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Review

Resilin: Protein-based elastomeric biomaterials ☆

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ABSTRACT

Resilin is an elastomeric protein found in insect cuticles and is remarkable for its high strain, low stiffness, and high resilience. Since the first resilin sequence was identified in *Drosophilia melanogaster* (fruit fly), researchers have utilized molecular cloning techniques to construct resilin-based proteins for a number of different applications. In addition to exhibiting the superior mechanical properties of resilin, resilin-based proteins are autofluorescent, display self-assembly properties, and undergo phase transitions in response to temperature. These properties have potential application in designing biosensors or environmentally responsive materials for use in tissue engineering or drug delivery. Furthermore, the capability of resilin-based biomaterials has been expanded by designing proteins that include both resilin-based sequences and bioactive domains such as cell-adhesion or matrix metalloproteinase sequences. These new materials maintain the superior mechanical and physical properties of resilin and also have the added benefit of controlling cell response. Because the mechanical and biological properties can be tuned through protein engineering, a wide range of properties can be achieved for tissue engineering applications including muscles, vocal folds, cardiovascular tissues, and cartilage.

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1. Natural resilin

The remarkable properties of resilin protein were first described by Weis-Fogh in the early 1960s [1–3]. Resilin, which is found in insect cuticle, is insoluble and heat stable [2]. Resilin forms a cross-linked network of protein chains, and the resulting material has rubber-like properties [1–3]. As such, Weis-Fogh found that natural resilin from dragonfly tendon has an elastic modulus of 600–700 kPa and can be stretched to three times its original length before breaking [2]. Furthermore, resilin is highly resilient and immediately recovers its original shape even after being stretched for weeks. Since the initial description of resilin derived from locusts and dragonflies, resilin has been found in a wide variety of insects including cicadas and cockroaches (further information available in Refs. [4,5]). Most recently, resilin has been identified in the clamp sclerites of monogenean fish parasites [6] and the opal teeth of copepod crustaceans [7].

The high resilience of resilin contributes to the mechanical properties and long lifetime of the structures in which they are found. For example, muscle alone cannot explain the jumping ability of fleas since it cannot generate the necessary force in the short duration of a jump [8]. Bennet-Clark and Lucey proposed that energy generated by muscle is stored in resilin, which serves as an efficient energy storage device to power jumping in a short time. Resilin is also found in the tymbal structures of cicadas, which generate sound, and acts to efficiently store and rapidly release energy over many cycles [9]. More recently, Burrows and coworkers calculated that resilin could not store sufficient energy to account for the jumping of froghoppers and locusts [10,11]. In these insects, resilin and chitinous cuticle form a composite material that serves as the energy storage device. Chitin, which is a relatively hard polysaccharide material, stores the energy, whereas resilin, which is a much softer material, protects chitin from fracture. Resilin also quickly returns the structure to its original shape, which allows the movement to be repeated. Their results raise an interesting question of whether chitin–resilin composites are a ubiquitous energy storage mechanism in insects and suggest that the jumping abilities of fleas, flea beetles, crickets, and other insects might need to be re-evaluated in this light.

2. Design and production of resilin-based proteins

2.1. Native resilin gene sequence

The *Drosophila melanogaster* gene product CG15920 was tentatively identified as encoding the resilin gene sequence by searching the *D. melanogaster* genome for sequences similar to tryptic peptide sequences derived from locust resilin (*Schistocerca gregaria*) [12]. The gene product CG15920 (i.e. a precursor of resilin) has

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620 amino acids consisting of a signal peptide sequence (residues 1–17) and three main regions (residues 26–602) (Fig. 1). After the signal peptide is removed, pro-resilin, which is uncrosslinked, is secreted into extracellular space. The three remaining domains are the N-terminal elastic domain (exon 1, residues 26–322), the chitin-binding domain (exon 2, residues 342–403), and the C-terminal elastic domain (exon 3, residues 412–602).

Exons 1 and 3 have repetitive sequences, which are a common feature found in other elastomeric proteins [13]. Indeed, these two exons contain a large number of repeating motifs that confer elasticity to resilin [14,15]. Elvin et al. [16] first showed that the exon 1 repeat motif confers elasticity to resilin function. Exon 1 is dominated by 18 repeats of a 15-residue sequence (GGRPSDSYGAPGGGN), whereas exon 3 contains 11 copies of a 13-residue repeating motif (GYSGGRPGGQDLG) [12]. Both exons are rich in proline and glycine residues, which provide high flexibility to resilin [17]. They also have tyrosine residues, which form intermolecular crosslinks through di- and tri-tyrosines connecting resilin polypeptides.

The central exon 2 domain is 62 amino acids in length and contains the Rebers–Riddiford (R–R) consensus sequence (specifically, the RR-2 type) [12]. There are three different types of R-R consensus sequences. The RR-1 and RR-2 types are mainly found in soft and pliable or hard and stiff cuticles, respectively. The RR-3 type is rarely observed [18]. The presence of the RR-2 type in exon 2 indicates that exon 2 is involved in forming a protein–cuticle composite by binding to chitin [19]. However, it has been reported that alternative splicing results in another variant of pro-resilin which does not contain the R–R consensus sequence and that natural resilin is a mixture of the two alternatively spliced variants [15,20].

