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Stabilization of Recombinant α-Amylase Using a Cellulose/Gold Hybrid Nanosupport

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Abstract

α-Amylase is one of the most widely used commercial enzymes across various industries. The demand for industrial enzymes is expected to continue growing due to the increasing global population, the depletion of natural resources, and the urgent need for environmentally sustainable alternatives in industrial processes. In this study, the gene encoding α-amylase from *Bacillus aquimaris* MKSC 6.2 (BaqA) was subcloned into the expression vector pET28a(+) and successfully expressed in *E. coli* BL21 (DE3). The synthesis of cellulose nanocrystals/gold nanoparticles (CNC/AuNPs) hybrid was accomplished using a hydrothermal treatment without toxic chemicals. Recombinant BaqA was covalently attached to a cysteine-modified nanosupport through a Schiff base reaction using glutaraldehyde linkages. The successful synthesis of the designed nanohybrid and the enzyme stabilization process were confirmed by FT-IR, DLS, intrinsic fluorescence, UV-Vis spectroscopy, FESEM, and EDX techniques. The enzyme in its free form exhibited maximum activity at pH 10 and a temperature of 70 °C. The optimal temperature for the immobilized enzyme increased to 80 °C, while the optimal pH remained unchanged. This catalytic platform significantly enhanced chemical and thermal stability, as well as enzyme stability under critical pH conditions. After a four-week storage period, the immobilized α-amylase retained 67.5% of its initial activity, whereas the free α-amylase only maintained 17% of its initial activity. The immobilized α-amylase demonstrated a catalytic efficiency of 0.488 mM^{-1} s^{-1}, which is more than double that of the free form, which had a catalytic efficiency of 0.254 mM^{-1} s^{-1}. Following eleven consecutive uses, the immobilized enzyme retained 75% of its initial activity. Based on the obtained results, the produced nanoenzyme could serve as a suitable candidate for industrial applications under harsh and critical conditions.

Key words: α-Amylase, Recombinant, Hybrid nanoparticles, Stability, Immobilization, Reusability