Journal of Colloid and Interface Science 390 (2013) 78–84

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

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A polyion complex nanogel

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ARTICLE INFO

Article history:
Received 6 July 2012
Accepted 9 September 2012
Available online 19 September 2012

Keywords:
Polyion complex
Nanogel
Dextran
Crosslinking
Salt bridge

ABSTRACT

Here, we synthesized dextrans modified with trivalent cationic or anionic groups. Aqueous solutions of the cationic and anionic dextrans were then mixed resulting in the formation of polyion complex nanogels (PIC-NGs), which have physically crosslinked salt bridges formed between the cationic and anionic groups. To prepare PIC-NGs in high yield, the content of ionic groups in the cationic and anionic dextrans should be low to avoid interparticle salt bridge formation. The structure of the PIC-NGs is easily affected by the ionic strength of the solution because of shielding of the charges in the ionic groups. However, the conversion of a small amount of the physical crosslinks to covalent crosslinks significantly improved the stability of the PIC-NGs. The covalent crosslinking also stabilized the PIC-NGs against pH change, which will destabilize the salt bridges. These results indicated that the conversion of the small amount of physical crosslinks to covalent crosslinks stabilized the other salt bridges.

1. Introduction

Nanogels (NGs) are submicron-size hydrogels composed of polymer chains crosslinked by covalent bonds or non-covalent interactions [1,2]. NGs have received much attention because of the following interesting characteristics. NGs respond to outer stimuli to change their volume and shape much more quickly than macroscopic gels [3–5]. NGs can accommodate various molecules in their inner free space [6–8]. The elasticity of the NGs significantly affects their biodistribution [9].

Most of the NGs reported so far employ covalent (chemical) crosslinking because of the stable nature associated with covalent crosslinking when compared with non-covalent (physical) crosslinking [10–13]. To design NGs with non-covalent crosslinking, interparticle crosslinking formation should be avoided because it results in macroscopic aggregation. Akiyoshi's group first reported physically crosslinked NGs based on hydrophobic interactions [14–16]. They successfully avoided interparticle crosslinking simply by modifying the hydrophobic groups with small amount. The small amount of modification reduced the chance to form the interparticle crosslinking. When highly hydrophobic cholesterol groups were employed to modify the hydrophilic polysaccharide main chain, the degree of modification of ~1.5 mol% was enough to form NGs, in which the main chains of the NGs were much contracted when compared with those of the original polysaccharide coils [17]. The less hydrophobic palmitoyl needed 5 mol%-substitution to form NGs [14,18]. These physically crosslinked NGs are promising materials as protein carriers [19–21]. Because of the reversible nature of the physical crosslinking, these NGs are able to accommodate large proteins, which are difficult to be accommodated in chemically crosslinked NGs.

The purpose of the present study is to design a new class of physically crosslinked NGs, referred to as polyion complex NGs (PIC-NGs), which are crosslinked by electrostatic interactions among the polycations and polyanions (Fig. 1). When the polycations and polyanions are mixed together, polyion complexes (PICs) are formed, which usually results in macroscopic coacervation [22,23]. We tried to avoid this macroscopic coacervation by minimizing the content of ionic groups in the polycations and polyanions. However, the minimized content of ionic groups was expected to weaken the salt bridges between the polycations and polyanions. To strengthen the salt bridges, we employed oligoionic groups to modify the main chain. We further studied the stabilization of the PIC-NGs by partial chemical crosslinking. The PIC-NGs may be applicable to drug delivery vehicles by electrostatically accommodating payload molecules. The present work will open up new areas of NGs research and will serve as the basis for designing PIC-NGs for specific applications.

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http://dx.doi.org/10.1016/j.jcis.2012.09.015

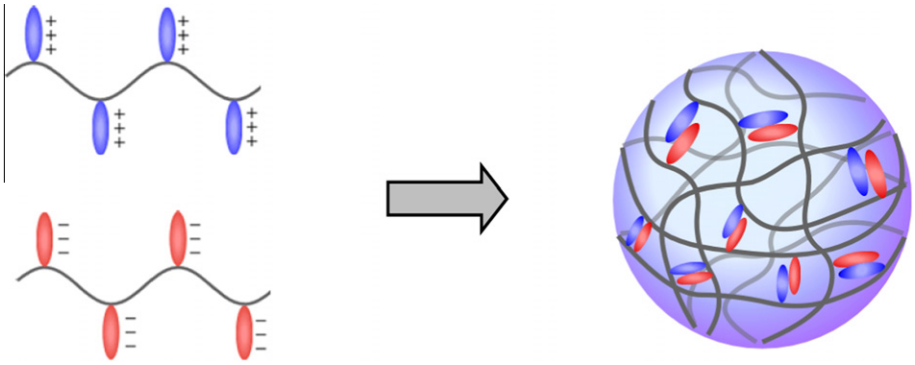

Fig. 1. Schematic representation of the formation of a PIC-NG.

