**Production of angiotensin I-converting enzyme (ACE) with reduced glycoform heterogeneity in insect cells**

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The renin-angiotensin aldosterone system (RAAS) plays an important role in the control of blood pressure and electrolyte balance. Angiotensin I-converting enzyme (ACE) is a critical component of the RAAS with current ACE inhibitors having a broad range of applications in the treatment of cardiovascular disease. Somatic ACE (sACE) consists of two homologous domains, the N and C domains. The germinal isoform (tACE) corresponds to the C domain. ACE is heavily glycosylated and both tACE and sACE have N- and O-linked oligosaccharides. The N-linked glycosylation sites are located on the surface of the molecule, causing potential interference during protein crystallization. Current expression-studies in our laboratory utilize CHO cells and the glucosidase inhibitor N-butyldeoxynojirimycin (NB-DNJ) for the expression of recombinant ACE, making the elucidation of tACE three-dimensional structure possible (1). Disadvantages associated with this approach include incomplete inhibition of a1,2-glucosidase I and lower protein yields (up to 50% less protein). These together with the high costs involved for NB-DNJ, emphasize the need for alternative ways of generating a minimally glycosylated form of recombinant ACE. Recombinant protein production in insect cells results in mannose-rich sugars, compared to the complex sugars produced by mammalian cells. To investigate the effect of recombinant produced insect-cell ACE on protein crystallization, both tACE and N-domain cDNA were cloned into pFastBac to facilitate expression in Sf9 and Sf21 cells via the Bac-to-Bac Baculovirus expression system. Results demonstrating the successful expression of tACE and N domain in insect cells will be presented.

1. Natesh, R.; Schwager, S.L.U.; Sturrock, E.D. & Acharya, K.R. (2003) Nature 421, 551-554.