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PolyAdenine cryogels for fast and effective RNA purification

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ABSTRACT

Cryogels are used effectively for many diverse applications in a variety of fields. The isolation or purification of RNA, one of the potential utilizations for cryogels, is crucial due to their vital roles such as encoding, decoding, transcription and translation, and gene expression. RNA principally exists within every living thing, but their tendency to denaturation easily is still the most challenging issue. Herein, we aimed to develop adenine incorporated polymeric cryogels as an alternative sorbent for cost-friendly and fast RNA purification with high capacity. For this goal, we synthesized the polymerizable derivative of adenine called as adenine methacrylate (AdeM) through the substitution reaction between adenine and methacryloyl chloride. Then, 2-hydroxyethyl methacrylate (HEMA)-based cryogels were prepared in a partially frozen aqueous medium by copolymerization of monomers, AdeM, and HEMA. The cryogels were characterized by using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), surface area measurements, thermogravimetric analysis (TGA), and swelling tests. RNA adsorption experiments were performed via batch system while varying different conditions including pH, initial RNA concentration, temperature, and interaction time. We achieved high RNA adsorption capacity of cryogels, with the swelling ratio around 510%, as 11.86 mg/g. The cryogels might be reused at least five times without significant decrease in adsorption capacity.

1. Introduction

Ribonucleic acid (RNA) is found in nature where it plays multiple important roles such as encoding, decoding, regulation and gene expression widely for life [1]. Together with deoxyribonucleic acid (DNA), RNA consists of all nucleic acids required for all known forms of life in its structure. Although DNA is responsible for heredity, some viruses, called as RNA viruses, also have genomic RNA encoding a protein sequence to move to a new host cell [2]. The viroid is the other group of pathogens, but they only consist of RNA, not code for any protein and are amplified by the polymerase of the host plant cell [2]. Moreover, the functions of RNA in the gene regulation are very remarkable for therapeutic potential [3]. Accordingly, RNA has a potential for developing new therapeutic agent and targeting the production of several RNA-based therapeutics [4]. Especially in criminal cases, very few amounts of DNA or RNA residues of these samples give the direction to reconstruct the events occurring in the crime field. Determination of this few RNA with high accuracy is quite vital for the origins of crime [5].

Due to these properties of RNA, the complete understanding of the important roles of RNA is very crucial for the diagnosis and treatment of many diseases or disorders, especially cancer, which trigger the attention on developing new materials and methods [6,7]. The standard methods used in the RNA preparation have some drawbacks. The removal of transcription end products becomes more difficult with increasing size of target RNA. Moreover, some techniques (i.e. denaturing PAGE purification) are time-consuming (2 or 3-day protocol) and not proper to apply for larger amounts of RNA analysis [8]. Sometimes misfolding of desired RNA might be observed at the end of the "heat/cool" techniques [9]. Separation processes may also adversely affect the use of RNA for the specific application such as, in particular, a UV shadowing visualization of RNA in the polyacrylamide gel and single molecule spectroscopy [10,11]. The most leading prerequisite for structural and functional analysis studies is the purity of the target molecule to be derived [12]. Advanced/hyphenated chromatographic techniques with high separation capacity are surpassing the conventional

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separation techniques, which depend on biological and physicochemical properties on gel filtration chromatography, ion exchange chromatography, affinity chromatography, and hydrophobic interaction chromatography [13,14]. Affinity chromatography is a fundamental method for diagnosis, isolation, separation and purification of biological molecules and on a highly specific molecular recognition [15–17].

The studies on the usage of super-macroporous cryogels as a selective chromatographic adsorbent for the purification or separation of biological molecules have attracted quite more attentions of the researchers in recent years due to their highly interconnected porous networks with the pore size of ranging between 5–100 μm. These structural features make cryogels ideal stationary phase with extremely well flow dynamics even working with high viscous solutions i.e. whole blood sample and wastewater etc. [18]. The porous morphology of cryogels also gains sponge-like flexible structure to the cryogel, which allows the researchers to develop them in different size and shape meanwhile making possible easy, efficient and fast elution strategies via structural deformation [19].

The main problems with benchmark RNA purification systems depend on multi-step processing, impropriety for continuous set-up, and requiring the use of harmful organic substances. Therefore, because of unique structural properties and flow-dynamics, the use of cryogels for RNA purification is the one of promising alternatives in the yielding and rapid DNA/RNA chromatography [20–24]. In this field, Kumar and coworkers developed boronic acid based cryogels for selective nucleic acid separation under mild conditions [20–22]. Although boronic acid based chromatography is mainly utilized for this purification protocol, it has pseudo-specificity due to the affinity between boronic acid group and not only nucleic acids but also all cis-diols found in saccharides and glycoproteins. Using amino acids [23] and dyes [24] as affinity ligand to isolate is another approaches. But, these ligands still have a group specificity, which causes a decrease in purity of final product even they suppose cheaper and milder working conditions. Herein, we supposed a new functional monomer to produce nucleic acid incorporated cryogels in single-step process to create a synergy between cryogels, specific and reversible nucleotide interactions, and Rotavirus. The conversion of biological building blocks into polymerizable derivatives is a smart bioinspired approach for directly creating biofunctional polymeric network [25]. By this way, it is also possible to involve nucleotides as a ligand in RNA chromatography to achieve highly selective and specific interactions between adsorbent and analytes via mimicking the natural interactions, i.e. adenine-thymine or uracil, guanine-cytosine. Because the selectivity and reversibility of the interactions, the final product could also be obtained with higher capacity and purity as well as in native form.