2. Experimental methods

2.1. Materials

Dextran (M_w 60,000–90,000) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Lithium chloride was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). These reagents were dried over diphosphorus pentoxide at 90 °C. *N,N′*-Bis-(3-aminopropyl)-1,3-propanediamine and 1,2,3,4-butanetetracarboxylic acid were purchased from Sigma (St. Louis, MO). *p*-Nitrophenyl chloroformate (PNC) was purchased from Wako Pure Chemical Industries (Osaka, Japan). 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Pyridine, dimethylsulfoxide (DMSO), and dimethylformamide were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Cationic dextran (CD)

The hydroxyl groups of dextran were activated with various concentrations of PNC following the literature method [24]. The content of *p*-nitrophenyl groups was determined by ^{1}H-NMR to be 9.5 and 41.2 mol% of glucose units. Then, the PNC-activated dextran (200 mg; containing 0.11 and 0.36 mmol nitrophenyl groups) was dissolved in DMSO (20 mL). This solution was added dropwise to a DMSO solution of *N,N′*-bis(3-aminopropyl)-1,3-propanediamine (230, 330 and 730 μL; 1.1, 1.6, and 3.6 mmol). The reaction mixture was stirred for 24 h at room temperature, and dialyzed with a Spectra/Pore 7 dialysis bag (MWCO 10,000) against diluted sodium hydroxide solution (pH = 11) for 2 days and distilled water for a day, and then lyophilized. The degree of substitution of the cationic dextran (CD) was determined by ^{1}H-NMR.

^{1}H-NMR (300 MHz, D_2O): δ 1.60 (6H, $(NHCH_2CH_2CH_2)_3NH_2$), 2.55 (10H, $NHCH_2CH_2CH_2(NHCH_2CH_2CH_2)_2NH_2$), 3.13 (2H, $NHCH_2$), 3.4–3.95 (6H, glucosidic protons without anomeric proton), 4.93 (1H, anomeric proton).

2.3. Anionic dextran (AD)

PNC-activated dextran (200 mg; containing 0.11and 0.36 mmol nitrophenyl groups) was dissolved in DMSO (20 mL). This solution was added dropwise to a DMSO solution of ethylenediamine (75, 110 and 240 μL; 1.1, 1.6, and 3.6 mmol). The reaction mixture was stirred for 24 h at room temperature, and dialyzed with a Spectra/Pore 7 dialysis bag (MWCO 10,000) against diluted sodium hydroxide solution (pH = 11) for 2 days and distilled water for a day, and then lyophilized. The degree of substitution of the ethylenediamine-modified dextran was determined by ^{1}H-NMR.

^{1}H-NMR (300 MHz, D_2O): δ2.66 (2H, CH_2NH_2), 3.12 (2H, $NHCH_2$) 3.42–3.95 (6H, glucosidic protons without anomeric proton), 4.93 (1H, anomeric proton).

The ethylenediamine-modified dextran (200 mg; containing 0.06 and 0.18 mmol amine groups) was dissolved in distilled water (20 mL). Then, 1,2,3,4-butanetetracarboxylic acid (140 and 430 mg; 0.6 and 1.8 mmol) was added to this solution, and pH of the solution was adjusted to 9 with sodium hydroxide solution. To this solution was added DMT-MM (49 and 150 mg; 0.18 and 0.55 mmol). The reaction mixture was stirred for 24 h at room temperature, and dialyzed with a Spectra/Pore 7 dialysis bag (MWCO 10,000), using diluted hydrochloric acid solution (pH = 4) for 2 days and distilled water for a day, and then lyophilized.

^{1}H-NMR (300 MHz, D_2O): δ2.18–2.63 (6H, protons of tetracarboxylic acid), 3.25 (4H, $NHCH_2CH_2NHCO$), 3.48–3.95 (6H, glucosidic protons without anomeric proton), 4.92 (1H, anomeric proton).

2.4. PIC-NG

CD and AD were dissolved in 10 mM phosphate buffer (pH 7.4) at 2.0 mg/mL. After these polymer solutions were mixed at various cation/anion (C/A) charge ratios, the mixture was sonicated for 30 min with an SU-9TH ultrasonic water bath to equilibrate the PIC formation. Macroscopic aggregates were removed by centrifugation (13,000*g*) for 30 min at 25 °C, and the supernatant was collected to obtain a PIC-NG solution. The yield of PIC-NG was determined using the phenol–sulfuric acid colorimetric method [25].

2.5. Crosslinked PIC-NG

An aqueous solution of DMT-MM (0.4 mg/mL) was added to 1 mL of 1.0 mg/mL PIC-NG in 10 mM phosphate buffer (pH 7.4). The reaction mixture was shaken for 24 h at room temperature and purified using a cellulose ultrafiltration filter (MWCO 3000).

2.6. Dynamic Light Scattering (DLS) and ζ-potential measurement

The diameters of the PIC-NGs were measured using Zetasizer Nanoseries (Malvern Instruments, Worcestershire, UK) at a detection angle of 173° and a temperature of 25 °C. The ζ-potential of the PIC-NGs was measured using a Zetasizer Nanoseries at a scatter angle of 17° and a temperature of 25 °C.

2.7. Static Light Scattering (SLS)

SLS analysis of PIC-NG (10 mM phosphate buffer (pH 7.4)) was conducted on DLS-7000 (Otsuka Electronics Co., Ltd., Osaka, Japan)