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Review

Developments in purification methods for obtaining and evaluation of collagen derived endogenous angioinhibitors

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ABSTRACT

Collagen constitutes one of the vital components of the basement membrane scaffolds. Non-collagenous domains (NC1) derived from collagens exhibit potent anti-angiogenic properties, thus attaining significance in regulation of angiogenesis promoted diseases. Individual NC1 domains essential for anti-angiogenic evaluations are generally obtained through purification of individual non-collagenous domains, which have undergone steady developments for enhancing the yields, purpose of biological evaluations and solubility based on the nature of different NC1 domains. This review focuses on the method developments in obtaining biologically active NC1 domains and for specific evaluations in different scenarios.

Contents

Introduction

Purification of individual proteins of interest to homogeneity for functional and structural characterization forms the basis for development of protein purification methodologies. Purification of individual proteins is affected by different factors such as occurrence of protein of interest as individual moiety or subunit, quantity of proteins present in biological systems and physico-chemical properties of the proteins including primary sequence, isoelectric values (pI),[1] solubility, size or relative molecular mass (Mr), charge and hydrophobicity. Each of these factors influence the functions

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[1] *Abbreviations used:* pI, isoelectric values; Mr, relative molecular mass; ECM, extracellular matrix; Gly-X-Y, glycine-proline-hydroxyproline; NC1, non-collagenous; EHS, Engelbreth-Holm-Swarn; GBM, glomerular basement membrane; GA, goodpasture antigen; HUVECs, human umbilical vein endothelial cells; DTT, dithiothreitol.

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of the target purified protein and are considered critical during optimization of purification methods, which are developed based on the factors effecting purification. Finally, the efficacy of purification methodologies applied for obtaining proteins of interest are indicated through the quantity and also the quality of purified proteins; evaluated through functional, biophysical and biochemical characterizations. Thus, protein purification methods are optimized for enhancement of quality and quantities of purified proteins of interest, which can be exemplified by considering the developments in the purification strategies applied for therapeutically important proteins such as anti-angiogenic non-collagenous domains obtained from collagens.

Purification methods and significance of NC1 domains

Collagens constitute vital components of the extracellular matrix (ECM) that forms the scaffold for tissue organization, along with other ECM components. Different collagen types (29 types) differing primarily in their compositions have been identified to date [1]. A common feature of collagens includes the typical triple helical conformation, arising from the glycine-proline-hydroxyproline (Gly-X-Y) repeats occurring in the characteristic filamentous domains of collagens [2]. Based on the type, collagens also contain non-collagenous (NC1) sequences or domains with sequences differing from repeated Gly-X-Y, that generally occur at the amino and carboxy-termini of the monomeric collagen subunits [3]. The common triple helical filamentous supra-molecular structure was considered to be the major functional moiety of collagens, until novel functional roles of NC1 domains were deciphered [4,5]. Non-collagenous domains of collagens were identified to possess structural, pathological and therapeutic properties; which were characterized through the isolation and purification of individual NC1 domains. Both the collagenous and non-collagenous sequences constitute different domains of the same monomeric chains of collagens (Fig. 1); warranting the need for isolation and purification of the individual non-collagenous domains for respective evaluations. Purification of individual non-collagenous (NC1) domains of collagens thus gained significance for evaluation of their structural, pathological and anti-angiogenic activities, which was achieved through the gradual developments in purification methodologies applied for enhancing the quality and quantity of these domains, which are highlighted in this review.

Purification of non-collagenous domains for biophysical and biochemical evaluations

Purification methods for isolation of non-collagenous (NC1) domains were initially applied for obtaining these domains to decipher their composition and structural significance in collagens. The methodologies used for such identification and characterization of non-collagenous (NC1) domains of collagen and for identification of role of NC1 domains in collagen structural assembly were based on the digestion of collagen obtained from tissues and subjecting them to either size exclusion or ion exchange chromatography. These methods enabled the identification and purification of NC domains for biochemical and biophysical characterizations. NC1 domains of type IV collagen were initially identified from the collagenase and protease resistant fragments of type IV collagen [6,7]. NC1 domains were prepared from the collagenase and peptidase treated samples of lens tissues and Engelbreth-Holm-Swarn (EHS) tumors for the identification and elucidation of role of NC1 domains in self-assembly of type IV collagens, respectively. The purification steps included size exclusion and ion exchange chromatographies for separation of NC1 domains of type IV collagen from collagenase digested samples [6–8]. Dimers of NC1 domains were obtained through the digestion of tumor derived type IV collagen with bacterial collagenases, followed by dialysis and Sephacryl-S-300 column chromatography. These isolated dimers of NC1 domains were shown to play a role in lateral assembly of type IV collagen, through turbidometric and rotary shadow analyses [8].

The NC1 domain's structural assembly into hexamers from dimeric and monomeric forms were deciphered by purification of globular NC1 domains from placental, ligament and tumoral tissues by collagenase digestion, followed by chromatography on HPLC [9]. Hexameric, dimeric and monomeric forms of the NC1 domains were also subsequently isolated from human placenta by collagenase and size exclusion methods, which lead to characterization of associations arising between NC1 domains [10]. Thus, purification of NC1 domains to their homogeneity was achieved through the protease and size exclusion or ion exchange chromatography methodologies, for enumerating the role of NC1 domains in collagen structural maintenance. However, novel functional roles of NC1 domains other than the role played by these domains in maintaining of collagen structure were also deciphered, which

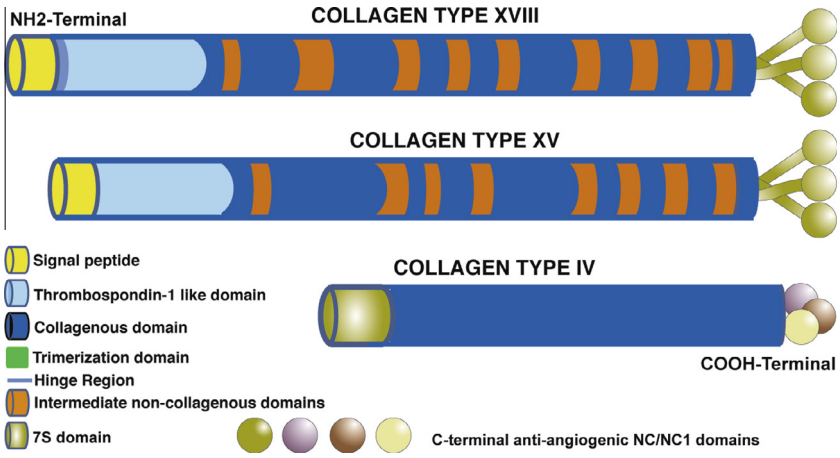

Fig. 1. Schematic illustration showing structures of type XVIII, XV and IV collagens. Structure of three collagens is presented with focus on the domains (highlighted in different colors for distinction). NC domains of three collagens play role in structural stability of collagens. Type IV collagen NC1 domains possess antigenic properties. Type XVIII and XV collagen contain homologous carboxy-terminal NC1 domains termed as endostatin and restin (endostatin-like domain), respectively. Six isoforms of type IV collagen NC1 domains are identified. Carboxy-terminal NC domains of three collagens exhibit anti-angiogenic and anti-tumor properties. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)