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Preparation of high-performance membrane adsorbers by surface-initiated AGET ATRP in the presence of dissolved oxygen and low catalyst concentration

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ABSTRACT

This contribution describes the first use of surface-initiated AGET (activators generated by electron transfer) ATRP (atom transfer radical polymerization) to prepare high-capacity adsorptive membranes for bioseparations. The present advancement greatly simplifies membrane development by overcoming the need for specialized catalyst handling and solution de-oxygenation steps that are required for standard ATRP methods. Furthermore, it enables modification to be done with ppm-level catalyst concentrations. Surface-initiated AGET ATRP from base cellulose membranes was used to prepare amine-based anion-exchange membranes. No precautions were taken to remove oxygen from the system, and catalyst concentrations were 50–200 ppm. A set of experiments was carried out to optimize the molar amount of reducing agent used in formulation. Catalyst concentration and polymerization time were used as independent variables to study the effects on protein binding capacity of the surface-modified membranes. Bind-and-elute measurements were done with an adsorptive membrane bed to measure dynamic protein binding capacities and to demonstrate the highly favorable transport properties of the membrane bed.

1. Introduction

Membrane adsorptive chromatography is a high-throughput alternative to resin column chromatography for protein purification [1–8]. Despite the many advantages of membrane chromatography, it has not been implemented broadly for the initial protein capture step due to the historically low volumetric capacities of membranes [1,4,5,8]. Thus, development of membranes with higher binding capacities is an active area of research.

Building adsorptive functionality into membranes by coating [9–12] or graft polymerization [13–19] is one strategy to increase binding capacities. While graft polymerization produces a large number of binding sites, control over the modification is needed to avoid pore blocking and hindered transport of large adsorptive compounds, such as proteins. Surface-initiated ATRP allows relatively fine and independent control over chain density and degree of polymerization for polymer chains grafted from the surface of base membranes [13–17]. Our recent work [14,15,19] has demonstrated many opportunities and advantages of surface-initiated ATRP for preparing membrane adsorbers.

ATRP is a redox-initiated polymerization reaction. The reaction is catalyzed by an organometallic catalyst complex comprising a transition metal coordinated to a ligand. The transition metal is susceptible to reaction with oxygen or other oxidizers, which promotes the metal to a higher oxidation state that cannot initiate the ATRP process. Therefore, to prepare surface-modified membranes with consistent performance properties, the preformed catalysts must be stored under an inert atmosphere, and experimental precautions are needed to maintain an oxygen-free environment throughout the process. Dissolved oxygen must be removed from solution prior to ATRP catalyst addition. The preparation of catalyst also must be done in a de-oxygenated solvent and under an oxygen-free environment to avoid the oxidation of catalyst. Therefore, the process of catalyst handling can be challenging and may become impractical at the industrial scale.

The present advancement overcomes these obstacles by forming the active catalyst in situ and removing dissolved oxygen by addition of a reducing agent into the solution. We use surface-initiated AGET (activators generated by electron transfer) ATRP, developed only recently by Matyjaszewski and coworkers [20–23]. In this method, the activating catalyst species Cu(I) is (re)generated from the oxidized Cu(II) form by electron transfer using environmentally acceptable reducing agents such as ascorbic acid (vitamin C) [24]. The reducing agent can be used in situ for reduction of Cu(II) to Cu(I) without formation of radicals in solution. The advantages of AGET ATRP over traditional ATRP are these: (i) catalyst concentration can be reduced significantly (down to 5–100 ppm) since the activating catalyst species can be regenerated

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continuously [22]. (ii) Polymerization can begin with the oxidatively stable Cu(II) species. (iii) It can tolerate headspace oxygen in the reaction chamber, and, therefore, does not require de-oxygenation of reaction mixtures or specialized preparatory equipment. Since no special de-oxygenation steps are needed prior to polymerization, the modification procedure is simplified greatly.

The objective of this research was to design a surface-initiated AGET ATRP protocol for membrane adsorber development [25]. Surface-initiated AGET ATRP from base cellulose membranes was carried out in sealed vials to prepare anion-exchange membranes for membrane chromatography. No precautions were taken to remove oxygen from the system, and ppm-level catalyst concentrations were used. A set of experiments was carried out to optimize the molar amount of reducing agent used in formulation. Catalyst concentration and polymerization time were used as independent variables to study the effects on protein binding capacity of the surface-modified membranes. Bind-and-elute measurements were done with an adsorptive membrane bed to measure dynamic protein binding capacities and to demonstrate the highly favorable transport properties of the membrane bed.

