Journal of Colloid and Interface Science 527 (2018) 117–123

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

Supramolecular proteinaceous biofilms as trapping sponges for biologic water treatment and durable catalysis

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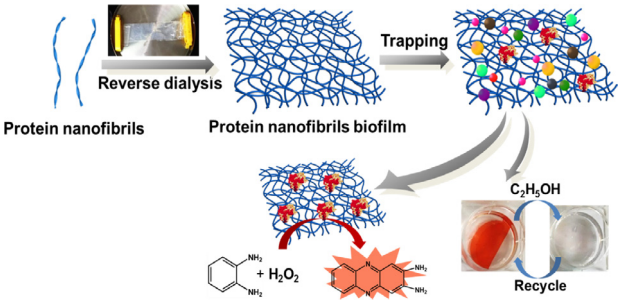
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 April 2018
Revised 9 May 2018
Accepted 14 May 2018
Available online 15 May 2018

Keywords:
Biofilm
Protein nanofibrils
Trapping foam
Ions adsorption
Catalytic activity

ABSTRACT

Inspired by the bacterial biofilms and chorions of living organisms which are made by proteinaceous assemblies and functional for multi-applications, various artificial protein fibrils-based nanoporous films are developed, and show their potential applications in multiple fields. Here, a simple and environmental friendly method was identified to produce bovine serum albumin (BSA) nanofibrils based biofilms, through a combination of protein fibrillation and reverse dialysis. BSA nanofibrils formed biofilms through intermolecular interactions, the resultant biofilms showed tunable thickness by altering the initial protein amount, good stability in organic and salty solvents, transparency and fluorescence properties, hold high capacity of trapping different substances (e.g. nanomaterials, organic dyes, heavy-metal ions and enzymes), and further enabled applications in biologic water treatment and enzyme stabilization. Taken o-phenylenediamine as substrate, the trapped horseradish peroxidase showed a catalytic activity 9–38 folds higher than free ones in organic phase, together with enhanced stability. These protein nanofibrils-based films offered an attractive biologic platform to hybridize diverse materials for on-demand functions and applications.

1. Introduction

Many bacteria secrete proteinaceous assemblies into hydrated biofilms and further accommodate diverse bio-macromolecules for invasion, colonization and protection from various physical and chemical stresses [1]. For example, curli fibrils, known as a class of ordered proteinaceous assemblies, make up ~80% of the biofilms of *Escherichia coli* and *Salmonella enterica*, and are able to combine a variety of functional exopolysaccharides and proteins [2]. Curli fibrils are structurally characterized by a typical amyloid architecture, in which repeating β-strand units orient perpendicular to the fibril axis. Besides bacterial biofilms, protein fibrils (e.g., amyloid) were also identified as the majority components of the

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https://doi.org/10.1016/j.jcis.2018.05.036
0021-9797/

protection chorions of silkmoth and killifish *Austrofundulus limnaeus*, and conserved over 200 types of functional proteins. This tightly ordered packing of cross "β-sheets" confers good physical stability, mechanic robustness and proteolytic degradation to biologic films [3]. As a matter of fact, in nature a broad range of proteins and peptides were found to assemble into fibrious structures associated either with neurodegenerative disorders or biological functions in living organisms [4,5]. Inspired by bacterial biofilms and the chorions of living organisms, protein fibrils may be promising to construct artificial biofilms and target specific functions by introducing different molecules or nanomaterials.

Technically nearly all types of proteins (e.g. zein [6], wheat gluten [7], lysozyme [8,9], β-lactoglobulin [10,11] and silk fibroin [12,13]) and peptides (e.g. $\alpha\beta$(1–42) [14]) could self-assemble into nanofibrils under certain environmental conditions [15]. With well-defined structural architecture, high aspect ratio, good mechanical performance, biodegradability and biocompatibility, these fibrils showed great potential as the reducing agents of metal nanomaterials [16], adhesive [17], and particularly the building blocks of biologic films applicable in tissue engineering, sensing, pigments and cosmetics, while methods to prepare protein nanofibrils-based nanoporous films usually include casting, filtration, spin coating, and self-assemble [3,18–20]. For example, lysozyme nanofibrils were attempted to produce anisotropic films with modulus of ~6.2 GPa via a conventional liquid-casting process [19], fibrous assemblies of silk were regulated precisely and engineered into ultrathin separation films through a vacuum-filtration process [18].

