

## Dual Role of Trigonelline in Targeting the Core Residues of HEWL: Inhibition of Fibrillation and Enhancement of Enzyme Activity

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### Abstract

Protein aggregation into amyloid fibrils is a pathological hallmark of numerous debilitating conditions, including Alzheimer's disease and Type II Diabetes. **Lysozyme**, a crucial defense enzyme is susceptible to fibrillation, often initiating at key hydrophobic core residues (e.g., Trp62, Trp63), which leads to severe functional loss. This study investigates the dual modulatory effect of **Trigonelline (TRG)**, a natural plant alkaloid, on **Hen egg white Lysozyme (HEWL)** fibrillation and function. We utilized a comprehensive approach combining biophysical (ThT fluorescence, Congo Red binding, Far-UV CD), microscopic (SEM), X-ray crystallography, and in silico docking, alongside a Lysozyme-specific functional assay. Our results confirm that TRG is a potent anti-fibrillative agent, dose dependently suppressing amyloid formation and successfully retaining the native alpha helical secondary structure. X-ray crystallography and fluorescence quenching revealed that TRG interacts directly with the core fibril-seeding site, specifically demonstrating a **sandwiched interaction between the Trp62 and Trp63 residues**. Crucially, TRG exhibited a significant dual role: it not only inhibited pathological aggregation but also **enhanced the native anti-microbial activity** of HEWL, successfully averting the functional impairment typically caused by fibrillation. These findings establish Trigonelline as a promising, naturally-sourced therapeutic lead, offering a unique dual mechanism for treating protein misfolding diseases by simultaneously suppressing aggregation and restoring/enhancing native protein function.

**Keywords:** *Trigonelline (TRG), Fibrillation, HEWL, Anti-fibrillation, Anti-microbial Activity, X-ray Crystallography*