyte. An increase in the concentration of erythrocytes from 0.1 to 0.6% increased the release of total hemoglobin followed by a gradual decrease, resulting in a bell-shaped release curve. However, the percentage of hemolysis decreased as increase in erythrocyte concentration at the constant amount of tolaasin. These results show that hemolysis is dependent on the amount of tolaasin and multiple binding of tolaasin is required for the hemolysis of single cell, suggesting a 'multi-hit model' in tolaasin-induced hemolysis. In order to study the number of tolaasin molecule or hit to lyse one erythrocyte, the logarithm of proportion of hemolysis was plotted versus the logarithm of the amount of tolaasin. The slope of the line for tolaasin-induced hemolysis was ten. These results show that the fraction of lysed erythrocytes was proportional to the tenth power of the amount of tolaasin, implying that tolaasin-induced hemolysis could be explained by the multi-hit model and it requires 10 hits of tolaasin.