Since the discovery of the resilin gene in *D. melanogaster*, there have been reports of putative resilin-like genes in other *Drosophila* species [21] as well as insects such as the beetle [21], honey bee [21], parasitic wasp [21], body louse [21], flea [22], buffalo fly [22], and dragonfly [22]. Of particular note, a resilin-like sequence (AQTPSSQYGAP) from the African malaria mosquito *Anopheles gambiae* was identified based on sequence homology of the YGAP tetrapeptide found in exon 1 of pro-resilin derived from *Drosophila* [23]. Unlike resilin sequences from the aforementioned insects, the consensus sequence from *A. gambiae* does not have a chitin-binding domain or high glycine content [22]. However, a resilin-like polypeptide (An16) composed of *A. gambiae* consensus sequences displays similar structure and mechanical properties to those of resilin [14,24].

2.2. Construction of recombinant DNA sequences

Resilin-like polypeptides have been manufactured by utilizing recombinant DNA technology, which confers numerous advantages in creating biomaterials. First, the DNA sequence of recombinant proteins can be tightly controlled at the molecular level to have the desired amino acid sequence, molecular weight, and order of functional domains. Also, material characteristics such as the mechanical and biological properties can be independently tuned since multiple functionalities can be fused into a single material [25]. Furthermore, recombinant DNA technology enables the design of new materials through inclusion of additional sequences and amino acid substitutions in the wild-type sequence.

Elvin and coworkers made the first attempt to clone a resilin-like polypeptide [16]. The first exon (residues 19–321) of the *D. melanogaster* CG15920 gene product was amplified from genomic DNA by polymerase chain reaction (PCR), and *Escherichia coli* was used as a host for protein expression. The first resilin-like protein, named rec1-resilin, is rich in glycine (35 mol.%) and proline residues (14 mol.%) but lacks hydrophobic residues with bulky side chains [16]. Later variations of recombinant resilin proteins included expression of full-length resilin containing exons 1, 2, and 3 from *D. melanogaster* [15].

Given that the elastic properties of naturally elastomeric proteins are attributed to their repetitive sequences [13], researchers have synthesized resilin-like polypeptides containing a number of repeating units based on four different species and have also combined them with other functional domains (Table 1). Initial attempts using PCR amplification to increase the number of repeating motifs were unsuccessful and resulted in gene mutations such as sequence truncation and modification [23]. Subsequent attempts successfully utilized molecular cloning to produce repetitive resilin sequences [23,26–29]. Recursive cloning, which is a useful strategy to multimerize sequences [30], has been used to produce genes encoding repetitive resilin-like polypeptides (Fig. 2A).

2.3. Expression of recombinant resilin-like polypeptides

Resilin-based polypeptides have been produced in *E. coli*, which is the most common host for expressing heterologous proteins due to its fast growth rate, cost-effectiveness, and low probability of post-translational modification [31,32]. Previous studies of resilin-based proteins have utilized the T5/T7 expression system using three different induction methods: isopropyl β-D-1-thiogalactopyranoside (IPTG) induction, autoinduction, and lactose induction (Table 1).

IPTG is an effective inducer and initiates transcription by binding to the *lac* repressor. However, the autoinduction method proposed by Studier is more convenient compared to the conventional IPTG induction method because there is no need to monitor bacterial growth to find the proper time point to add the inducer [33]. In addition, the autoinduction method is economical because protein expression is regulated by carbon and energy sources, such as glucose and lactose, in the growth medium and an expensive inducer such as IPTG is not needed. Previous studies reported that the culture density and the yield of target protein (rec1-resilin) were 3-fold higher when using autoinduction compared to IPTG induction [34]. Lactose induction is an alternative method to produce resilin-like polypeptides. In large-scale fermentation, a combination of lactose and IPTG induction resulted in

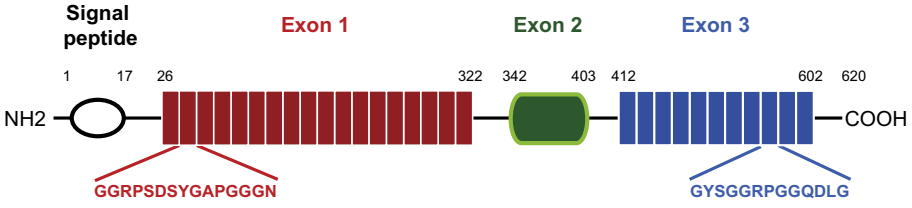

Fig. 1. The putatative resilin sequence from the *Drosophila melanogaster* CG15290 gene product. The sequence consists of a signal peptide and three different exons (exons 1–3). The signal peptide is removed before secretion into the extracellular space. Exons 1 and 3 include 18 repeats of GGRPSDSYGAPGGGN and 11 copies of GYSGGRPGGQDLG, respectively. The sequence in exon 2 is involved in binding of chitin.

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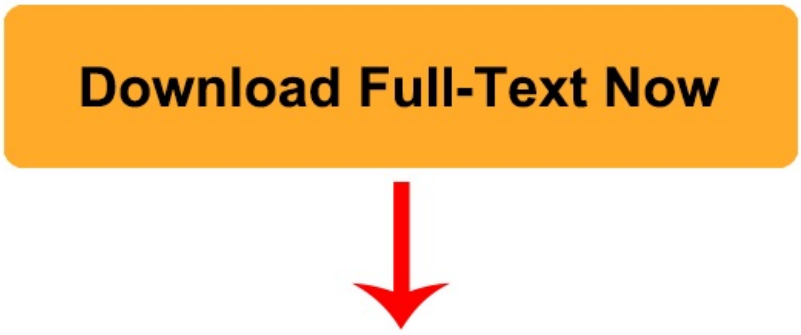

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