The Effects of Active Site Mutation in Anopheles gambiae Tranglutaminase 3(AgTG3)

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Wild-type (WT) AgTG3 produced by the male accessory glands (MAGs) of the malaria mosquito Anopheles gambiae catalyzes cross-linking of a substrate called Plugin. The resulting coagulated mass, the mating plug, is transferred to a female mosquito during mating. Formation of the mating plug via WT AgTG3 is necessary for efficient sperm storage by the female mosquito, which has a direct consequence on fertility. Inhibition of this seminal fluid transglutaminase could diminish its catalytic activity, thereby suppressing Plugin cross-linking. This study hypothesized that a mutation at Cys323 in WT AgTG3 would abolish cross-linking catalytic activity in the Plugin substrate. To test the research hypothesis, a C323A mutant AgTG3 protein was expressed, purified, and assayed for enzymatic activity using two orthogonal methods: cross-linking SDS-PAGE and plate-based fluorescence detection. Concurrently, WT AgTG3 as a control was expressed, purified, and assayed by the same methods. Assays yielded average fluorescence intensities of 17.5, 39.125, 17.375, 17.74, and 18.5, for Buffer-only, WT AgTG3 in calcium, WT in iodoacetamide (IA), the C323A mutant in calcium, and the C323A mutant in IA respectively. With a F-value of 318.56 and a p-value of <0.001, the results were significant enough to reject the null hypothesis. A Tukey Honestly Significant Difference post-hoc test showed that WT AgTG3 with Calcium was the only treatment significantly different from the other treatments and control. Additional biophysical experiments demonstrate the behavior of the C323A mutant protein resembles that of WT AgTG3 in solution, which suggests the mutation does not affect other properties of the enzyme in solution, thus supporting the hypothesis.