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Crosslinked ELP-based nanoparticles, using the strain promoted azide–alkyne cycloaddition

Ferdinanda C.M. Smits, Wilke W.A. Castelijns, Jan C.M. van Hest *

Radboud University Nijmegen, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

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ABSTRACT

A well-defined elastin-like polypeptide (ELP) block copolymer was synthesized via protein engineering for the formation of shell-crosslinked micellar particles. The block copolypeptide consisted of two domains with different transition temperatures (T_t), with the more hydrophilic block positioned at the N-terminus. At this protein end additional lysines were introduced to facilitate crosslinking. Upon raising the temperature above the T_t of the more hydrophobic block particles were formed. Two methods of crosslinking were investigated. Using genipin the lysines were directly used for particle stabilization. To be able to crosslink and functionalize the nanoparticles in an orthogonal and efficient fashion, another crosslinking strategy was developed, making use of the strain promoted azide–alkyne cycloaddition (SPAAC) reaction. For this purpose the block polypeptide was azidated making use of a diazotransfer reaction. Subsequent assembly and crosslinking using a bis-cyclooctyne reagent resulted in the formation of stable nanoparticles. Encapsulation of a hydrophobic dye showed these nanoparticles to have potential as nanocarriers.

1. Introduction

Biodegradable polymers have been extensively studied for their potential use as biomaterials in medicine [1,2]. Most of these biomaterials are based on synthetic polymers. In order to be applied in biomedicine, polymers have to meet stringent demands regarding chemical structure and composition. This has motivated scientists to develop better defined methods for polymer synthesis, of which the use of genetically engineered polymers is one of the most advanced. An example of such well-defined protein polymers are elastin-like polypeptides (ELPs). Due to the high control over composition, low toxicity and biodegradability, ELP-based materials have gained a lot of interest for their use in the development towards nanomedicine applications [3,4].

ELPs are a special class of protein-based polymers, as they are stimulus-responsive. These ELPs show lower critical solution temperature (LCST) behavior, meaning that upon heating through their transition temperature (T_t) they will switch from a water-soluble to an insoluble hydrophobic state, resulting in aggregation. This LCST behavior is fully reversible and can be varied by the composition of the amino acids, the molecular weight and the ionic strength of the solution.

Much research has been performed in creating nanoparticles composed of ELP-based block copolymers. By employing block copolymers with significantly different transition temperatures, amphiphilicity is induced, upon heating through the transition of the ELP block with the lowest T_t, which results in self-assembly into micellar structures [5–11]. Also conjugates of ELPs have been developed, for example with other polypeptide blocks, like poly(aspartic acid) [12], silk-like polypeptides [13] and capsid proteins [14], or non-peptide based blocks such as

* Corresponding author. Tel.: +31 24 36 53204.
E-mail address: j.vanhest@science.ru.nl (J.C.M. van Hest).

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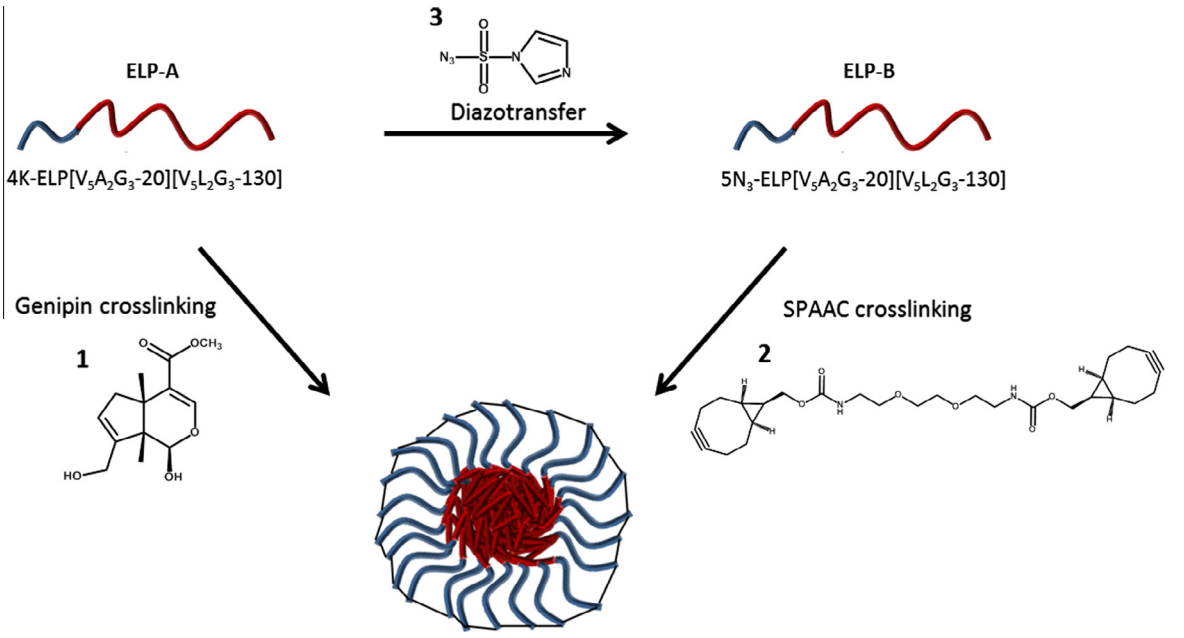
PEG [15], small compounds [16], oligonucleotides [17] and poly(acrylic acid) [18].

