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Revealing the impact of poly(ethylene oxide) blocks on enzyme activable coatings from peptide–polymer conjugates

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ABSTRACT

Peptide–polymer conjugates composed of a poly(ethylene oxide) (PEO) block and a precursor segment from mussel foot protein-1 (mefp-1) are enzymatically oxidized by tyrosinase. A functional transition from weak/reversible binders to strong/irreversible adsorption onto aluminum oxide surfaces is observed. A set of mefp-1-*block*-PEO bioconjugates with PEO-block lengths of 850, 3200 and 5200 g/mol is synthesized and investigated to elucidate effects of PEO-block length on the enzyme activable formation of antifouling coatings on aluminum oxide surfaces. The variation of PEO-block length systematically affects the activation kinetics of the bioconjugates by tyrosinase, the adhesion behavior of the activated bioconjugates, the stability of the resulting aluminum oxide coatings and the antifouling properties of coated aluminum oxide surfaces. Mefp-1-*block*-PEO_{3200} exhibits the best compromise as enzyme activation and adhesive properties showed excellent behavior. Stable coatings on aluminum oxide are formed in the activated state, which reduce albumin protein interactions in a practically quantitative manner. The coating appears to be sufficiently dense and stable to reach similar antifouling properties as covalently "PEGylated" aluminum substrates.

1. Introduction

Combination of sequence-defined peptides and synthetic polymers lead to a versatile class of precisely definable, multifunctional block copolymers, which are termed peptide–polymer conjugates [1–3]. Within the last decades, these biohybrid polymers contributed significantly to expand the functional and structural space available for synthetic polymer sciences [4–11]. On the one hand, advances in soft-matter structure formation processes lead to novel types of self-assembled structures and mechanisms to precisely regulate the assembly/disassembly processes [12–20]. Furthermore, bioconjugates proved to be valuable tools to adjust interaction capabilities and define chemical interfaces, generating specific drug transporters, sequence specific coatings to engineer nanoparticle surfaces or adjustable condensation additives for DNA delivery [4,7,21–25]. Besides, these relevant contributions in the field of biomedicine, peptide–polymer conjugates enabled the realization of functional macromolecules for molecular electronics, engineering of crystals by biomimetic crystallization and activable adhesives or self-forming hierarchical composites [26–34].

Biomaterials provide a source of inspiration and challenges, which have been addressed by exploiting peptide–polymer conjugates [1,35]. For instance, the adhesive system of marine mussels adhere rapidly onto various surfaces from wood to Teflon® and thus promised new materials that outperform state of the art adhesives, glues or coatings [36–39]. l-3,4-dihydroxyphenylalanine (l-dopa) residues have been identified as one of the key

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components in the byssal adhesive system, contributing to cohesion between the concerted set of mussel foot proteins (mefp) and adhesion by generating effective interfaces to various surfaces [38–41]. l-Dopa is generated in a post-translatory enzyme processing step by tyrosine oxidation in adhesive protein precursors [42]. Several aspects of the byssal formation process could be mimicked with synthetic polymers, leading to mussel-glue inspired polymers [38,43–46].

More recently, tyrosinase could be applied to activate adhesion properties of bioconjugates composed of poly(ethylene oxide) (PEO) and a precursor segment of the mussel foot protein 1 of *mytilus edulis* (mefp-1) (Fig. 1) [47]. During activation, the tyrosine residues are oxidized in a two-step mechanism. First oxidation of tyrosine residues leads to an *ortho*-dihydroxyphenole derivate (catechol) followed by the second oxidation step, which forms the corresponding quinone.

Minor follow-up-reactions such as cross-linking might occur depending on the applied reaction conditions, as has previously been observed [47]. Probably these reactions are progressively reduced with increasing molecular weight of the PEO blocks in the bioconjugates.

Enzyme activated mefp-1-*block*-PEO bioconjugates proved to effectively adhere to stainless steel surfaces generating a robust coating. The non-covalent surface "PEGylation" (PEO surface attachment) of steel substrates exhibited antifouling properties, as adsorption of bovine serum albumin (BSA) could be suppressed. The effective suppression of non-reversible protein adsorption might be of great interest for biomedical applications.

