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Review

Applications of elastin-like polypeptides in drug delivery

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A B S T R A C T

Elastin-like polypeptides (ELPs) are biopolymers inspired by human elastin. Their lower critical solution temperature phase transition behavior and biocompatibility make them useful materials for stimulus-responsive applications in biological environments. Due to their genetically encoded design and recombinant synthesis, the sequence and size of ELPs can be exactly defined. These design parameters control the structure and function of the ELP with a precision that is unmatched by synthetic polymers. Due to these attributes, ELPs have been used extensively for drug delivery in a variety of different embodiments—as soluble macromolecular carriers, self-assembled nanoparticles, cross-linked microparticles, or thermally coacervated depots. These ELP systems have been used to deliver biologic therapeutics, radionuclides, and small molecule drugs to a variety of anatomical sites for the treatment of diseases including cancer, type 2 diabetes, osteoarthritis, and neuroinflammation.
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1. Introduction

Stimulus-responsive biomaterials are a promising resource with applications in controlled drug delivery. Elastin-like polypeptides (ELPs) are one such biomaterial whose composition is inspired by the repetitive hydrophobic domains of human tropoelastin [1]. ELPs are biopolymers composed of short repeating peptide motifs, of which the most common is the pentapeptide VPG*X*G where *X* is a guest residue that is any amino acid except proline. ELPs exhibit stimulus-responsive lower critical solution temperature (LCST) phase transition behavior. They are soluble at temperatures below a characteristic cloud point temperature (T_t) (also known as the inverse transition temperature) and aggregate into micron scale coacervates above the T_t [2]. This phase transition occurs over a short time scale and is typically reversible, such that ELP coacervates will resolubilize when returned to a temperature below the T_t.

ELPs are useful materials in a variety of applications since their stimulus-response is highly tunable [3]. The T_t is precisely controlled by intrinsic parameters including the composition and molecular weight (MW) of the ELP (Fig. 1) and is also influenced by extrinsic factors such as concentration, solutes, and, for some ELP sequences, by solution pH. The hydrophobicity of the guest residue is a primary intrinsic parameter that controls the T_t, as hydrophobic guest residues depress the T_t, while hydrophilic guest residues elevate the T_t [2,4,5]. The MW of the ELP is inversely related to the T_t, such that an increase in MW leads to a decrease in the T_t [6]. The concentration of an ELP solution also influences the T_t in an inverse relationship, such that an increase in ELP concentration results in a lower T_t [6]. The ELP T_t is additionally influenced by cosolutes, such as salts. Most notably, kosmotropic salts (Cl^- and higher on the Hofmeister scale of anions) depress the ELP T_t, with increasing concentration of salt enhancing this effect [7]. Furthermore, the local pH can influence the T_t of ELP sequences that include ionizable guest residues [8,9].

ELPs are typically produced recombinantly from synthetic genes, whose construction requires specialized methods for assembling repeating genes with precise control over gene length. Concatemerization is the oldest technique to create a library of repetitive ELP genes with varying lengths. In concatemerization, genes with cohesive ends self-ligate to create oligomers within a receiving cloning vector. Although this provides a rapid method to assemble ELP genes of varying length, the genes created by concatemerization have a distribution of lengths, which does not ensure that a desired gene length will be achieved. Concatemerization is therefore less desirable when an exact MW of an ELP, and thus an exact ELP gene length, is required. Furthermore, successful insertion of concatemerization products into the cloning vector is biased to a low degree of polymerization (typically $\leq$30 pentapeptides), such that this technique is less useful for applications where a higher degree of polymerization is required.

Concatemerization is useful, however, when paired with other assembly methods to quickly build ELP genes with a precise length. Recursive directional ligation (RDL) is one such method that involves the stepwise oligomerization of short ELP genes derived from concatemerization [10]. Recursive gene addition builds the length of the encoded ELP in an existing vector by using a single cut site for the insertion of an ELP gene with complementary sticky ends. However, RDL runs the risk of self-

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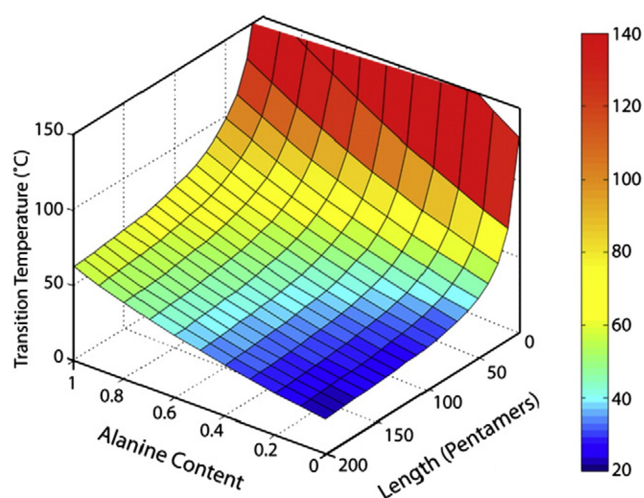

Fig. 1. Intrinsic design parameters of ELP length and guest residue composition (alanine guest residue titration into a 100% valine guest residue sequence) provided precise control of the T_t. Quantitative modeling of $(VPGXG)_n$, with variables $X =$ guest residue of alanine and valine (expressed as fraction of alanine content) and $n =$ chain length in pentapeptides, allowed prediction of the T_t over a broad temperature range for ELPs at 25 μM in PBS. Adapted with permission from [3]. Copyright 2013 American Chemical Society.

ligation of both the receiving plasmid and the ELP gene insert, which decreases the efficiency of this method.

