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"Stabilization of photoprotein aequorin through mutagenesis and Deep Eutectic Solvents"

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Abstract

Aequorin, a calcium-regulated photoprotein, has diverse applications in biosensing and imaging [1-2]. However, its stability limits its broader usage, particularly under harsh conditions [3]. This study investigates the impact of two types of deep eutectic solvents (DES), choline chloride-glycerol (ChCl-Gly) and choline chloride-urea (ChCl-Urea), on enhancing of structural and thermal stability of G14A mutant of aequorin. so far, there has been a steady increase in utilization of these solvents on protein stabilization [4]. The G14A mutation, because of local increase in the number of van der Waals interactions, is hypothesized to influence its stability and folding properties [5]. The G14A variant of aequorin was expressed in *E. coli* BL21(DE3) cells and purified using affinity chromatography. Structural analysis was performed using Far-UV circular dichroism (CD) spectroscopy, while intrinsic fluorescence measurements and thermal stability assays were employed to assess the protein's structural integrity and heat tolerance in the presence of DESs. The stability of G14A aequorin was evaluated by monitoring changes in secondary structure and fluorescence intensity after exposure to DES solutions at different time intervals (5, 10, 15, 30, and 60 minutes) at 70 °C. Far-UV CD spectra revealed that both ChCl-Gly and ChCl-Urea significantly increased the secondary structure content of G14A aequorin compared to the control. The intrinsic fluorescence intensity of this mutant in the presence of ChCl-Gly was significantly decreased compared to the control, while ChCl-Urea caused a minor increase in emission. Thermal stability assays showed that G14A in the presence of both ChCl-Gly and ChCl-Urea buffers exhibited improved stability over

time at 70 °C. Both DES buffers facilitated increased protein stability compared to the control, indicating a protective effect on the protein's conformation. The G14A mutation, in combination with DES buffers, enhances the stability of aequorin, making it more robust and applicable for a broader range of biotechnological and analytical applications.

Key words: Aequorin, deep eutectic solvents, choline chloride-glycerol, choline chloride-urea

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