Design of Collagen-Like Proteins to Study Structure-Function Relationships in Vascular Ehlers-Danlos Syndrome

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Statement of Purpose

Vascular Ehlers-Danlos Syndrome (vEDS) is a rare, genetic connective tissue disorder that can result in fatal arterial dissections and organ rupture. vEDS is primarily caused by single Gly substitutions (G→X) in collagen, a primary structural component of the extracellular matrix in blood vessel walls. Within the media layer of a blood vessel, smooth muscle cells (SMCs) interact with collagen-III via integrin-binding sites (IBS) to maintain blood vessel strength and structure and regulate cell function. In particular, the collagen-III sequence GROGER is a high-affinity IBS and is in a significantly overrepresented region in reported vEDS cases. G→X mutations within this IBS may impair the critical cellular functions of SMCs that bind to the sequence in addition to the structure and strength of collagen fibers.

The research aims to utilize bacterially-derived collagen-like proteins (CLPs) to examine the effect of vEDS-specific mutations on collagen-integrin interactions. Bacterial CLPs form a stable triple helix, similar to mammalian collagen. Moreover, they provide a biological "blank-slate" to insert specific human collagen ligand binding sites, allowing us to characterize the effects of specific Gly mutations at any location while controlling the surrounding sequence. This abstract presents our approach for the design, expression, and purification of CLPs harboring single G→X mutations.

Methods

Different CLP sequences with Gly substitutions within and outside the IBS have been designed using Benchling [Biology Software] (San Francisco, California). The designed CLP sequences were cloned into a pCold-I plasmid, expressed with a His-tag using *E.coli* BL21-DE3, and purified using a Ni-NTA purification system. The protein purity was verified using SDS-PAGE, and circular dichroism (CD) spectroscopy was used to investigate the triple helix structure and stability. Cell adhesion assays were performed to confirm cell binding function of the designed CLPs. Human aortic SMCs were cultured in complete DMEM media. A 96-well plate was coated with multiple concentrations of CLPs overnight at 4°C, followed by BSA blocking for 2 hours at room temperature. Collagen I and bovine serum albumin (BSA) were used as positive and negative controls, respectively. Cells (10,000 per well) were seeded onto the coated plates and incubated at 37°C with 5% CO_2. After 24 hours, the unbound cells were removed by washing with PBS. The bound cells were fixed with 4% paraformaldehyde and the F-actin cytoskeleton stained with TRITC-Phalloidin.

Results and Discussion

In the present study, recombinant CLP containing the wild-type (WT) IBS, i.e., GROGER sequence, was expressed using the pCold vector system, which allowed for selective induction of the target protein at low temperatures. Protein expression was compared in different *E.coli* hosts, multiple culture media compositions, different induction temperatures, and post-induction incubation time. Conditions that produced optimal results included using *E.coli* BL21 DE3 bacterial strain, terrific broth (TB) media, and a combination of 25°C and 15°C temperature for protein induction and expression.

The purified CLP, confirmed by a single band with SDS-PAGE (Fig 1A), self-assembled into a triple helical structure with a characteristic CD spectrum, i.e., a positive peak at 220 nm and a negative peak at 198 nm (Fig 1B). Additionally, cell-adhesion assays (Fig 2) demonstrate the cell-specific adhesion of CLPs attributed to interactions with the inserted GFPGER IBS.

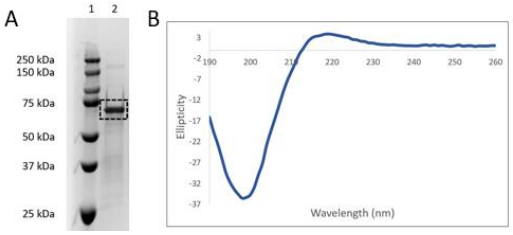

Figure 1: A, SDS-PAGE of the purified samples. B, CD Spectra of the purified CLPs shows a characteristic triple helical spectrum.

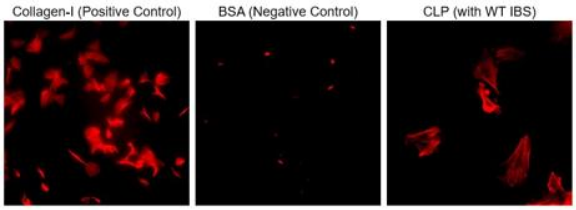

Figure 2: Adhesion of SMCs after 24 hours on TC plates coated with Collagen-I (0.5 mg/mL), BSA (4% w/v), and CLPs (1 mg/mL).

Conclusion

This work successfully demonstrates the expression and purification of bacterial CLPs that self-assemble into a triple helix. We are currently optimizing the protein expression of mutant CLPs that contains different G→X mutations. In the future, we will utilize the purified CLPs to develop CLP-PEGDA hydrogels for 3D cell culture to investigate the effect of Gly mutations on tissue structure and function.

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