Title : Exploration of a Functional Sugar Synthase Activity Assay Method and Evaluation of Stabilizing Agents Effects on Protein Content and Enzyme Activity

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Major : Biochemistry and Biochemical Innovation

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Enzyme stabilization is crucial for preserving the native three-dimensional structure of proteins and ensuring their optimal catalytic performance. Because enzymes are highly sensitive to environmental factors such as temperature, pH, and various chemical agents, appropriate stabilization strategies are required to prevent conformational changes, structural degradation, and loss of enzymatic activity. Stabilizing additives, particularly polyols and sugars, are widely employed to enhance enzyme stability. Among these, mannitol, a highly polar polyol compound, has gained considerable attention as an effective protein stabilizer. Owing to its strong hydrophilicity and capacity to form extensive hydrogen bonds with surrounding water molecules, mannitol promotes preferential hydration at the protein surface. This effect reduces protein unfolding and aggregation, thereby preserving the native conformation of enzymes and maintaining their catalytic activity during storage and under stress conditions. This study aimed to explore the optimal condition to reveal the enzyme activity for functional sugar production and to investigate stabilizing agents capable of preserving protein content and enzymatic activity in crude enzyme. In this experiment use the functional sugar synthesizing enzyme. Initial substrate concentrations of 0.2%, 5%, and 10% and Incubation times of 30, 40, 50, and 60 minutes were evaluated by DNS method. The results showed that a substrate concentration of 10% with a incubation time of 30 minutes yielded the highest enzymatic activity. Therefore, these conditions were selected for subsequent enzyme activity analysis. The investigation of stabilization began with, supplementing with five stabilizing agents including glycerol, ascorbic acid, glutamic acid, mannitol, and glutathione at various concentrations. The crude enzyme solution were stored at 4 °C for 20 days and the protein concentration and enzyme activity were

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detected by Bradford assay and DNS method, respectively. The results demonstrated that the remaining 78% of protein content in crude enzyme occurred when supplemented by mannitol as 0.5 M of final concentration. Furthermore, showing the the enzymatic activity was the highest as 185% and the high conversion yield as 127% was displayed using 30% (w/w) sugar as initial substrate for 24 hours of incubation time.

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Enzyme stabilization is a crucial process for maintaining the three-dimensional structure of proteins in a functional state. Since enzymes are highly sensitive to environmental factors such as temperature, pH, and various chemical substances, appropriate control conditions and stabilizing agents are required to prevent structural degradation and loss of enzymatic activity. This study aimed to determine the optimal conditions for measuring the activity of a functional sugar synthesizing enzyme and to investigate and compare stabilizing agents capable of preserving protein integrity and enzymatic activity over an extended period. The optimal conditions for enzyme activity measurement were evaluated by varying substrate concentrations (0.2%, 5%, and 10%) and reaction times (30, 40, 50, and 60 minutes). The results indicated that a substrate concentration of 10% with a reaction time of 30 minutes yielded the highest enzyme activity and was therefore selected for subsequent experiments. In the stabilization study, the enzyme was suspended in phosphate buffer and supplemented with different stabilizing agents, including glycerol, ascorbic acid, glutamic acid, mannitol, and glutathione at various concentrations, compared with a control condition. The findings demonstrated that the enzyme suspended in buffer containing 0.5 M mannitol exhibited the highest enzymatic activity and showed the greatest stability, suggesting its suitability for potential industrial applications.

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