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Review

Tropoelastin: A versatile, bioactive assembly module ☆

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ABSTRACT

Elastin provides structural integrity, biological cues and persistent elasticity to a range of important tissues, including the vasculature and lungs. Its critical importance to normal physiology makes it a desirable component of biomaterials that seek to repair or replace these tissues. The recent availability of large quantities of the highly purified elastin monomer, tropoelastin, has allowed for a thorough characterization of the mechanical and biological mechanisms underpinning the benefits of mature elastin. While tropoelastin is a flexible molecule, a combination of optical and structural analyses has defined key regions of the molecule that directly contribute to the elastomeric properties and control the cell interactions of the protein. Insights into the structure and behavior of tropoelastin have translated into increasingly sophisticated elastin-like biomaterials, evolving from classically manufactured hydrogels and fibers to new forms, stabilized in the absence of incorporated cross-linkers. Tropoelastin is also compatible with synthetic and natural co-polymers, expanding the applications of its potential use beyond traditional elastin-rich tissues and facilitating finer control of biomaterial properties and the design of next-generation tailored bioactive materials.

1. Introduction

Elastin is the dominant component of mature elastic fibers, which provide strength and persistent elasticity to a range of important tissues. Its structural and cell signaling roles have classically been recognized within large blood vessels, lungs and the skin [1], making elastin mimicry sought after for biomaterials applications and tissue repair. Elastin-derived materials have shown great promise, but continue to evolve as more is understood about the drivers of elasticity, details of structural features, the elastin assembly process and the capacity for hybrid material formation. These insights, in combination with integrated design approaches [2], promise the ability to tailor constructs, carefully matching both the physical and biological requirements of each elastin-rich tissue to be replaced.

The soluble monomer of elastin is tropoelastin (TE), highly expressed during development, but in adults only in response to injury. The elastin gene encodes a ~72 kDa polypeptide and, depending on alternative splicing and protein maturation, yields a mature protein of at least 60 kDa. A dominant feature of the tropoelastin amino acid sequence is its well-characterized alternating hydrophobic and hydrophilic domain structure. Hydrophobic regions of tropoelastin feature non-polar amino acids such as glycine, valine, proline and leucine [3], often arranged in repetitive motifs, the most common being the repeating sequence VGVAPG [4]. Overall, hydrophobic residues account for 82% of the primary sequence [5]. The hydrophilic domains are rich in lysine and alanine, which participate in cross-linking.

Large quantities of highly purified tropoelastin have only become available relatively recently. Isolation of the monomer directly from in vivo sources is difficult and inefficient, due to the speed at which extracellular, expressed tropoelastin is cross-linked and incorporated into the growing elastic fiber [6]. Recombinant expression in an *Escherichia coli* bacterial system [7] and subsequent optimization of a synthetic elastin gene [8] have allowed for the production of a recombinant tropoelastin that is

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identical to the naturally secreted human form, displaying assembly and coacervation properties expected for native elastin [9].

Access to tropoelastin has facilitated rapid progress in understanding its structure, cell signaling and assembly properties. Techniques previously incompatible with mature insoluble elastin have now been employed to probe the monomer and better understand its relationship to elastin and the elastic fiber. As more is learned, there is a growing appreciation of the relationship between the shape, flexibility and cell interactive regions of the monomer, how these translate in properties of elastin and how these can be manipulated for the production of biomaterials. While this review will focus on TE-based materials, better knowledge of the TE monomer has also enhanced the utility of recombinant elastin-like protein polymers for tissue engineering applications, as recently described [10].

2. Fundamental understanding of tropoelastin

2.1. Structural features

Because pure, full-length tropoelastin has only recently become available, the earliest structural insights were from elastin derivatives. Infrared absorption and circular dichroism (CD) studies on α-elastin, a heterogeneous product derived from acid hydrolysis of elastin [11], indicated that solubilized elastin has a predominantly disordered structure. Nuclear magnetic resonance studies reveal that the majority of backbone carbonyl carbon atoms in elastin-based polypeptides are highly mobile [12]. Sequence-based structure prediction algorithms likewise estimated ~75% disorder in the structure of the tropoelastin monomer [13]. Subsequent CD and Raman spectroscopy studies carried out directly on recombinant tropoelastin and elastin-like peptides also confirm a large proportion of highly disordered regions, which are attributed to the hydrophobic domains [13,14].

The abundance of disordered structures within tropoelastin, defined as a preferential adoption of transient local structural motifs over stably bonded secondary structures, is manifested in the intrinsic flexibility of the molecule [15]. Elastin-based polypeptides alternate between different conformations in equilibrium, giving rise to a conformational ensemble that includes β-turns, β-strands and polyproline II (PPII) structures [16–19]. PPII structures are defined by an absence of intramolecular and intermolecular hydrogen bonds and can therefore interconvert between conformations [17].

