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Insulin Concentration-driven Amyloid Aggregation

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Insulin, a peptide hormone essential for blood sugar regulation, forms insoluble amyloid fibrils under specific conditions, impacting industrial production and therapeutic use. This study examines insulin amyloid aggregation at varying concentrations (10 – 300 μM) at given experimental conditions (100 mM NaCl-HCl buffer, pH 1.6, 65 °C, and 700 rpm shaking) using a 96 half-volume well plate and ClarioStar plate reader. Aggregation was monitored with Thioflavin T fluorescence readings at 3- and 10-min intervals. Atomic force microscopy and circular dichroism assessed fibril morphology and secondary structure. Higher insulin concentrations (250–300 μM) accelerated amyloid formation by shortening the lag phase with a distinct growth phase mechanism. Fibrils exhibited similar β-sheet content across all concentrations but were longer and more numerous at higher concentrations. The 3-min interval of fluorescence detection provided more detailed kinetic data, improving sigmoidal growth curve analysis. Insulin concentrations above 50 μM consistently formed longer fibrils with high reproducibility, with 75 μM insulin at a 3-min shaking interval identified as the optimal condition for kinetic studies.

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