Tetrahedron 73 (2017) 4979–4987

Contents lists available at ScienceDirect

Tetrahedron

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

Native protein hydrogels by dynamic boronic acid chemistry

Christiane Seidler [a, b], David Y.W. Ng [a, **], Tanja Weil [a, b, *]

[a] Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
[b] Ulm University, Albert-Einstein-Allee 11, 89081 Ulm, Germany

ARTICLE INFO

Article history:
Received 18 May 2017
Received in revised form
27 June 2017
Accepted 28 June 2017
Available online 29 June 2017

Keywords:
Dynamic covalent chemistry
Stimulus responsive
Cytochrome C
Boronic acid
Hydrogel

ABSTRACT

Bioactive protein-based materials are unique functional systems that incorporate the specificity and intelligent characteristics of biomolecules within synthetic polymers. Herein, we take advantage of the boronic acid/salicyl hydroxamate molecular recognition strategy to crosslink an apoptosis inducing enzyme, cytochrome c, by dynamic covalent interactions to form a bioactive and responsive hydrogel for controlled administration. The material exhibits attractive rheological properties with high storage modulus and modulus crossover despite the perceived conformational sensitivity of the proteins. Upon acidification, the hydrogel matrix dissociated and release active enzymes into A549 cells, which initiates apoptosis. In combination, the chemical strategy facilitates the integration of native enzymes structurally into the hydrogel scaffold while simultaneously providing biological activity under a stimulus controlled release mechanism.

1. Introduction

The evolution of chemistry – from static to dynamic, where molecular bonds or supramolecular interactions are able to reorganize and exchange in a programmable fashion, has been realized as a fundamental feature in creating intelligent materials on multiple length-scales.[1–3] This strategy has been used by Nature and refined to near-perfection which we now bear witness to its many creations. In all these biologically complex systems, both dynamic covalent chemistry and supramolecular chemistry play a central role in all networks and processes from the transport of small molecules to hierarchical superstructures of macromolecular assemblies.[4,5]

Supramolecular chemistry provides intrinsic and geometric variations based on hydrogen, ionic bonds and π-π networks, whereas dynamic covalent chemistry is to a large extend found in disulfide bridges of proteins and peptides. Although deceptively simple in terms of chemistry, disulfide bonds dictate bioactivities that require a change in protein conformation/folding as well as in defining the energy landscapes of many structures.[6] Hence, disulfide bridges are the most conserved (>95%) functionality across the proteome.[7] The importance of this functional moiety is further emphasized by protein disulfide isomerase, a molecular chaperone that bridges cysteine residues in proteins, which accounts for up to 0.8% of total cellular protein.[8] Based on the number and placements of cysteine residues, mechanical properties of proteins (i.e. keratin, collagen),[6] its functions (i.e. vascular endothelial growth factors, integrin)[9] as well as entire systems (i.e. virus maturation)[10] are engineered within the dynamics of disulfide chemistry.

Specifically, dynamic covalent chemistry is used to create entropically driven folding and allows the existence of multiple stable conformations of proteins for their function. In this way, dynamic covalent structure formation ensures that misfolded peptide sequences are not self-assembled into toxic aggregates.[11] As such, dynamic covalent bonds impart both chemical flexibility and stability in the entire biological system. Similarly, dynamic covalent bonds currently available in synthetic chemistry are rather limited in variety as stimulus responsive groups are typically irreversible in order to drive the reaction forward. Among those that are reversible, the selection narrows further if compatibility towards the physiological conditions is required. Herein, we select a strategy based on boronic acids that is i.e. non-toxic, biorthogonal, rapid, stable and therefore fulfils the stringent

* Corresponding author. Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany.
** Corresponding author. Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany.
E-mail address: weil@mpip-mainz.mpg.de (T. Weil).

http://dx.doi.org/10.1016/j.tet.2017.06.066

demands as responsive chemical functionality for biomedical applications.[12]

Boronic acids form stable and reversible complexes with *cis*-diols in a pH-dependent manner under aqueous conditions.[13] The stability of these boronic esters relies on 1) the steric bulk of the diol to resist hydrolysis, 2) electron orbital overlap thus favouring O-donors compared to N-donors, 3) molecular geometry to fit the small boron center.[14] Hence, several systems of boronic acid interactions have been reported including the complexation with various carbohydrates, catechol derivatives as well as salicyl hydroxamates.[15] Among these substrates, salicyl hydroxamates represent the highest binding affinity and their use with boronic acids ranges from a click-like ligation of peptides[16] to the construction of pH-responsive macromolecular systems.[17,18] Extending the application repertoire of the reaction, we investigate the use of this dynamic complexation in building native protein hydrogel systems.

In general, hydrogels are typically made from covalently cross-linked or supramolecular polymers. Based on the type of intramolecular linkage, the mechanical properties can be customized. Recently, proteins have received increasing attention as scaffold materials[19] and/or crosslinkers[20,21] to form biocompatible and responsive materials. However, the use of therapeutically relevant enzymes within the gel matrix was not reported before. Enzymes are catalytic proteins that are extremely sensitive and most chemical techniques often cause denaturation of the protein. The advantage of using enzymes as a matrix component means that the exact stoichiometry is precise compared to a typical encapsulation procedure where the enzyme is trapped within a cross-linked polymer. Local administration of apoptosis (programmed cell death) inducing proteins in a hydrogel matrix provides great opportunities to control growth of e.g. tumor or bone-degrading cells for therapy.[22]

We report herein the formation of a native protein hydrogel by crosslinking cytochrome c (CytC), an important enzyme that initiates apoptosis, with poly(ethyleneglycol) (PEG) using boronic acid-salicyl hydroxamate (PBA-SHA) chemistry. By incorporating these components, we aim to create a dynamic hydrogel that is biocompatible, enzymatically active, pH-responsive and self-healing. This combination provides a new perspective where sensitive (bio)molecules can now be easily integrated as matrix components in an elegant way.