In a hydrated environment, tropoelastin presents a highly flexible form [13]. The structural dynamics of the elastin backbone is mainly achieved by the proportion of rigid proline and flexible glycine residues [20]. Steric constraints imposed by proline and the entropic disadvantage of glycine confinement determine the possible backbone conformations [15,21]. The placement of recurring proline and glycine residues promotes the formation of labile β-type turns, which contribute significantly to flexibility within the protein [22]. Accordingly, skewing the ratio of proline to glycine in elastic-like polypeptides away from the natural sequence promotes the formation of β-structure-rich amyloid-like fibrils, which show a greater degree of conformational restriction than the native elastin structure [21].

In addition to PPII and other labile structures, the hydrophobic repeat units in tropoelastin also form more compact motifs such as short β-sheets or β-turns [14,23–25]. In contrast, the hydrophilic domains are associated with a low content of α-helices in the free protein [25–28]. The presence of independent clusters of structure in tropoelastin is consistent with the gradual unfolding transition observed upon urea treatment [13].

Obtaining a definitive high-resolution tertiary structure of tropoelastin has been hindered by its inability to form resolvable crystals. In addition, the insoluble nature of polymerized elastin precludes the use of classical spectroscopic techniques [29]. Nevertheless, the binding of hydrophobic probes to tropoelastin and the ability of hydrophobic sequences to be cleaved in protease mapping experiments indicate the presence of surface-exposed hydrophobic regions, suggesting that tropoelastin hydrophobic domains are not restricted to the core, as is expected for folded globular proteins [13,30]. Furthermore, a portion of hydrophobic regions remain partially accessible in cross-linked elastin [31].

Small-angle X-ray and neutron scattering analyses of recombinant human tropoelastin in solution revealed an extended asymmetrical shape with a long narrow rod from one terminus that branches into a larger region at the other terminus [32]. By comparing the solution structures of full-length tropoelastin and mutant isoforms truncated at various lengths from the N-terminus, specific domains were assigned to distinct spatial locations within the model. An elongated coil region encompasses the N-terminus to domain 18, and leads downstream to a spur containing domains 20–24 that corresponds to a predicted hinge region [33,34]. The model shows a protrusion around domains 25–26 that is assigned to a bridge region, which connects to an open foot region at the C-terminus (Fig. 1A).

The uniqueness of the tropoelastin structure directly accounts for the elastomeric properties of the protein [29,35,36]. The flexibility of the polypeptide chains contributes to the high entropy of the relaxed state. When elastin is stretched in an aqueous environment, the hydrophobic sequences, most likely those in the coil region [32], become more exposed to the solvent and constrain the local order of water molecules [14]. This decrease in entropy acts as a driving force for spontaneous elastic recoil back to the native disordered state [37–39].

2.1.1. The N-terminal region

The solution structure of human tropoelastin comprises a distinct narrow (2–3 nm), elongated segment that persists for ~11 nm before branching into the bridge and foot regions [32]. Structural comparison of the full-length species with constructs truncated at various distances from the N-terminus allowed the assignment of this linear section to the tropoelastin N-terminal region encompassing domains 2–18. The spring-like coil region is proposed to account for tropoelastin elasticity, which is characterized by the ability of the single molecule to reversibly extend to eight times its resting length [32]. The N-terminal region displays the same structural motif organization as in the full-length molecule, comprising alternating hydrophobic and hydrophilic domains [40]. Mechanistically, the elasticity of tropoelastin molecules arises from the substantial entropy associated with the flexibility of hydrophobic domains in the relaxed state [38,39]. Extending the monomer increases order by restricting the range of accessible conformations [41]. Furthermore, the increased solvent exposure

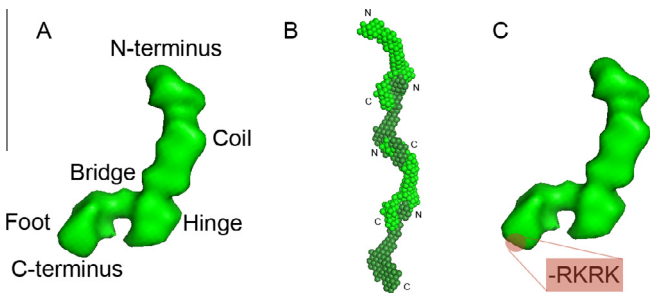

Fig. 1. (A) Structural features of the tropoelastin monomer; (B) cell interactive C-terminal sequence – RKRK; and; (C) assembly model showing the N- to C- terminal interaction. Adapted from Ref. [32].