Biosensors and Bioelectronics 99 (2018) 56–61

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Streptavidin-hydrogel prepared by sortase A-assisted click chemistry for enzyme immobilization on an electrode

Takuya Matsumoto[a], Yuki Isogawa[b], Tsutomu Tanaka[b,*], Akihiko Kondo[a,b]

[a] Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657-8501, Japan
[b] Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada, Kobe 657-8501, Japan

ARTICLE INFO

Keywords:
Sortase A
Streptavidin
Hydrogel
Bioanode

ABSTRACT

A tetrameric streptavidin (SA)-appended LPETG tag was site-specifically linked to azido-containing tri-glycine via sortase A catalysis and the resulting azido-modified SA (SA-N3) was retained in the biotin-binding pocket. SA-N3 was polymerized with dibenzylcyclooctyne-modified branched poly(ethyleneglycol) (DBCO-PEG) using azido-modified branched PEG (N3-PEG) as a spacer via copper-free click chemistry. The resulting SA-based hydrogel exhibited gel-like mechanical properties and could immobilize biotin-modified molecules through biotin-SA affinity. Glucose dehydrogenase (GDH) was immobilized in the SA-based hydrogel, and the hydrogel was then coated on a glassy carbon electrode (GCE) and used for the biocatalytic oxidation of glucose. The designed GCE exhibited better performance and stability compared with GDH chemically adsorbed onto a GCE. In addition, the designed GCE anode and a Pt-carbon cathode were assembled into a glucose/O_2 fuel cell that provided a maximum power density and open circuit voltage of 11.8 ± 0.56 μW cm^{-2} and 0.17 V, respectively.

1. Introduction

Enzyme immobilization is an important technology for various biological systems and applications, including industrial and analytical applications (Ansari and Husain, 2012; DiCosimo et al., 2013), and for the generation of protein microarrays (Wong et al., 2009), biosensors, and biofuel cells (Willner et al., 2009; Rasmussen et al., 2016; Wang et al., 2016a). There has been significant research on methods for immobilizing enzymes on material surfaces, including crosslinking, adsorption, and entrapment (Ansari and Husain, 2012; DiCosimo et al., 2013; Homaei et al., 2013). Enzyme entrapment in hydrogels is one of the important approaches that retains the activity and conformation of the enzyme in a physiological environment (Kojuharova et al., 1988; Shiroya et al., 1995; Kim et al., 2011). Biomaterials such as chitosan and hyaluronic acid (Tang et al., 2014; Sun et al., 2015), or chemical materials such as poly(ethyleneglycol) (PEG) (Bayramoglu and Arica, 2014), are often used to provide a hydrogel base. The capacity of these materials for immobilized enzymes is increased by crosslinking, co-polymerization, or functionalization (Bayramoglu and Arica, 2014; Tang et al., 2014; Sun et al., 2015). However, an immobilized enzyme in a hydrogel is prone to leaking out of the gel because the enzyme is typically simply encapsulated within the small pores of the gel. Functional hydrogels that address this issue have recently been demonstrated and use functional building blocks such as protein (Guan et al., 2013; Kim et al., 2013; Ramirez et al., 2013). For example, a multimeric protein was used as a bridge site in a hydrogel backbone in order to utilize the protein's function. The artificial protein hydrogels use the trimeric protein CutA as a cross-linker. CutA and a self-assembling polypeptide co-polymer were mixed and formed a hydrogel that exhibited high stability in solutions at a wide range of pH values and temperatures (Guan et al., 2013; Ramirez et al., 2013). In addition, these protein hydrogels contain a motif for immobilizing enzymes and are therefore used as a scaffold for immobilizing enzymes. Another approach involves direct self-assembly and hydrogelation using three self-assembling multimeric dehydrogenases that form a hydrogel that, coated on an electrode, is useful in enzymatic methanol fuel cells (Kim et al., 2013). An alpha-helical leucine zipper domain and a random structure soluble peptide domain were genetically introduced into each dehydrogenase to promote heteromolecular self-assembly. The hydrogel-coated electrodes in the fuel cells provided power and current densities comparable to those previously reported for similar enzyme cascade systems (Palmore et al., 1998). These kinds of protein hydrogel retain high activity due to encapsulation of the enzyme by self-assembling blocks and protein blocks that act as a scaffold to immobilize the enzyme. An alternative approach is hydrogels constructed by direct self-assembly of the enzyme of interest.

