Journal of Controlled Release 240 (2016) 93–108

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Elastin-like polypeptides: Therapeutic applications for an emerging class of nanomedicines

Jordan Despanie [a], Jugal P. Dhandhukia [a], Sarah F. Hamm-Alvarez [a,c], J. Andrew MacKay [a,b,*]

[a] Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90033-9121, USA
[b] Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA
[c] Department of Ophthalmology, University of Southern California, Los Angeles, CA, 90033, USA

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 6 November 2015
Accepted 9 November 2015
Available online 11 November 2015

Keywords:
Nanomedicine
Elastin-like polypeptides
Drug delivery
Biomedical engineering

ABSTRACT

Elastin-like polypeptides (ELPs) constitute a genetically engineered class of 'protein polymers' derived from human tropoelastin. They exhibit a reversible phase separation whereby samples remain soluble below a transition temperature (T_t) but form amorphous coacervates above T_t. Their phase behavior has many possible applications in purification, sensing, activation, and nanoassembly. As humanized polypeptides, they are non-immunogenic, substrates for proteolytic biodegradation, and can be decorated with pharmacologically active peptides, proteins, and small molecules. Recombinant synthesis additionally allows precise control over ELP architecture and molecular weight, resulting in protein polymers with uniform physicochemical properties suited to the design of multifunctional biologics. As such, ELPs have been employed for various uses including as anti-cancer agents, ocular drug delivery vehicles, and protein trafficking modulators. This review aims to offer the reader a catalogue of ELPs, their various applications, and potential for commercialization across a broad spectrum of fields.

1. Introduction

While ambitious visions for nanomedicine have outpaced their practical applications, nanomedicine has had a significant impact on drug delivery systems. First envisioned in 1906 by Paul Ehrlich [1], these novel drug delivery systems are aimed at improving clinical outcomes through innovations in particle size, shape, multifunctionality, site-directed delivery, and the reduction of toxicity [2]. The therapeutic index, defined as a ratio between toxic and effective doses, is one measure by which nanomedicines may be optimized. Drug toxicity depends on duration, concentration, and total exposure to drug. In particular, many chemotherapeutics have low therapeutic indices caused by dose-limiting side-effects in normal tissues. While raising the dose of an approved free drug can improve efficacy, off-target effects render this unsafe. Thus, a major rationale for exploring nanomedicines is that they can enhance the therapeutic index compared to their free drug counterpart.

One potential advantage of nanomedicine includes the capacity to construct materials that ferry diverse drugs to sites of disease for controlled release and/or site-directed delivery. By tuning the size and architecture of nanoparticles, for instance, it may be possible to enhance drug residence times and augment the therapeutic index over what could be achieved with free drug alone. Breakthroughs have been most prominent in the oncology space with the approval of nanoformulations, such as liposomal doxorubicin and albumin-bound paclitaxel, aimed at exploiting the enhanced permeability and retention (EPR) effect sometimes seen in tumors [3,4]. Alternatively, with over 50% of all anti-cancer drugs doubling as substrates of the P-glycoprotein efflux pump, nanoparticles are a compelling solution to overcome multidrug resistance [5].

Extensive efforts to expand the utility of nanoformulations have relied on grafting polymers to their surface. Liposomes are among the best-characterized nanomedicine platforms [6]. For early liposomal formulations, rapid opsonization and detection by the mononuclear phagocyte system, which removes foreign bodies from the blood, hampered the mean residence time and therapeutic efficacy [7]. To prevent opsonization, protective polymers were later developed to sterically shield nanoparticles, which propelled liposomes to clinical approval twenty years ago [8]. Based on data from liposomes and other similarly sized particles, the surface properties for new carriers should be optimized to minimize opsonization, prevent complement activation, and eliminate clearance by the mononuclear phagocyte system. All of these factors can be strongly influenced by composition and architecture of polymers at the nanoparticle surface. Thus, advances in nanomedicine have been closely tied to the development of polymers for biological applications.

