Expression, Purification and Crystallization of Human Thrombomodulin Domains

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Thrombomodulin (TM) is a multifunction glycoprotein expressed on the epithelial cell surface. This glycoprotein is structurally organized into 5 distinct domains. From the N-terminus to C-terminus, TM has an N-terminus C-type lectin domain, an EGF-like repeats, and a serine/threonine-rich region which were on the extra-membrane, a transmembrane domain and a short cytoplasmic tail on the intra-membrane. Each of the distinct domains has different biological functions that impact on coagulation, fibrinolysis, inflammation, cell Adhesion, and cell proliferation. To elucidate how the single molecule can play different import role by each distinct domain, we expect to obtain the crystal structure of each TM domains. Here, we use the *Pichia Pastoris* expression system to express different domains of TM: the TMD-1 construction contains the C-type lectin domain, the TMD-23 construction contains the EGF-like repeats and the serine/threonine-rich region. Both *Pichia Pastoris* expressed TMD-1 and TMD-23 were glycosylated proteins. The unequal glycosylation of TMD-1 may influence protein crystallization, therefore we use *E.coli* to express TMD-1. The *Pichia Pastoris* expressed TMD-23 was purified through Nickel affinity column. By using vapor diffusion method, we test some crystallization conditions of TMD-23 (12 mg/mL，25 mg/mL，36 mg/mL). Unfortunately, there is very low resolution protein crystal (20-40Å). The expression of TMD-1 in *E. coli* results in the formation of inclusion bodies. We use the on-column refolding to obtain the soluble form of TMD-1 and test some crystallization conditions of soluble TMD-1 (5 mg/mL), but the TMD-1 is instable, it often aggregated itself.

By using the MALDI-TOF and Gel filtration, we expect the Mr of TMD-23 is 41 kDa, and TMD-23 may become a dimmer.

Figure 1. The Functions of the Thrombomodulin Domains

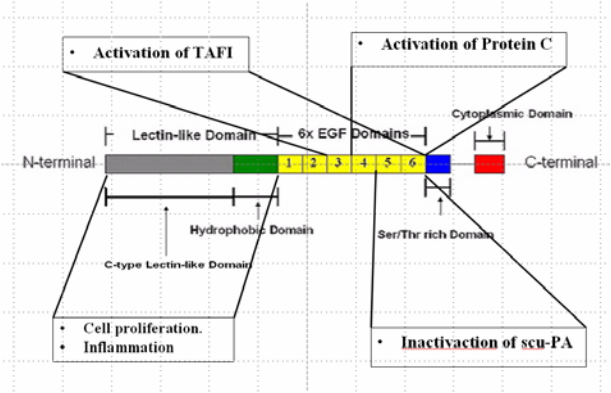

Figure 2. Crystallization Results

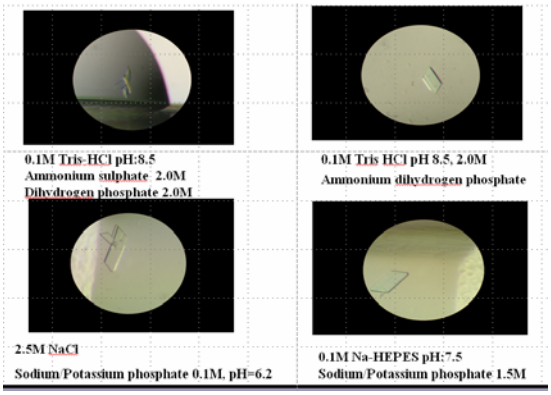

Figure 3. X-ray Diffraction Test