In case ELP nanoparticles are to be employed under physiological conditions one has to choose one of the ELP-blocks to have a T_t below 37 °C or one needs to apply a crosslinking strategy in order to keep the nanoparticles assembled, as the ELP phase transition is reversible. Many crosslinking methods have been used for ELP-based materials, which are either based on chemical crosslinking [10,19–22], enzymatic crosslinking [23] or reversible self-crosslinking by use of cysteine residues [11,24]. Many of the chemical crosslinking methods rely on the introduction of lysines into the ELP. The location of crosslinking is in most cases within the core of the particle. This results in stable nanoparticles which can be used at any desired temperature.

In this paper an ELP-based nanoparticle is described that has crosslinkable anchors at the outer shell of the particle, leading to particles with a large reservoir for possible drug loading. (Scheme 1) To allow peripheral crosslinking 4 additional lysine residues were introduced at the most outer part of the nanoparticle. The crosslinking chemistry employed should be fast, selective and not lead to modification of any possible cargo. Most chemical crosslinkers used up to date are not orthogonal. This brought us to the idea to use the recently developed spontaneous and bio-orthogonal strain promoted azide–alkyne cycloaddition (SPAAC) reaction for crosslinking [25–28]. In order to be able to perform the SPAAC crosslinking reaction, the lysine residues and the N-terminus were chemically converted into azide units. To compare the crosslinking using SPAAC with another already existing crosslinking method, it was decided to use genipin (**1**), an FDA-approved natural crosslinker that reacts with amine functionalities to form crosslinked networks [29]. It was shown that crosslinking using SPAAC was much faster than using genipin (**1**). Subsequent loading of a hydrophobic model dye shows these nanoparticles to have potential as nanocarriers.

2. Experimental

Unless stated otherwise all chemicals were obtained from Sigma–Aldrich and used without further purification. Standard molecular biology protocols were used for gene synthesis and oligomerization. Digested vectors and inserts were purified by gel electrophoresis (Promega Wizard SV Gel and PCR Clean-Up System). Clones were maintained in *Escherichia coli* XL1-Blue and TOP10 (Invitrogen). Synthetic oligonucleotides were purchased from BioLegio (Nijmegen, the Netherlands). Ampicillin was purchased from MP Biomedicals. PBS, LB and 2xTY medium was provided by the Radboud Institute for Molecular Life Sciences (RIMLS). Genipin (**1**) was purchased from Wako Chemicals. BCN-$(POE)_3$-BCN (**2**) and BCN-DY495 were purchased from SynAffix BV. Ultrapure Milli-Q water (MQ) was obtained from a WaterPro PS polisher (Labconco, Kansas City, MO) set to 18.2 MΩ/cm. Protein mass characterization was performed by electrospray ionization time-of-flight (ESI-TOF) on a JEOL AccuTOF. Deconvoluted mass spectra were obtained using MagTran 1.03b2. Isotopically averaged molecular weights were calculated using the "Protein Calculator v3.3" at http://www.scripps.edu/~cdputnam/protcalc.html. Dynamic light scattering (DLS) experiments were performed on a Zetasizer Nano S (Malvern Instruments Ltd., England). Prior to DLS measurements, the self-assembled samples were centrifuged (1 min at 10,000*g*). For transmission electron microscopy (TEM), TEM grids (CF200-Cu, EMS) were glow-discharged using a Cressington Carbon coater and power unit. 5 μL



Scheme 1. Outline of the development of ELP-A and ELP-B based nanoparticles that are crosslinked at the outer shell using either genipin (**1**) or BCN-$(POE)_3$-BCN (**2**).