* Corresponding author.
E-mail address: tanaka@kitty.kobe-u.ac.jp (T. Tanaka).

http://dx.doi.org/10.1016/j.bios.2017.07.044
Received 20 April 2017; Received in revised form 14 July 2017; Accepted 18 July 2017
Available online 19 July 2017

Here, we demonstrate a streptavidin (SA)-based hydrogel inspired by the former strategy. SA is a tetrameric protein and is used as a bridge site in the hydrogel backbone. In addition, SA has four biotin-binding pockets that can be used to immobilize other molecules, including enzymes of interest (Green, 1990). We therefore anticipated that SA would be the ideal protein building block for constructing a protein hydrogel as it would act both as a bridge site and an immobilization site. Four-branched PEG was chosen to form the backbone of the SA-hydrogel. Biotin-binding by SA was retained by using a methodology for self-assembling SA and PEG without disrupting the biotin-binding pockets of SA: specifically, sortase A (SrtA)-mediated protein ligation, and a bio-orthogonal chemical reaction for self-assembling SA and PEG. Sortase A from *Staphylococcus aureus* is a transpeptidase that sorts enzymes for displaying secreted proteins on the cell surface (Mazmanian et al., 1999). SrtA recognizes the C-terminal LPXTG motif of a protein, and subsequently links a glycine oligomer to the C-terminus through a native peptide bond. This ligation reaction has been used to prepare a variety of bioconjugates (Mao et al., 2004), to immobilize proteins (Ito et al., 2010; Hata et al., 2015), and to image cells (Tanaka et al., 2008). Although SrtA-mediated ligation is an attractive approach for tethering two different molecules, active SrtA is typically retained within the system after the SrtA-mediated reaction, thus requiring another step to remove SrtA (Witte et al., 2015). This complication can be addressed by combining SrtA-mediated ligation with bio-orthogonal cross-linking. To avoid the need for an extra step, target proteins are pre-modified with the bio-orthogonal group using SrtA-mediated site-specific ligation (Haridas et al., 2014). Click chemistry is a versatile bio-orthogonal reaction involving a cycloaddition between an azido group and a terminal alkyne (Bernardin et al., 2010). Click handles can be site-specifically incorporated into target molecules using site-specific SrtA-mediated ligation (Witte et al., 2013; Haridas et al., 2014; Wang et al., 2016b).

Herein, we prepared a SA-based hydrogel by SrtA-mediated click ligation. SA with a genetically-appended LPETG tag (SA-LP) was site-specifically tethered to an azido-GGG peptide using sortase A. The resulting azido-modified SA (SA-N3) was self-assembled with dibenzylcyclooctyne-modified branched PEG (DBCO-PEG) and azido-modified branched PEG (N3-PEG) as a spacer via copper-free click chemistry. The resulting SA-based hydrogel exhibited gel-like mechanical properties and could immobilize biotin-modified molecules through biotin-SA affinity. Here, the application of this hydrogel was demonstrated by immobilizing enzyme in the SA-based hydrogel, then coating this gel on a glassy carbon electrode (GCE). GDH was chosen as a model of immobilizing enzyme, which has been utilized for constructing glucose fuel cell (Willner et al., 2009). The prepared GCE exhibited biocatalytic activity (glucose oxidation). This SA-based hydrogel provides a new way of immobilizing an enzyme of interest on a bioelectrode, and will also be useful for other biological applications.

2. Materials and methods

2.1. Materials

Four-branched PEG SUNBRIGHT PTE-200PA (MW 20,000 Da) was purchased from YUKA SANGYO CO., LTD. (Tokyo, Japan). Dibenzylcyclooctyne (DBCO)-Fluor545 was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). N3-NHS-Ester (azido-dPEG$_4$-*N*-Succinimidyl Ester ester) was purchased from QUANTA BIODESIGN, LTD. (Montgomery, AL, USA). DBCO-NHS-Ester was purchased from Jena Bioscience GmbH (Thüringen, Germany). EZ-Link Sulfo-NHS-LC biotin was purchased from Thermo Fisher Scientific Inc. (Kanagawa, Japan). H-GGG-K(N3)-OH*HCl was purchased from Iris Biotech GmbH (Marktredwitz, Germany). PQQ-dependent glucose dehydrogenase was purchased from Toyobo Co., Ltd. (Osaka, Japan). KOD FX DNA polymerase, pCold IV vector, TALON metal affinity resin, a BCA protein assay kit, and *Escherichia coli* BL21(DE3) strain were purchased from Takara Bio Inc. (Shiga, Japan). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Protein expression and purification