Moreover, protein fibrils also showed their superiority in combining different substances for specific applications, as the chemical accessibility of functional groups at the biomolecule surface provides possibilities for numerous binding sites. For example, β-lactoglobulin fibrils combined with nanomaterials (e.g. graphene [10], apatite [21], Au nanosheets [16] and activated carbon[22]) into composite films through vacuum-filtration. As cellular scaffolds, fibrils of amyloidogenic fragment 2097–2108 from the α1 chain of mouse laminin attached active biomolecules to control cell growth, proliferation and differentiation [23]. Protein fibrils also served as carriers for viruses and nanoparticles (e.g. iron nanoparticles [24]) as well as controlled drug release [3]. Furthermore, the adsorbed substance on the protein membranes could undergo diverse secondary reactions to build membranes with multiple designed functions, e.g. amino acids as natural reduction sites could reduce the absorbed metal ions to inorganic nanoparticles for further catalytic applications [22,25].

Based on these, in this study, an innovative and simple method to prepare protein nanofibrils-based nanoporous biofilms was presented, through a combination of protein fibrillation and reverse dialysis of bovine serum albumin (BSA). BSA nanofibrils formed biofilms through intermolecular interactions, the biofilms were free-standing, biodegradable and nontoxic, with tunable thickness, high transparency, endurance and large area. The biofilms showed high capacity as multifunctional trapping foams for different substances, e.g. nanomaterials, organic dyes, metal ions and enzymes. Their powerful trapping ability offered a good opportunity of functionalization for multiple applications, e.g. in biologic water treatment, thermal sensing, recycling ions of noble metals and improved catalytic activity.

2. Experimental section

2.1. Materials

Bovine serum albumin (BSA, purity > 96%) was bought from Sangon Biotech (Shanghai) Co., Ltd. The cellulose dialysis bag (1000 Da cut-off dialysis membrane) and Proteinase K (50 u/mg) were bought from Solarbio Science & Technology (Beijing) Co., Ltd. Horseradish peroxidase (HRP, Rz > 1.5) was purchased from Majorbio Biotech Company, USA. 4-Aminoantipyrine (purity > 98 %) was bought from East China Normal University Chemical Factory. α-Zein was obtained from Tokyo Chemical Industry Co., Ltd.. Tetrachloroauric acid ($HAuCl_4$, 48% Au basis) was purchased from Sigma. Other reagents were obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. All solutions were prepared by ultra-pure water (resistivity: 18.2 MΩ cm^{-1}).

2.2. Fibrillation of BSA

Typically [26], 1.0 g BSA protein was dissolved in 40 mL of 50 mM phosphate buffer (pH 7.4) to get a clear solution. After adding 60 mL of ethanol, the solution (1 wt% of BSA) was incubated at 65 °C for 24 h and then at room temperature for 2 days to get the BSA nanofibrils suspension.

2.3. Reverse dialysis

For the preparation of biofilms with thickness of 95 μm, 30 mL of the BSA fibrils suspension (1 wt%) was sealed in a cellulose dialysis bag (1000 Da cut-off), then dialyzed against a 20% (w/v) aqueous solution of polyethylene glycol (20 kDa) at 25 °C for 10 h. Without further specification, the dialysis bag was 20 cm in length, and 4.4 cm in width. The BSA biofilm formed in the dialysis bag after completely reverse dialysis. By adding H_2O directly into the dialysis bag, the biofilm was easily washed out without collapse. In order to remove free molecules and nanofibrils, the resultant biofilm was immersed and washed alternately by H_2O and 60% (v/v) ethanol for three times, then kept in H_2O at 4 °C. All the mentioned applications (e.g. trapping capacity, hybridization, and catalytic activity) were performed using the hydrated biofilms directly.

2.4. Evaluation of adsorption capacity

The biofilm (formed with 30 mL of the BSA fibrils suspension, thickness of 95 μm) was cut into circular ones using a circular mold (diameter of 2.4 cm). A circular biofilm (thickness of 95 μm, and diameter of 2.4 cm) was immersed in the solutions of dyes (1 mM, 4 mL), metal ions (10 mM, 3 mL), enzymes (0.5 mg/mL, 4 mL) and nanomaterials (0.25 mg/mL, 4 mL), respectively, for 2 h at room temperature. After the adsorption, the biofilms were taken out using tweezers, the solutions were collected to determine the adsorption capacity, by taking away the amount of unbound materials (dyes, enzymes, nanomaterials) in the solutions from the initial amount of materials added. The adsorption capacity was evaluated by UV–vis spectrophotometer (for dyes, enzymes and nanomaterials) and inductively-coupled Plasma Optical Emission Spectrometer (for metal ions), 3 parallel experiments were conducted simultaneously to calculate the average adsorption capacity.

2.5. Trapping and reducing ions of noble metals into biofilms

Briefly, a circular BSA biofilm (95 μm of thickness, 2.4 cm of diameter) was immersed in 3 mL of $HAuCl_4$ or $AgNO_3$ (10 mM) for 2 h, respectively. The ions-rich biofilms were then incubated at 60 °C for 6 h to get the hybrid films.

2.6. Evaluation of catalytic activity of enzymes in aqueous phase

The relative activity of enzymes was evaluated through the phenol removal efficiency combined with a colorimetric assay.