

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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