Poster Presentation

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Expression and purification of human Afamin for structure/function analysis

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Protein glycosylation plays an important role in protein stability, folding, and secretion but presents a major challenge for protein crystallization. Crystallization of highly glycosylated proteins is often difficult because of conformational and chemical inhomogeneity of the glycan decorations. Therefore, it is almost always necessary to modify the material to obtain a conformational homogenous protein that can crystallize. (1) We have explored several avenues in the case of the human 87 kDa glycoprotein afamin. Afamin (AFM), a member of the albumin gene family, is mainly expressed in the liver and secreted into the bloodstream. (2) Elevated afamin plasma concentrations are associated with major diseases such as metabolic syndrome and cancer;(3) however, pathophysiological functions are largely unknown. Therefore, we are pursuing the crystal structure of various forms of recombinantly expressed human afamin (rhAFM) for structure guided exploration and analysis of its function. Multiple variants of rhAFM are pursued: 1) fully glycosylated wild-type rhAFM (expressed in CHO cell lines); 2) rhAFM complexed with Fab fragments of two anti-AFM mAbs, 3) enzymatic deglycosylation of rhAFM with PGNaseF, 4) partially glycosylated rhAFM (expressed in glycosylation-deficient Lec1-CHO cells); and 5) glycosylation-free rhAFM, obtained from HEK293 cells virally transfected with a cDNA mutant lacking all 5 glycosylation sites by replacement of ASN with ASP. Yields of up to 2 mg/mL glycosilation free mutant rhAFM in HEK273 cells have been comparable to native rhAFM. C-terminal His6-tagged rhAFM was captured by Ni-IMAC from serum-free cell culture supernatants. All variants and Fab-AFM complexes were polished via SEC yielding pure products per SDS-PAGE and immunoblot analysis. Crystals have been obtained from Fab fragments and crystalline spherulites which are useful for microseeding from Fab-AFM complexes.

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