In this study, we aimed to develop a novel polymeric material containing nucleotide (adenine) in cryogel form and to investigate the rapid isolation of RNA with high selectivity and efficiency. For this aim, we synthesized our nucleotide-based functional monomer, adenine methacrylate (AdeM) via substitution reaction between methacryloyl chloride and adenine at the first step. Subsequently, we characterized the resulting monomers by nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopies. At the second, we copolymerized AdeM with 2-hydroxyethyl methacrylate (HEMA) to synthesize the HEMA-based cryogels. After the characterization of the cryogels via FTIR, scanning electron microscopy (SEM), surface area measurements, thermogravimetric analysis (TGA), and swelling tests, RNA adsorption was performed via batch-wise while varying different conditions including pH, initial RNA concentration, temperature and interaction time. As the final step, we used the cryogel developed for fast and efficient purification of RNA molecules from rotavirus homogenates.

2. Material and methods

2.1. Materials

We purchased the basic monomer (2-hydroxyethyl methacrylate, HEMA), cross-linker (ethylene glycol dimethacrylate, EGDMA), and sodium lauryl sulfate (SLS) from Sigma (St. Louis, MO, USA) and stored them as received at 4 °C until use. We kept RNA (R6625-25G, Type VI, Torula Yeast) supplied from Sigma at −20 °C. We purchased initiators, *N*,*N*,*N*',*N*'-tetramethylethylene diamine (TEMED) and ammonium persulphate (APS) from BioRad (Hercules, CA, USA).

2.2. Methods

2.2.1. Preparation of functional monomers

The Adenine methacrylate (AdeM) monomer used in this study was synthesized according to the literature (Fig. 1a) [26]. For this purpose, as a first step, benzotriazole methacrylate synthesis was carried out by dissolving benzotriazole (0.01 mol, 1.19 g) and triethylamine (TEA) (0.011 mol, 1.11 g) in dry toluene and the solution was then cooled to 0–5 °C. The adenine (0.01 mol, 1.35 g) solution in 1 M NaOH was dropwise added to the solution at constant stirring speed. After that, the mixture was allowed to react at room temperature for 5 h. After the filtering of the salt slurry, the functional monomer (adenine methacrylate, AdeM) was finally obtained as an oil-like yellowish solid by distillation of the solvent to remove out the unreacted TEA and H-benzotriazole under reduced pressure.

2.2.2. The synthesis of cryogels

General procedure for synthesizing the cryogel could be summarized as 2.45 mL of HEMA and AdeM monomer (50, 75 and 100 mg separately) were dissolved in 2.55 mL of deionized water (DIW), and the mixture was stirred on a magnetic stirrer until obtaining a homogeneous solution. Meantime, 0.5 g of sodium lauryl sulfate (SLS) and 0.6 mL of ethylene glycol dimethacrylate (EGDMA) were dissolved in 9.40 mL of DIW. After mixing of both phases, we cooled it in an ice bath for 20 min. After that, 10 mg of APS and 50 μL of TEMED were introduced into the mixture as a redox-initiator to start the polymerization reaction. Subsequently, the mixture was poured between two glass plates with covered three edges and allowed to complete polymerization at −12 °C for 24 h. The cryogels obtained were washed extensively to remove unreacted monomer and surface-active agent, SLS after cutting them into the circular shape (o.d. 0.8 cm). The cryogels were stored at 4 °C in the fridge in the solution including sodium azide (0.1%, w/v) as anti-microbial agent until use. The monomer ratios and cryogel notations were summarized and given as table in the Supporting Information file (Table SI-1). The characterization methods applied and other experimental methods were given in Supplementary file in details.

3. Results and discussion

In recent years, although the purification of RNA has been performed via enzymatic, electrophoretic and chromatographic methods [20–24], there was no satisfactory yield achieved due to the structural sensitivity of RNA molecules. In this respect, we focused our attention on developing a novel material for fast and efficient RNA purification. In this study, we proposed a bioinspired approach to synthesize poly(HEMA) based cryogels including the polymerizable derivative of adenine as functional monomer (AdeM) for affinity separation of RNA molecules. After characterization of the materials, we optimized adsorption conditions through evaluating the affecting factors from aqueous solutions. At the final step, we achieved RNA purification from rotavirus under optimized conditions. We also applied the kinetic