Recursive directional ligation by plasmid reconstruction (PRe-RDL) solves the problem of self-ligation by introducing restriction sites that deconstruct two parent plasmids containing ELP genes, such that reconstruction of a functional plasmid is only achieved by successful ligation of the two plasmid fragments containing the ELP-encoding components [11]. Due to the step-wise nature of both RDL and PRe-RDL, these methods can be used to create ELPs with complex architecture at their sequence level, such as multi-block copolymers. These techniques also allow insertion of peptide or protein sequences at the N- and C-terminals of an ELP gene, providing chemical or biological activity for drug delivery applications. However, these techniques require multiple cloning steps to create an ELP gene with a high degree of polymerization.

Rapid synthesis of ELP genes with a high degree of polymerization can be achieved in one step with overlap extension rolling circle amplification (OERCA) [12]. This assembly method uses a circular ssDNA that encodes a desired repeat unit of the ELP gene. Rolling circle amplification (RCA) is first carried out to create a linear ssDNA containing several copies of the circular template. RCA is followed by polymerase chain reaction (PCR) with primers that are complementary to the ends of the product from the RCA reaction, resulting in dsDNA and further lengthening of the genes by overlap extension. OERCA is a powerful method for multiplexed synthesis of repetitive genes, whose size range is tunable and is typically longer than that achieved with concatemerization. We note, however, that OERCA is limited to the synthesis of homopolymer ELPs and thus cannot be used to create genes that encode alternative architectures, such as block copolymers.

ELP genes are typically expressed in *E. coli*, although ELPs have also been expressed in yeast [13–15], fungus [16], and plants [17–19]. Purification of ELPs from *E. coli* lysate is achieved by exploiting the ELP's thermal responsiveness using a non-chromatographic separation method called inverse transition cycling (ITC) [20]. ITC has four sequential steps: (1) selective aggregation of the ELP by raising the solution temperature above the T_t or by depressing the T_t below the solution temperature with the addition of kosmotropic salts; (2) centrifugation above the T_t to pellet the aggregated ELP and discard soluble contaminates in the supernatant; (3) recovery of soluble ELP with dissolution of the pelleted material by reversing its phase transition in a buffer at a temperature below the T_t; and (4) centrifugation below the T_t to pellet insoluble contaminants and collect purified soluble ELP in the supernatant. Repeating cycles of centrifugation above and below the T_t increases the purity of the ELP product. ITC is a powerful alternative to chromatography that allows easy purification of ELPs with equipment found in most biology laboratories.

As biologically inspired recombinant materials, ELPs have distinct properties that make them useful for applications in drug delivery. ELPs are biocompatible and are therefore suitable for local and systemic administration, as they induce minimal inflammatory and immune effects in animal models [21–23] and can be administered to humans without eliciting an adverse immune response [24]. Additionally, the genetically encoded design of ELPs permits exact control over the sequence of the ELP, which can be exploited to precisely specify the location at which a biological drug—peptide or protein—is fused to an ELP, or the location at which a reactive residue is placed for covalent conjugation of the ELP with small molecule drugs. Furthermore, their genetically encoded design leads to perfectly monodisperse polymers. As MW influences important biological parameters, like circulation clearance, this monodispersity allows improved prediction of the ELP behavior *in vivo*, as compared to alternative polydisperse materials.

The biological composition of ELPs also ensures their biodegradation, permitting their safe break down into peptides and amino acids that can be easily cleared from the body. The kinetics of ELP degradation have been analyzed both *in vitro* and *in vivo* with ^{14}C-labeled ELPs, where the degradation products were visualized by SDS-PAGE and quantified by radioactivity [25]. A degradation rate of approximately 2.5 wt.%/day was observed following intravenous administration of a 59.4 kDa ^{14}C-labeled ELP [26]. This degradation rate suggests that ELPs can strike a good balance between *in vivo* stability, over the time course required for many drug delivery applications, and clearance over several weeks. Although all ELPs are susceptible to eventual clearance by mechanisms of degradation, it is important to consider the effect of ELP aggregation on degradation kinetics for ELPs that are used *in vivo* in their aggregated coacervate phase. *Ex vivo* experiments have shown that soluble ELPs below their T_t were enzymatically degraded by both elastase and collagenase. However, a decrease in enzymatic degradation by collagenase was observed at temperatures above the T_t of a homopolymer ELP, in which the ELP was aggregated into micron-scale coacervates, or at temperatures above the critical micelle temperature of diblock copolymer ELPs, at which the hydrophobic ELP domain was sequestered in the micelle core [27]. This differed from degradation by elastase, which was efficient regardless of ELP aggregation. These experiments demonstrated that aggregation can be a factor in the degradation rate of ELPs and their higher order assemblies, and that these effects are protease specific.

2. Architectures and assemblies of ELP drug carriers

2.1. Soluble ELP unimers

Due to their recombinant design and stimulus-response, ELPs can serve as useful carriers for drug delivery in a variety of sequences, architectures, and higher order assemblies (Table 1). ELPs in their soluble state—which we term unimers to distinguish them from their self-assembled or aggregated counterparts—are useful materials for enhancing the size and stability of appended cargo and conferring stimulus-responsive behavior to these conjugates. These ELPs are typically designed as homopolymers (wherein the guest residue is a single amino acid) or as pseudorandom ELPs (wherein the guest residue is a mixture of residues) that are chosen to impart a T_t above body temperature so that they are soluble *in vivo*. These features, in combination with the ease of ELP purification by ITC and their genetically encoded design, make ELPs excellent purification tags for fusion partners such as recombinant peptide and protein drugs [28]. ITC can be used for the purification of these genetic fusions because the ELP retains its LCST behavior in the fusion, although the T_t of the ELP fusion may vary from the T_t of the ELP alone [29]. The simplicity and low cost of ITC purification provides

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