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# Mechanism of $Zn^{2+}$ Inhibition on Tolaasin Channel Activity

Kwang-Hyun Cho*, Sook-Jin Kim, and Young-Kee Kim

Department of Agricultural Chemistry, Chungbuk National University

Tolaasin is a 1.9 kDa peptide produced by *Pseudomonas tolaasii* and causes a brown blotch disease on cultivated oyster mushrooms. These molecules form channels in the plasma membranes of various cells including red blood cells and destroy cellular structure, known as 'colloid osmotic lysis'. In order to understand the molecular mechanism of tolaasin-mediated channel formation, the effect of $Zn^{2+}$ was investigated on hemolysis and channel formation since $Zn^{2+}$ has been known to block the tolaasin activity. $Zn^{2+}$ inhibited the tolaasin-induced hemolysis in a dose-dependent manner and Ki value was 170 μM. The inhibitory effect of $Zn^{2+}$ was reversible since the inhibition was removed by the subsequent addition of EDTA. The elucidation of $Zn^{2+}$ effect will be very useful to understand the structure of tolaasin channel. The channel formation of tolaasin requires the multimerization of tolaasin, membrane insertion of multimerized molecules, and formation of pore. $Zn^{2+}$ probably inhibits the one of these three processes. When $Zn^{2+}$ was added after the channel formation of tolaasin in lipid bilayer, it was able to block the channel gating. In hemolysis experiments, tolaasin was added and incubated for 10 min, the time that starts to appear hemolysis. The addition of $Zn^{2+}$ after 10 min of incubation suppressed the initiation of hemolysis. Subsequent addition of EDTA initiated hemolysis. These two findings indicate that $Zn^{2+}$-induecd inhibition occurs after channel formation. In order to measure the pore size of tolaasin channel, the effect of various pore blocks was investigated. We found that the size of molecules higher than 2,000 Da blocks the hemolytic activity of tolaasin, representing that the diameter of pore is ~1 nm. Since the ionic diameter of hydrated $Zn^{2+}$ is much smaller than the size of pore, $Zn^{2+}$ probably blocked the channel by binding to the mouth of pore rather than by plugging up the tolaasin channel.