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Hydrazone self-crosslinking of multiphase elastin-like block copolymer networks

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ABSTRACT

Biosynthetic strategies for the production of recombinant elastin-like protein (ELP) triblock copolymers have resulted in elastomeric protein hydrogels, formed through rapid physical crosslinking upon warming of concentrated solutions. However, the strength of physically crosslinked networks can be limited, and options for non-toxic chemical crosslinking of these networks are not optimal. In this report, we modify two recombinant elastin-like proteins with aldehyde and hydrazide functionalities. When combined, these modified recombinant proteins self-crosslink through hydrazone bonding without requiring initiators or producing by-products. Crosslinked materials are evaluated for water content and swelling upon hydration, and subject to tensile and compressive mechanical tests. Hydrazone crosslinking is a viable method for increasing the mechanical strength of elastin-like protein polymers, in a manner that is likely to lend itself to the biocompatible in situ formation of chemically and physically crosslinked ELP hydrogels.

1. Introduction

Recombinant elastin-like protein polymers (ELP) represent a promising new class of biomaterials that may be formulated as gels, films, nanofibers or micelles with potential applications in drug delivery, tissue engineering or as components of implanted medical devices [1–8]. We have recently described ELPs with hydrophilic, elastomeric midblock sequences flanked by self-associating, hydrophobic endblocks in an ABA triblock format [1,9,10]. Designs with individual block sizes in excess of 35 kDa have resulted in protein-based biomaterials demonstrating structural polymorphism, providing the opportunity to broadly tune mechanical responses and drug elution rates [11–15]. Notably, due to the self-association of endblock sequences, triblock ELPs form physical, non-covalent crosslinked gel networks in aqueous, physiologic environments (pH 7.4, 37 °C), as reviewed elsewhere [1]. Physical crosslinking is reversible and, in principle, eliminates the need for inflammatory or cytotoxic compounds associated with chemical crosslinking schemes. ELP properties, including physical crosslinking, depend upon repeat sequences of the pentapeptide [(Val/Ile)–Pro–Xaa–Yaa–Gly]. The polarity of the fourth residue (Yaa) dictates the coacervation or inverse temperature transition (T_t) of the polypeptide in aqueous solution. The identity of the third residue (Xaa) influences block mechanical properties, with the consensus glycine enhancing elasticity and the substitution of alanine contributing to plastic mechanical behavior. Consequently, ELP endblocks with a significant fraction of (VPAVG) sequences tend towards plastic behavior and lower T_t while midblocks containing (VPGEG) repeats contribute to elasticity and elevated T_t. Sequences are designed so that cold, aqueous ELP solutions transition to elastomeric gels through endblock self-assembly as they are warmed to physiologic temperature, with T_t in the range of 10–15 °C.

Despite the advantages of physical crosslinking, self-assembled domains can be disrupted at lower mechanical stresses than covalent crosslinks. The majority of reported methods for covalent crosslinking of ELPs rely upon amino groups, and employ either chemical or enzymatic approaches. Chemical methods including isocyanates, NHS-esters, phosphines, aldehydes or genipin have been reported [16–23], whereas enzymatic approaches have consisted of transglutaminase and lysyl oxidase [24]. In addition, we have investigated solid-state crosslinking of recombinant elastin using both UV and visible light-activated photoinitiators [25].

Strategies for combined chemical and physical crosslinking may result in significant reinforcement of properties arising from self-assembled, multiphase polymer network structure. In an ELP designated LysB10, lysine residues were included at the block interfaces, and crosslinked with glutaraldehyde (GTA) after endblock self-assembly [10]. This modification successfully stabilized

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