As nanomaterials, high molecular weight polymers can solubilize hydrophobic drugs [9,10]. Polymer-drug conjugation involves the

* Corresponding author at: Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, 1985 Zonal Avenue, Los Angeles 90033-9121, USA.
E-mail address: jamackay@usc.edu (J.A. MacKay).

http://dx.doi.org/10.1016/j.jconrel.2015.11.010
0168-3659/

appending of a water-soluble polymer onto the drug of choice [11]. Polymers with large hydrodynamic radii can prevent renal filtration and extend the drug mean residence time. If these polymers are amphiphilic, they can also directly mediate nanoparticle assembly [12,13]. As such, vast arrays of polymeric species, ranging from natural to synthetic, have been studied as drug carriers. Chemical polymerization produces mixtures of polymers with differing chain length, which necessitates statistical definitions of their polydispersity. The polydispersity index (PDI) is defined as the ratio of the mass-average molecular weight (M_w) to the number-average molecular weight (M_n) and characterizes the heterogeneity in a system. A monodisperse polymer possesses a PDI approaching 1 while polydisperse polymers have PDI greater than 1. The PDI is highly dependent on the mechanism of the polymerization reaction [14]. As polymers of differing molecular weights have various fates in the body, it is evident that the PDI should be controlled to the greatest extent possible [15]. Additional aspects of polymer nanomaterials to be considered include immunogenicity, biodegradability, efficiency of encapsulation and drug loading, as well as stability on the shelf and in the body.

Synthetic polymers, *e.g.* polyethylene glycol (PEG) and polycaprolactone (PCL), have seen widespread use as biomaterials owing to their low immunogenicity and high biocompatibility. Drawbacks related to polydispersity, linkage stability, and limited carrying capacity [16,17] unfortunately limit the delivery applications of these platforms. More recent advances have since focused on the fabrication of hybrid nanocarrier systems. Block copolymers, for instance, can spontaneously self-assemble into nanostructures in the form of micelles, electrostatic complexes, and polymersomes due to their amphiphilic properties [2].

Compared to conventional polymers, biomaterials derived from recombinant proteins may serve as viable alternatives. The molecular techniques underpinning these 'protein polymer' technologies were first detailed in 1986 [18,19]. Archetypal peptides include leucine zippers [20], collagen-like polymers [21], extended recombinant polypeptide (XTEN) polymers [22], silk-like polypeptides (SLPs) [23], and elastin-like polypeptides (ELPs) [24]. These protein polymers, comprised of repeating motifs sourced from either natural or *de novo* engineered amino acid sequences, are generated *via* genetic engineering and recombinant biosynthesis. This offers an exquisite level of precision and tunability with regards to the length, molecular weight, sequence, and monodispersity of the resulting material. Owing to their origin as natural polypeptide chains, protein polymers can be biocompatible and biodegradable, leaving only small peptides and amino acids as their metabolic byproducts [25].

Genetic encoding, meanwhile, grants structural and functional control over molecular features such as secondary structures, targeting motifs, and drug conjugation sites. This provides a compelling rationale for the systematic construction of libraries varying by specific amino acid residues. The addition of natural peptides and protein domains to these polymers creates fusion proteins which often maintain the function and activity of the parent macromolecule [26]. Finally, the low costs of large-scale production in biological systems render recombinant protein polymers amenable to process scale-up [27].

1.1. Elastin-like polypeptides

Elastin is a polymeric extracellular matrix (ECM) protein, found in tissues as varied as the skin, lungs, blood vessels, and cartilage, which underpins the protractible nature of vertebrate tissues [28–30]. Though only one gene encodes the ~60 kDa soluble precursor, tropoelastin, it exists as polymorphs containing repetitive hydrophobic motifs, largely valine and alanine, denoting elastomeric domains. Other amino acids present at significant levels include glycine and proline, which disrupts alpha helix and beta-sheet formation. These elastomeric domains occur between distinct peptides that are involved in crosslinking other tropoelastin monomers through the action of lysyl oxidase on lysine residues [31]. As biosynthesis and extrusion into the ECM proceed, the final products generated are insoluble elastin fibrils. Pioneering studies first revealed the temperature-sensitive nature of hydrolyzed α-elastin [32]. The protein remained soluble below 25 °C, but when heated to 37 °C, elastin phase-separated into a secondary amorphous phase known as a coacervate. Interestingly, the investigators further noted that this process was completely reversible. Those findings eventually facilitated the first chemical synthesis of an elastin-like polypeptide prior to the emergence of molecular biology as a discipline [33].

