Production of Recombinant Bacterial Collagens for Use as New Materials for Biomedical Applications

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Introduction:

Collagen has proven safe and effective in numerous medical products, and is used in tissue engineering. In mammals, there are some 28 different collagen types, all of which include a characteristic triple-helical structure with a $(Gly\text{-}X\text{-}Y)_n$ repeating sequence (Ricard-Blum S. Cold Spring Harb Perspect Biol 2011, 3:a004978.).

Collagen is usually extracted from animals, but typically only the most abundant collagen, type I collagen, is available in sufficient yield for biomedical applications. However, as with all clinical products which include animal derived material, there is a concern of transmissible diseases. This introduces the potential use of recombinant systems to produce collagens in a disease-free and reproducible format. However, recombinant mammalian collagens require the functional co-expression of prolyl 4-hydroxylase to achieve collagen stability.

Recently the presence of $(Gly\text{-}X\text{-}Y)_n$ repeating collagen-like sequences that form stable triple-helical structures has been characterised for several bacteria (Xu C. et al. Biomacromol 2010, 11:348-356.). These collagens lack hydroxyproline, yet form stable triple-helices at 35-38 °C. This stability without the need for hydroxyproline means that they can be readily produced in microbial systems, including in *E. coli*.

In the present study we have examined constructs based on the ~237 amino acid, collagen-like protein Scl2 from *S. pyogenes.* The production yields and properties of the Scl2 protein have been compared with constructs containing inserts of specific functional domains from animal collagens, and with constructs containing multiple repeats (up to 4) of the Scl2 domain.

Methods:

Constructs: Constructs were made using Scl2 that included the registration domain (V), the collagen domain (CL), but without the C-terminal non-collagen tail.

Site directed mutagenesis was used to introduce heparin (H) and integrin (I) binding sequences. Constructs containing multiple CL domains, $V\text{-}(CL)_{1\text{-}4}$ were made using standard protocols. Selected DNA clones were subcloned into *E. coli* expression system pColdIII and transformed into *E. coli* host BL21 for expression.

Fermentation: Recombinant bacterial collagens were produced in 2 L stirred tank bioreactors connected to a Biostat B (Sartorius Stedim Germany) control system. A defined media was used with glucose as the carbon source. Various cell densities and activation temperatures were used. Samples were analysed by SDS-PAGE.

Results:

The base VCL construct and those containing H, I and HI inserted domains were readily expressed in *E. coli*, and prepared as pure proteins (Fig. 1). The heparin binding function was shown by binding of FITC-heparin, while the integrin binding function was shown through binding of murine L929 cells.

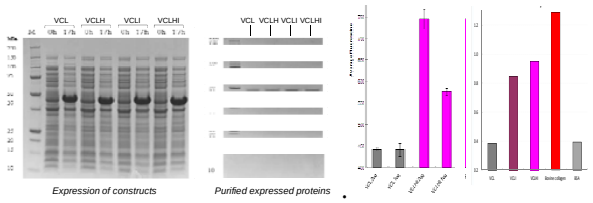

Figure 1: Production, purification and assay of functional inserts

The VCL and VCLH constructs were examined for larger scale production, as this would be key for biomedical applications. Comparisons were made between low and high cell density production. This showed that good yields, >19 g/L could be obtained from high cell density production using defined media (Table 1).

Construct	Production	Final OD	Cell paste (g/L)	Product (g/L)
VCLH	25 °C for 10 h	106	108	8.2
VCL	25 °C for 10 h	88	113	9.5
VCL	15 °C for 24 h	65	112	10.0
VCL	25 °C for 10 h & 15 °C for 14 h	96	n.d.	19.3
VCL	25 °C for 10 h & 15 °C for 14 h	105	137	13.0
VCL	25 °C for 10 h & 15 °C for 14 h	144	148	19.0

Table 1: *Fermentation conditions and protein yields for VCL & VCLH, using fed batch defined medium with pre induction at 37 °C for 24h.*

Using the preferred production protocol that gave the best yields, the production of constructs of increasing length, $V\text{-}(CL)_{1\text{-}4}$, was examined. These data showed that as the size of the construct increased, the cell production was reduced and this in turn also lead to decreasing product yields (Fig. 2). The products were, however, all stable and could be readily resolved by SDS-PAGE (Fig. 2).

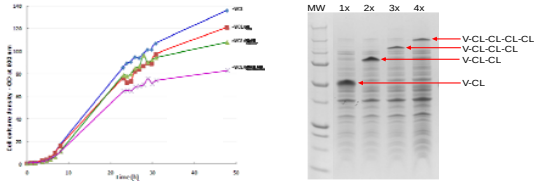

Figure 2: Production and preparation for multiple CL domains

Conclusions:

The CL sequence, previously shown to be non-immunogenic and non-cytotoxic, shows limited biological interactions. We have successfully introduced functional, animal derived sequences into the CL domain and have also prepared constructs containing multiple CL domains. The constructs can be produced in 2L fermentation using fed-batch defined medium, giving sufficient yields, of up to >19 g/L, for use in biomedical products.