2. Results and discussion

Both hydrogel components, the CytC protein and PEG, were modified correspondingly with boronic acid and salicyl hydroxamate moieties (Fig. 1, Scheme 1). CytC is a 12.4 kDa protein that contains 20 amino groups in its sequence, allowing modification with 4-carboxyphenylboronic acid succinimidyl ester. The modification of the protein was accomplished efficiently in phosphate buffer (pH 8.0, 50 mM) with an excess of 4-carboxyphenylboronic acid succinimidyl ester (18 eq.). The boronic acid modified CytC (CytC-BA) was purified by ultrafiltration (5 kDa MWCO) and the number of boronic acid residues was found to be 11 (MALDI-TOF MS, Supporting Fig. S1).

In order to cross-link CytC-BA, PEG_{2000} was selected as it is a hygroscopic scaffold that retains water and at the same time extremely flexible to dynamically exchange within the matrix. PEG_{2000}-$(NH_2)_2$ was first transformed into PEG_{2000}-$(N_3)_2$ by an azido transfer reaction. Subsequently, PEG_{2000}-$(N_3)_2$ underwent a copper-catalyzed azide-alkyne cycloaddition using Trt-protected ethynyl salicyl hydroxamic acid and acid deprotection of the intermediate affords the target crosslinker PEG_{2000}-$(SHA)_2$ in good yields. A longer analogue using PEG_{3000} was synthesized to study the effect of chain length and hence the amount of trapped water molecules on the system. In addition, a 4-arm PEG_{2000} was also selected as an investigation towards how multivalent binding events affect rheological properties while keeping the relative number of water molecules constant.

2.1. Hydrogel formation and rheological properties

The hydrogel was first constructed by varying the stoichiometry of the crosslinker (0.5, 1.0, 1.5 mol eq. with respect to each boronic acid/salicyl hydroxamate binding event). With a 8 wt% CytC-BA, a hydrogel was instantaneously formed upon the addition of the cross-linker for all three stoichiometry at pH 7.4. The kinetics of gelation was monitored by a rheometer through the increase of both the storage modulus (G′) and the loss modulus (G″) with incubation time. As a representative example, Fig. 2A shows the steady increase of G′ as well as the G′/G″ ratio as the components gel, implying that the dynamic covalent bonds between the boronic acid and the SHA are formed over time attributing the material its strength and stiffness. Comparing across different stoichiometry of

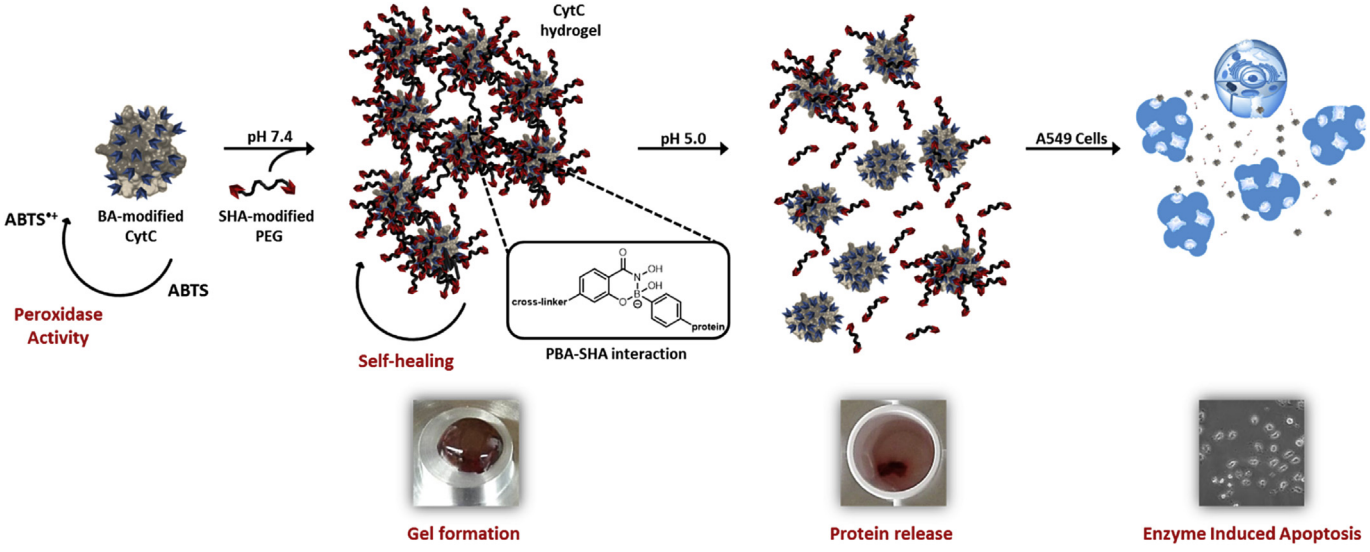

Fig. 1. Schematic of a pH-responsive hydrogel synthesized using cytochrome c as a bioactive protein precursor.