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Macroporous, responsive DNA cryogel beads

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ABSTRACT

Biocompatible soft materials that are macroporous and tough are in demand for a range of applications. Here, we describe the preparation of macroporous DNA cryogel beads by crosslinking DNA in frozen aqueous solution droplets at −18 °C. Ethylene glycol diglycidyl ether was used as the crosslinker and N,N,N′,N′-tetramethylethylenediamine as catalyst. The beads swell in 4.0 mM NaBr 74–212 times their dry weights and exhibit moduli of elasticity around 0.5 kPa. In dry state, they contain irregular large pores of 10^1–10^2 μm in sizes due to the ice crystals acting as a template during the gelation reactions. DNA beads can be compressed up to about 80% strain without any crack developments. They also exhibit reversible swelling–deswelling cycles in water and acetone, respectively, undergoing a discrete phase transition in aqueous acetone solutions containing 51% acetone. The ability of the beads for the removal of carcinogenic agents from aqueous solutions was also demonstrated using phenanthrene as a model compound. The sorption capacity of the beads was found to be 420 μg phenanthrene/g DNA.

1. Introduction

All living cells contain deoxyribonucleic acid (DNA) molecules carrying genetic information in their base sequences. In its native form, DNA is a semi-flexible polymer with a double-helical (ds) conformation stabilized by hydrogen bonds between the amine bases [1]. When a DNA solution is subjected to high temperature, the hydrogen bonds holding the two strands together break and the double helix dissociates into two single flexible strands having a random coil conformation [2]. Due to the unique structure of DNA, chemical compounds having aromatic planner groups such as benzopyrine and phenanthrene intercalate between adjacent base pairs of ds-DNA and result in mutation and endocrine disruption [3,4]. This fact also suggests that, after insolubilization of DNA, it can be utilized as an adsorbent for such toxic materials from waters.

Several techniques were developed to prepare water-insoluble DNA compounds for the removal of toxic materials. Yamada et al. prepared water-insoluble and nuclease-resistant DNA films by UV radiation [5], while Yamamoto and co-workers prepared DNA liquid crystal gels by the reaction with metal cations [6,7]. DNA/nanoparticle hybrids [8], DNA-immobilized nonwoven cellulose fabric [9], DNA complexes with lipids [10], cationic surfactants [11,12], DNA immobilized in gels [13,14], semi-interpenetrating networks [15], DNA films [16], and hydrogels containing DNA strands as grafts [17], or as crosslinks [18] are among materials containing water-insoluble DNA. Although such materials can be used as specific sorbent for the removal of carcinogens, they have the general drawback that they are nonporous, and mechanically weak. An efficient sorbent material should have a macroporous structure allowing a fast liquid transport through the continuous macropores [19]. In addition, a fast response against the external stimuli and a good mechanical performance are also requirements for efficient materials.

DNA hydrogel is a network of chemically crosslinked DNA strands swollen in aqueous solutions [20]. Such soft materials are a good candidate to make use of the characteristics of DNA such as coil-globule transition, biocompatibility, selective binding, and molecular recognition [16,18]. DNA hydrogels were prepared starting from branched DNA molecules via ligase-mediated reactions [21]. These hydrogels can also be prepared by crosslinking DNA in semi-dilute solutions using a chemical crosslinker such as ethylene glycol diglycidyl ether (EGDE) in the presence of N,N,N′,N′-tetramethylethylenediamine (TEMED) catalyst [22]. EGDE contains epoxide groups on both ends that can react with the amino groups on the nucleotide bases to form a three dimensional DNA network [23]. DNA hydrogels are responsive systems exhibiting drastic volume changes in response to the external stimuli, such as the composition of aqueous solutions of acetone [22,24], or polyethylene glycol [25], concentrations of inorganic salts [26–29], polyamines [27,28], cationic macromolecules [30], or surfactants [27,28,31]. We have recently focused on developing responsive DNA hydrogels with a wide range of tunable properties such as the conformation of the network strands [25], viscoelasticity [32], and nonlinear elasticity (strain hardening) [33]. Gelation reactions conducted at

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50 °C show that a high concentration of DNA such as 9.3 w/v% stabilizes double-stranded (ds) DNA conformation while, at lower concentrations, single stranded (ss) DNA gels were obtained [25]. No gel formation was observed at DNA concentrations as low as 5%. Gels formed from ss- or ds-DNA strands were highly transparent indicating that they are nonporous in dry state.

Preparation of DNA hydrogels with a macroporous structure has, to our knowledge, not been previously reported. Creating an interconnected pore structure within the crosslinked DNA network would result in the formation of fast responsive DNA hydrogels. In the present study, we describe the preparation of macroporous DNA hydrogels in the form of millimeter-sized beads suitable as specific adsorbent for carcinogenic compounds. Our strategy to prepare such gel beads is to conduct the crosslinking reactions of ds-DNA (about 2000 base pairs long) using EGDE crosslinker within the droplets of frozen DNA solutions at −18 °C. This low temperature gelation technique known as cryogelation is a simple route for the preparation of macroporous gels [34–40]. During the freezing of an aqueous polymer solution containing a chemical crosslinker, the polymer chains and the crosslinker molecules expelled from the ice concentrate within the channels between the ice crystals, so that the crosslinking reactions only take place in these unfrozen liquid channels. After crosslinking and, after thawing of ice, a macroporous material (cryogel) is produced whose microstructure is a negative replica of the ice formed. As will be seen below, the crosslinking DNA in aqueous frozen solution droplets at −18 °C produces spherical, macroporous, tough cryogel particles with fast-responsivity. The characteristics of polyelectrolyte hydrogels such as the volume phase transition can be observed in the cryogel beads in a very short period of time and in a reversible manner. Here, we also show that macroporous DNA beads can effectively be used in the adsorption processes of carcinogenic compounds such as dilute aqueous solutions of phenanthrene.

