A Novel Intein-Based System for Simultaneous Production and Purification of Insulin Chains

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With 400 million diabetic patients in the world, diabetes is a global epidemic that can lower life expectancy by a decade, quadruple the risk of heart disease, and cause kidney failure or adult-onset blindness. The number of diabetic patients is projected to grow to 600 million in the next twenty years, leading to a significant increase in the demand for insulin and its analogs. The current insulin production method in E. coli is complex and involves separate steps of proinsulin synthesis and isolation, cleavage by protease digestion, and purification by chromatography. In this project, I designed a novel intein-based system that can simultaneously produce and purify both A and B chains of insulin. Inteins are protein motifs that are able to excise themselves from a protein. Many natural inteins are relatively large and not optimal for recombinant protein production. By aligning multiple intein sequences, I have designed a small intein of 135 amino acids. The A and B chains were linked to the ends of the intein. A chitin binding domain was also inserted into the intein to facilitate insulin chain purification. The DNA sequence for the whole protein was inserted into a plasmid and transfected into E. coli. My data indicate 1) the expressed whole protein can be captured by a chitin affinity column, 2) treatment with a thiol agent results in simultaneous cleavage and purification of the insulin A and B chains from the whole protein, while the intein and chitin binding domain remain with the column.

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