2. Experimental

2.1. Materials

Regenerated cellulose membranes (RC 60) with 70 μm thickness, 47 mm diameter, and 1.0 μm average effective pore diameter were purchased from Whatman, Inc. The following chemicals and solvents were purchased from Sigma–Aldrich, with purities given in wt.%: albumin from bovine serum (further purified fraction V, ~99%, $M_r \sim 66$ kDa), L-ascorbic acid (>99%), 2-bromoisobutyryl bromide (2-BIB, 98%), copper(II) chloride (99.999%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%), ethanol (anhydrous, ≥99.5%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%), hydrochloric acid (HCl, ACS reagent, 37%), methanol (≥99.9%), neutral activated aluminum oxide, 2-propanol (≥99.8%), sodium chloride (NaCl, >99.5%), sodium hydroxide (≥98%), tetrahydrofuran (THF, anhydrous, ≥99.9%), triethylamine (TEA, ≥99%), tris(hydroxymethyl)aminomethane (Tris–base, ≥99%), and water (ACS reagent grade, HPLC). Prior to polymerization, DMAEMA was passed through a column of the aluminum oxide to remove inhibitor compounds.

Serum vials, rubber stoppers, aluminum caps and vial crimper were purchased from Voigt Global Distribution Inc. Loading buffer A (20 mM Tris–base, adjusted to pH 7.8 with HCl) and elution buffer E (1 M NaCl in buffer A) were used for protein binding capacity measurements. Buffers were prepared using distilled water that had been passed through a Milli-Q® Ultrapure (Millipore, Bedford, MA) purification system. All buffers were degassed by ultrasonication immediately prior to use.

2.2. Membrane surface modification

2.2.1. Surface modification of regenerated cellulose membranes was carried out in two steps

In the first step, RC60 membranes were activated with ATRP initiator groups using a protocol described in detail previously [14,15]. AGET ATRP was used in the second step to graft poly(DMAEMA) from the initiator sites. A typical polymerization procedure follows. Monomer, DMAEMA, was added to the solvent, 2-propanol, to prepare a 2.0 M monomer stock solution. A catalyst was formed in situ by addition of copper(II) chloride (50 or 200 ppm) and amine ligand, HMTETA (100 or 400 ppm), to the monomer solution. Next, this mixture was placed on a magnetic stir plate for 15 min until it became homogeneous, indicating the formation of a fully soluble catalyst complex. An initiator-activated membrane was placed inside a 40 mL serum glass vial and 35 mL of the polymerization solution was added. The glass vial was sealed using a rubber stopper and aluminum crimp cap. No precautions were taken to remove headspace oxygen or dissolved oxygen. The reducing agent, ascorbic acid, was dissolved into 2-propanol solvent using a second serum glass vial. This vial was placed into an ultrasonic bath for 15 min until ascorbic acid was dissolved. A known volume of the ascorbic acid solution was injected by syringe into the serum glass vial containing the membrane and polymer solution. The temperature was raised to 40 ± 2 °C by placing this vial into a constant temperature shaker bath. Polymerization was terminated by removing the membrane from the reaction mixture, and washing it thoroughly with methanol and HPLC water.

In a first set of experiments, the concentrations of the reducing agent and catalyst were varied at constant polymerization time and temperature. In the second set of experiments, polymerization time was used as the independent variable for one catalyst/reducing agent formulation.

2.3. Protein binding capacities of modified membranes

Bovine serum albumin (BSA) was used as model protein to measure static protein adsorption capacities. BSA was dissolved in a loading buffer A to prepare 1.0 and 3.0 mg/mL protein solutions. Prior to use, the solutions were filtered through disposable cellulose acetate syringe filters with 0.2 μm pore diameter (Puradisc 30, GE Healthcare Bio-Sciences) to remove protein aggregates. Breakthrough curves at two different volumetric flow rates were generated to measure the membrane dynamic binding capacities.

2.3.1. Static binding capacity

An anion-exchange membrane (47 mm dia.) was placed in a 40 mL glass bottle (I-Chem*short, wide-mouth, Fisher Scientific) with 10 mL of 3.0 mg/mL BSA solution for 20 h to reach equilibrium protein binding in a shaker bath at 22 ± 1 °C. Binding capacities were calculated using the mass balance method described previously [15].

2.3.2. Dynamic binding capacity

Dynamic binding capacity measurements were done using an ÄKTA Purifier 100 chromatography system (GE Healthcare Bio-Sciences, Uppsala, Sweden). Membranes that had been surface modified with anionic polyelectrolyte were cut into small diameter (16 mm) discs and equilibrated with 20 mL of loading buffer A prior to loading them into a membrane holder. A stack of 7 membrane discs was placed between sheets of filter paper (Grade-6, 3 μm pore size, Whatman Inc.,) and loaded into a Mustang® Coin Unit (Pall Corporation, Port Washington, NY) to prepare a membrane adsorber. Each run started with passage of 20 column volumes (CVs) of loading buffer to equilibrate the membrane adsorber bed. Next, 1.0 mg/mL BSA solution was delivered. Following complete loading, the bound BSA was eluted with elution buffer E until a stable baseline was observed with UV detection. After every run, the membrane bed was cleaned and regenerated with 5 CVs of 0.5 M sodium hydroxide solution, followed by 20 CVs of 1 M NaCl solution, and finally rinsed with 20 CVs of loading buffer to prepare the bed for the next run. The system dead volume and dynamic binding capacities were calculated at 10% breakthrough and 50% breakthrough using a method described previously [19].

3. Results and discussion

Husson and coworkers [14,15,17,19] have demonstrated that surface-initiated ATRP can be used to prepare ion-exchange