Application of Supercharged Protein to Allow Secretion-Based Production of a Broad Range of Recombinant Proteins through the ABC-Transporter System

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Recent research and industrial trends indicate the importance of economical and efficient methods for mass production of recombinant proteins. The Pseudomonas fluorescens expression host system is a platform with an ABC transporter, which enables effective secretion of proteins for continuous recombinant protein production in a gram-negative host. We utilized supercharging methods to modify the amino acid sequence that result in a significant change in overall protein charge. We used two methods to modify the charge of the proteins; the AvNAPSA method, a customized version of an algorithm created to prevent protein aggregation and the Activity Focused Supercharge method to preserve the activity of the secreted protein. We have found that negative supercharging allowed the secretion of a number of proteins with high isoelectric points. While a prior publication reported that the isoelectric threshold for protein secretion is 5.5, we have found that protein secretion is not likely to be dependent on its overall isoelectric point but rather on the charge of shorter regions within the protein that actually interact with the transporter. Our results can be used to improve the efficiency of protein secretion in various cell-based protein production systems with potential industrial applications.