2. Experimental

2.1. Materials

Cryogels were made from deoxyribonucleic acid sodium salt from salmon testes (DNA, Sigma). According to the manufacturer, the% G–C content of the DNA used is 41.2%, and the melting temperature is reported to be 87.5 °C in 0.15 M sodium chloride plus 0.015 M sodium citrate. The molecular weight determined by ultracentrifugation is 1.3×10^6 g/mol, which corresponds to approximately 2000 base pairs. The crosslinker ethylene glycol diglycidyl ether (EGDE, 50%, technical grade, Fluka), N,N,N′,N′-tetramethylethylenediamine (TEMED, Merck), phenanthrene (Fluka), and sodium bromide (NaBr, Merck) were used as received. Stock solutions of EGDE and TEMED were prepared by dissolving 2.61 mL EGDE and 0.568 mL TEMED in 10 and 20 mL 4.0 mM NaBr, respectively. DNA and TEMED concentrations in the gelation solutions were expressed as DNA% and TEMED%, respectively, which are the mass of DNA and the volume of TEMED in 100 g reaction solution. The crosslinker (EGDE) content of the reaction solution was expressed as EGDE%, the mass of EGDE added per 100 g of DNA.

2.2. Gelation reactions

The crosslinking reaction of DNA was carried out at −18 °C in the presence of 50% EGDE. The pH of the reaction solution was set to 11.0 by the addition of 0.44% TEMED [25]. DNA was first dissolved in 4.0 mM NaBr at 35 °C for 1 day. After addition of EGDE and stirring for 1 h, TEMED was included into the reaction solution. Note that the solutions containing more than 1% DNA were too viscous and could not be dropped into the continuous phase to obtain spherical beads. Therefore, they were heated to 50 °C and held at this temperature for 10 min to partially denature DNA and thus, to decrease the viscosity of the solutions. Two techniques were used for the preparation of macroporous DNA cryogel beads:

Technique A: DNA solution containing EGDE and TEMED was added dropwise into an excess of liquid nitrogen to create small frozen droplets. As each droplet touches the liquid nitrogen, nitrogen starts to boil while the droplet spins on the liquid surface until it falls down into the liquid nitrogen. After obtaining frozen droplets, they were transferred into paraffin oil as the continuous phase at −18 °C and the reactions were conducted for 3 days.

Technique B: DNA solution was directly dropped into paraffin oil at −7 °C and after complete addition, the temperature of the oil phase was decreased to −18 °C and the reactions were conducted for 3 days at this temperature. Note that droplets of DNA solutions in paraffin oil cannot be obtained if the temperature was initially set to −18 °C due to the high viscosity of the oil. Preliminary experiments conducted under different experimental conditions showed that −7 °C is the optimum temperature for the addition of the droplets into the oil phase.

After 3 days, the gel beads were removed from the oil phase, washed several times with acetone. Thereafter, they were placed in an excess of 4.0 mM NaBr solution and the solution was replaced many times.

DNA cryogels were also prepared in the form of cylinders of about 4.5 mm diameter. The reaction solution containing DNA, EGDE, and TEMED prepared as described above was transferred into plastic syringes. Half of the syringes were immediately frozen in liquid nitrogen to prevent denaturation of DNA due to the effect of EGDE-TEMED pair [25]. The other half of the syringes were heated in an oven at 50 °C for 20 min to completely melt DNA, following quenching in liquid nitrogen to fix ss-DNA conformation. Indeed, hyperchromicity measurements (see below) showed 90 ± 10% denaturation of ds-DNA in these samples All syringes were then transferred into a freezer at −18 °C to conduct the gelation reactions for 3 days. In this way, cryogels consisting of mainly ss-DNA and ds-DNA strands were prepared.

For comparison, hydrogels of DNA were also prepared by conducting the gelation reactions at 50 °C both in plastic syringes and between the parallel plates of the rheometer (Gemini 150 Rheometer system, Bohlin Instruments) equipped with a Peltier device for temperature control. The upper plate (diameter 40 mm) was set at a distance of 500 μm before the onset of the reactions. During all rheological measurements, a solvent trap was used to minimize the evaporation. Further, the outside of the upper plate was covered with a thin layer of low-viscosity silicone oil to prevent evaporation of solvent. A frequency of ω = 1 Hz and a deformation amplitude γ = 0.01 were selected to ensure that the oscillatory deformation is within the linear regime.

2.3. Hyperchromicity measurements

For the hyperchromicity measurements, gelation reactions were conducted, as described above, except that the frozen droplets or the reaction solutions were transferred into empty glass vials at −18 °C instead of paraffin oil. After 5–10 min of the reaction time, samples were taken and after thawing, they were diluted to a concentration of 26 mg/L with 4.0 mM NaBr. The degree of denaturation was estimated from the optical absorbance at 260 nm measured with a T80 UV–visible spectrophotometer. The results were presented as the normalized absorbance A_{rel} with respect to that measured from starting DNA solution. Because melting of