Here, we summarize our study analyzing the adsorption of a set of enzyme activable mefp1-*block*-PEO bioconjugates on construction aluminum (aluminum oxide) surfaces (Fig. 1). The bioconjugates exhibit the same mefp-1 precursor sequence but differ in PEO-block lengths. Counteracting effects of the PEO-block on activability and coating properties on aluminum surfaces are observable, making an optimum PEO-block length compared to the peptide segment expectable (Fig. 1). High molecular weight PEO-blocks seem preferable to achieve an optimized surface shielding, effective antifouling coatings and reduced costs for effective peptide parts. However, disfavored effects on increased PEO-block length such as reduction of enzymatic mefp-1 activation in the bioconjugates and reduced interactions of the peptide segments with the surface might be foreseen.

2. Results and discussion

In nature, *M. edulis* foot protein mefp-1 serves as a protective coating of the mussel byssus [39]. A minimal adhesive sequence has been identified, exhibiting the primary sequence AKPS**Y**^{5}PPT**Y**^{9}K [48]. This important functional section provides two processable tyrosine residues, Y5 and Y9, which can be enzymatically oxidized to l-dopa. Remarkable adhesive properties of the activated minimal mefp-1 sequence onto stainless steel surfaces have already been described [49,50]. A set of AKPSYPPTYK-poly(ethylene oxide) conjugates (**II a–c**) and the corresponding non-conjugated precursor adhesion sequence (**I**) were synthesized by solid-phase supported peptide synthesis (SPPS) and are summarized in Table 1. **II a–c** could be accessed by SPPS with PEO block lengths of $M_{n,PEO}$ = 850, 3200 and 5200 by following inverse conjugation strategies [51]. Both the peptide and the bioconjugates could be isolated in a fully deprotected manner and characterization by means of mass spectrometry (ESI-MS, MALDI-TOF-MS) and ^{1}H NMR proved the chemical identities (cf. SI).

To investigate the impact of the different C-terminally conjugated PEO-blocks on tyrosinase substrate characteristics, UV–vis activation assays were performed for each of the conjugates (**II a–c**) and the non-conjugated peptide **I** (cf. Fig. 2). For this purpose, solutions of the different samples were oxidized by 100 u/mL tyrosinase and the

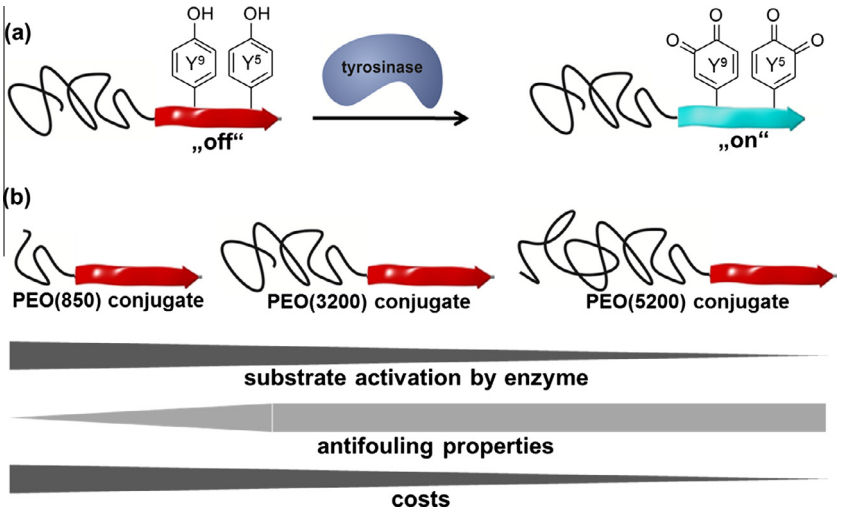

Fig. 1. Illustration of the tyrosinase activation of peptide–polymer conjugates (a). The bioconjugate exhibits a mefp-1 precursor segment which constitutes a weak adhesion domain (left) but a tyrosinase substrate and hence can be enzymatically processed leading to di-dopa carrying mefp-1 domain with activated adhesive properties (right). Property profiles of peptide-PEO conjugates, changing along with the increase of the PEO-block length ratio from $M_{n,PEO}$ = 850, 3200 and 5200 (b).