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Novel affinity membranes with macrocyclic spacer arms synthesized via click chemistry for lysozyme binding

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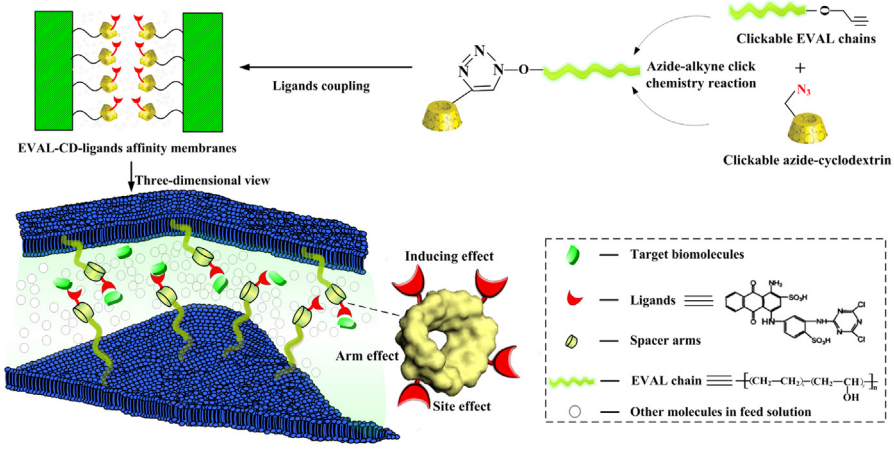
HIGHLIGHTS

- Macrocyclic spacer arms has been proposed for high performance affinity membrane.
- Inducing effect, arm effect and site effect render the enhanced performance.
- Effective binding behavior for lysozyme has been confirmed.

GRAPHICAL ABSTRACT



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ABSTRACT

Affinity membrane has great potential for applications in bioseparation and purification. Disclosed herein is the design of a novel affinity membrane with macrocyclic spacer arms for lysozyme binding. The clickable azide-cyclodextrin (CD) arms and clickable alkyne ethylene-vinyl alcohol (EVAL) chains are designed and prepared. By the azide-alkyne click reaction, the EVAL-CD-ligands affinity membranes with CD spacer arms in three-dimensional micro channels have been successfully fabricated. The FT-IR, XPS, NMR, SEM and SEM-EDS results give detailed information of structure evolution. The abundant pores in membrane matrix provide efficient working channels, and the introduced CD arms with ligands (affinity sites) provide supramolecular atmosphere. Compared with that of raw EVAL membrane, the adsorption capacity of EVAL-CD-ligands membrane (26.24 mg/g) show a triple increase. The study indicates that three effects (inducing effect, arm effect, site effect) from CD arms render the enhanced performance. The click reaction happened in membrane matrix in bulk. The effective lysozyme binding and higher adsorption performance of affinity membranes described herein compared with other reported membranes are markedly related with the proposed strategy involving macrocyclic spacer arms and supramolecular working channels.

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1. Introduction

The rapid development of biotechnology requires more efficient separation techniques. The traditional column chromatography is usually carried out by packed beds, which have several major limitations such as high pressure drop, relatively slow intra-bead mass transport, difficulty in column packing and complicated scale up procedures, etc [1,2]. During recent years, affinity membrane has emerged as a promising alternative and gained growing attention. During membrane filtration, the ligands in affinity membrane matrix can bind the desired biomolecules in feed solution by specific adsorption, and such process exhibits short diffusion path, low pressure drop, easy online operation, etc [3–7]. As a competitive way for the quick separation and purification of biomolecules, the attractive properties of affinity membrane are related with the considerable microporous structure in membrane matrix, as well as the specific interactions between the ligands and target species. The target molecules such as bovine serum albumin (BSA) protein [8], lysozyme (LZY) [9], monoclonal antibody [10], human immunoglobulin (HIgG) [11], lactoferrin [12], viruses [13], tryptophan [14], DNA [15], etc., have been reported.