Elastin-like polypeptides (ELPs) are an artificial, biomimetic class of protein polymers inspired by the recurring hydrophobic motifs of tropoelastin [34]. Due to their broad range of applications, including in drug delivery and tissue engineering, ELPs have attracted attention throughout the scientific community [35–37]. The canonical ELP unit consists of a hydrophobic, five amino acid motif (Val-Pro-Gly-X_{aa}-Gly)$_n$ where the guest residue, X_{aa}, specifies any amino acid and n determines the number of pentapeptide repeats. Proline is usually avoided at the fourth residue since its presence can interfere with coacervation. It should be noted, however, that inclusion of amino acid side chains capable of enhancing functionality does not necessarily interfere with ELP phase behavior. The addition of tyrosine to facilitate spectrophotometric analysis [38] or lysine for crosslinking [39,40] are two examples. Furthermore, ELPs with other repeat motifs beyond the one described have similar properties. Examples may range from other pentapeptides (*e.g.* IPGVG) to heptapeptide (*e.g.* LGAGGAG) and nonapeptide (*e.g.* LGAGGAGVL) repeat sequences [41].

A primary aspect of ELP biomaterials involves their ability to reversibly form coacervates following temperature changes (Fig. 1a). This feature is known as the critical transition temperature (T_t) and can be explained thermodynamically in terms of the Gibbs free energy ($\Delta G = \Delta H - T\Delta S$). If ΔG during a temperature transition (ΔG_t) is zero, then $\Delta H_t = T_t \Delta S_t$ which can be rearranged to $T_t = \Delta H_t / \Delta S_t$. The increase in order of ELPs at T_t might appear to contradict the second law of thermodynamics—namely that the order of a system has an inverse relationship with temperature—but the complete system consisting of protein polymer and water must be considered. In the absence of a

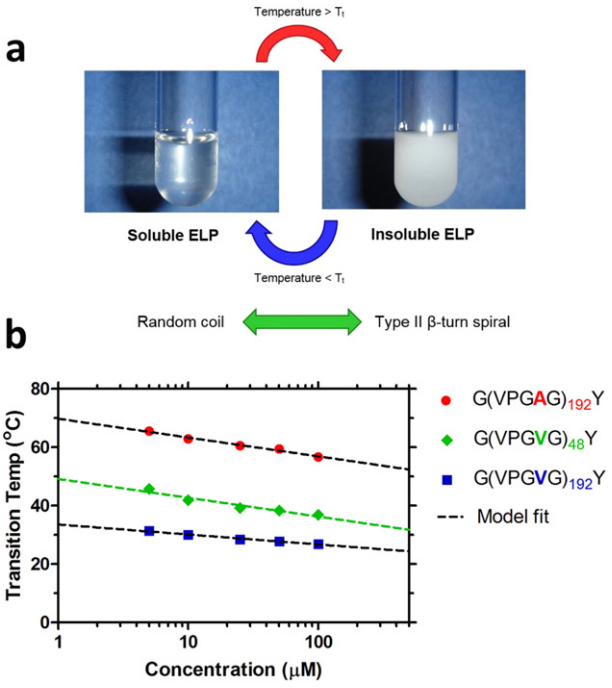

Fig. 1. Depiction of reversible phase separation by elastin-like polypeptides (ELPs). a. ELPs are soluble below a transition temperature, T_t, but undergo coacervation at temperatures above T_t. b. The linear relationship between T_t and concentration can be studied by measuring optical density as a function of temperature. Three different ELPs of varying length and hydrophobicity phase separate above the indicated lines.