KOD FX DNA polymerase was used for the polymerase chain reaction (PCR). The gene encoding SA-LP was obtained by PCR using pColdI-SA-LPETG (Matsumoto et al., 2011) as the template with 5′-AGGTAATACCATATGATGAATCACAAAGTGCATCATCATCATCATCA-TGCCGAGGCCGGCATCACCGGC -3′ as the 5’ primer and 5′-ATTTACCTATCTAGACTAGCCGCCTGTTTCTGGCAAGGAGGCGGCG-GACGGCTTCAC-3′ as the 3′ primer. The amplified fragment was subcloned into the *NdeI*/*XbaI* sites of the pCold IV vector to yield pCold IV-SA-LP, which was then introduced into *Escherichia coli* BL21 (DE3). Cells were grown in Luria-Bertani medium at 37 °C to an optical density (OD; 600 nm) of 0.5, and then the cells were incubated for an additional 30 min at 15 °C. Protein expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.5 mM. After growth for an additional 24 h at 15 °C, the cells were harvested by centrifugation. The cell pellets were resuspended in 20 mM phosphate buffer (pH 8.0) containing 150 mM NaCl and then lysed using sonication. SA-LP was purified from the soluble fraction using TALON metal affinity resin according to the manufacturer's protocol and then dialyzed against 10 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl. Additionally, His_6 tag appended SrtA (His_6-SrtA) was expressed and purified according to a previous report (Matsumoto et al., 2011). The concentration of purified proteins was determined using a BCA protein assay kit.

2.3. Preparation of azido-modified streptavidin

The modification reaction was performed in 50 mM Tris–HCl, 150 mM NaCl, 10 mM $CaCl_2$, containing 0.003 mM His_6-SrtA, 0.03 mM Stav-LPETG, and 0.6 mM H-GGG-K(N3)-OH*HCl for 2 h at 37 °C (pH 7.5, up to 600 μL). The unreacted H-GGG-K(N3)-OH*HCl and His_6-SrtA were removed by centrifugation in a viva spin 30 K unit (30 kDa molecular weight cutoff). To verify the introduction of the azido group, the resulting SA-N3 solution was reacted with DBCO-Fluor545 (2 mM, 1 μL). The mixture was then mixed with Sodium dodecyl sulfate-Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) sample buffer (50 mM Tris–HCl, 2% SDS, 6% 2-mercaptoethanol) and the samples were subjected to SDS-PAGE. The gels were stained with Coomassie brilliant blue R-250 (CBB) or analyzed using an LAS imager.

2.4. Construction of streptavidin-based hydrogel

The azido or DBCO group was modified with PEG by reacting N3-NHS or DBCO-NHS with four-branched amino-PEGs. Four-branched amino-PEG had four amino groups on termini, and amino-reacting NHS group was utilized for introducing N3 group or DBCO group in four termini of four-branched amino-PEG. SA-N3 solution (32 μM, 100 μL), N3-PEG (167 mg mL^{-1}, 30 μL) and DBCO-PEG (167 mg mL^{-1}, 30 μL) were mixed for one hour at RT to form the hydrogel. Rheometer measurements were performed using a Paar-Physica MCR-301 parallel plate rheometer (Anton Paar, Ashland, VA, USA) with a 25 mm plate fixture (PP25).

2.5. Preparation of the bioanode and biofuel cell

Biotinylated GDH was prepared by mixing PQQ-GDH (80 μM) and NHS-biotin (400 μM) for 2 h at RT. Unreacted NHS-biotin was removed by centrifugation using a viva spin 30 K unit, then the SA-N3 solution and the biotinylated (or non-biotinylated) GDH (two times of the stoichiometric molar equivalence of SA-N3) were added and