However, the performance of affinity membrane is still far from satisfactory for large-scale application [16,17]. How to increase the adsorption performance has become the most critical problem in the field of affinity membrane. To tackle this challenging issue, many strategies have been implemented, such as designing new ligands, development of membrane modules, optimizing operating conditions, new coupling methods and designing functional surface, etc [18–21]. For example, the glucose ligands were reported to be bound on the nanofiber for glycosylated surface of affinity membrane, which showed the merits of large surface area and high porosity as well as good specificity with lectin [22]. And a variety of matrix materials including polyvinylidene fluoride, cellulose and its derivatives, chitosan, polyamide and its derivatives, polysulfone and its derivative, modified polyethylene, polyethylene, polypropylene, ethylene vinyl alcohol and polyvinyl alcohol, etc., have been developed [14,17]. Particularly, the considerable research focuses on the selection and design of ligands since the membrane performance is significantly influenced by the specific affinity function [23,24]. A variety of types of ligands, such as protein G, antigen and antibody ligands, dye ligands and ion exchange ligands, have been developed in order to obtain higher separation efficiency [25,26]. Dye ligands have been considered as one of the important alternatives to natural counterparts for specific affinity chromatography. Dye ligands are able to bind most types of proteins. They are commercially available, inexpensive, and can easily be immobilized, especially on matrices bearing hydroxyl groups. Despite all the efforts, the problem is still not completely solved. In fact, the effective affinity role between the ligands (even those inexpensive dye ligands) and biomolecules has been extensively proved [27]. We hold the opinion that the challenge for efficient adsorption is how to assure the adsorption sites (ligands) working in an efficient way. Traditionally, the ligands in membrane matrix work in an inert or waiting manner, which is unfavourable for the executive ability to be exploited to the largest extent.

Here, we present a new strategy based on macrocyclic compound with specific supramolecular structure. It is well known that the cyclodextrin, which is a typical supramolecular host with macrocyclic architecture, which has inclusion and inducing effect for the guest molecules [28,29]. During recent years, there are a few reports on the spacer arms such as diaminooctane [30,31], diaminohexane [32], diglycidyl ether [33,34], 3,6-dioxa-1,8-octanedithiol, triazole ring [35], etc. As far as we know, the report about the macrocyclic arms for affinity membrane is quite insufficient.

In this work, the cyclodextrins are introduced into membrane matrix as spacer arms, which are expected to show three effects (inducing effect, arm effect, site effect) for the enhanced membrane performance. The cyclodextrin arms and ligands will be introduced into the EVAL membrane matrix based on click chemistry, which is extremely selective and high yielding while utilizing very mild reaction conditions, and has become efficient way for material modification [36–40].

Fig. 1 shows a schematic diagram of the formation strategy of EVAL-CD-ligands affinity membranes with CD spacer arms. The clickable azide-cyclodextrin arms (β-CD-N_3) and clickable alkyne-EVAL (EVAL-alk) chains are designed and prepared based on molecular design. By the azide-alkyne click chemistry reaction, the CD spacer arms are introduced into the EVAL membrane matrix. After ligands coupling process, the EVAL-CD-ligands affinity membranes with three-dimensional micro channels are fabricated. The abundant pores in membrane matrix provide three dimensional pathway and channels for efficient work, and the CD arms with ligands (adsorption sites) provide supramolecular atmosphere. Such supramolecular atmosphere from CD arms can result in three effects (inducing effect, arm effect, site effect) for an expected enhanced membrane performance, which will be discussed later. The molecular structure and morphology will be investigated based on FT-IR, XPS, NMR, SEM and SEM-EDS results. Based on the detailed analysis on microporous channels and adsorption performance, the effects of CD arms will be discussed.

2. Experimental

2.1. Materials

Ethylene vinyl alcohol was from Kuraray Chemicals Manufacture. Polyethylene glycol was dried under vacuum overnight at room temperature prior to use from Tianjin Kemiou Chemical Reagent Corporation. The α-Cyclodextrin, β-Cyclodextrin, Dimethyl sulfoxide, Reactive dye, Dimethylformamide, Triethylamine, Toluenesulfonyl chloride, Methylene chloride and 3,5-dimethylphenol was obtained from Sinopharm Chemical Reagent Corporation. Other reagents were from Sinopharm Chemical Reagent Corporation. All chemicals used were of analytical reagent grade and used without further purification.

2.2. Preparation of EVAL-CD membranes

The experimental details on the preparation of clickable alkyne-EVAL (EVAL-alk) and clickable azide-cyclodextrin arms (β-CD-N_3) are provided in Supplementary data. To give an efficient working place, the porous EVAL-CD membranes were prepared by click chemistry and subsequent phase inversion technique. The experimental details on the fabrication of EVAL-CD-ligand membranes are provided in Supplementary data.

2.3. Characterization and measurement

A field emission scanning electron microscope (FESEM, Hitachi S-4800) was used for the morphology characterization. The surface morphologies and roughness of membranes were analyzed by an atomic force microscopy (Veeco, NanoScope IIIa Multimode AFM). Roughness was obtained through the software Nanoscope. The infrared spectra of membranes were presented by BRUKER VECTOR-22 FT-IR. All NMR spectra were obtained on a Bruker AM-300 spectrometer. Sample concentrations were about 0.7% (w/v) in