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Short Communication

Molecular weight determination of a newly synthesized guanidinylated disulfide-containing poly(amido amine) by gel permeation chromatography

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ABSTRACT

A cationic gene delivery vector, guanidinylated disulfide-containing poly(amido amine) (CAR-CBA), was synthesized by Michael addition reaction between N,N′-cystaminebisacrylamide (CBA) and guanidine hydrochloride (CAR). Gel permeation chromatography (GPC) was used to evaluate the molecular weight of synthesized CAR-CBA. Polyethyleneimine (PEI) with molecular weight of 25 kDa was adopted as a reference, and polyethylene glycols (PEG) with different molecular weights were used to establish a standard curve for determining the molecular weight of CAR-CBA. The effects of two critical factors, namely columns and eluents, on the molecular weight measurement of CAR-CBA were investigated to optimize the GPC quantitative method. The results showed that Ultrahydrogel columns (120, 250) and HAc–NaAc (0.5 M, pH 4.5) buffer solution were the optimal column and GPC eluent, respectively. The molecular weight of the synthesized CAR-CBA was analyzed by the optimized GPC method and determined to be 24.66 kDa.

1. Introduction

The application of non-viral gene delivery vectors, especially cationic polymers, in gene delivery field has aroused extensive attention due to their various superiorities over viral vectors, such as non-immunogenicity, no integration of exogenous genes into host chromosomes, and convenience of manufacturing and handling [1]. However, the relatively high cytotoxicity and low transfection efficiency [2] are the main weaknesses of cationic polymers as gene delivery vectors.

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Many cationic polymers have been studied as gene delivery vectors, including HPAA [3], HPAMAM [4], PEG-PEI [5] etc. Recently, a series of biodegradable cationic polymers with disulfide linkages in the backbone have been reported [6–8]. These disulfide bonds are stable in extracellular matrices, and can be rapidly cleaved in the reductive environment of the cytosol through disulfide bond reduction [9], thus resulting in increased release of DNA from the complexes and decreased toxicity . In addition, polymers conjugated with arginine were reported to show substantial improvement of cell-penetrating ability [10]. It was proved that the guanidine groups in arginine functioned most importantly for improving gene transfection efficiency. And simple chemical modification of polymers with guanidine could lead to a significant enhancement in transfection efficiency [11]. Taken together, a novel cationic polymer introduced disulfide linkage and guanidine group is expected to provide great benefits in gene delivery systems.

The transfection efficiency and toxicity of cationic polymers have been reported to be closely related to their molecular weights (Mw) [12]. For example, polyethyleneimine (PEI), a widely used polycation gene vector, exhibited high transfection efficiency and high cytotoxicity with a higher molecular weight, e.g., 25 kDa, while PEI with lower molecular weight, e.g., 800 Da, showed much less transfection efficiency and negligible cytotoxicity [12]. Therefore, the molecular weight determination of cationic polymers is of great importance to their transfection efficiency and toxicity.

Gel permeation chromatography (GPC) is one of the most commonly used methods for determining the molecular weight of polymers, where macromolecules are separated according to their molecular sizes as sample solution flows through a packed bed of porous gels. However, in the case of molecular weight determination of cationic polymers, issues such as aggregation [13] and ion exclusion [14] often appear, leading to less accurate molecular weight results [15]. Thus, it is critical to investigate different factors affecting the molecular weight measurement of cationic polymers, so as to obtain more accurate molecular weight results.

In this study, we synthesized a novel water-soluble, cationic polymer with disulfide linkages and guanidine groups (CAR-CBA), aiming to obtain a gene delivery vector with favorable transfection activity and low toxicity. N,N′-cystaminebisacrylamide (CBA) and guanidine hydrochloride were selected, and Michael addition reaction was utilized in the synthetic processes. A quantitative GPC method was used to determine the molecular weight of CAR-CBA. Effects of different types of columns and eluents on the molecular weight measurement of CAR-CBA were investigated, and then the molecular weight of the synthesized CAR-CBA was determined.

2. Materials and methods

2.1. Materials

N,N′-cystaminebisacrylamide (CBA) was purchased from Alfa Aesar Co., Ltd., Shanghai, China. Branched PEI (bPEI, waterfree) with molecular weight of 25 kDa was purchased from Sigma Aldrich Co., Ltd., St. Louis, USA. Guanidine hydrochloride (CAR) was purchased from Sinopharm Chemical Reagent Co., Ltd., China. Trifluoroacetic acid (TFA), triisopropylsilane (TIS) and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl chloride (Pbf-Cl) were obtained from GL Biochem Ltd., Shanghai, China. The Polyethylene glycol (PEG) standards were purchased from ZZBIO Co., Ltd., Shanghai, China. Acetone and N,N-dimethylformamide (DMF) were purchased from Dikma Technologies Inc., China.

2.2. Synthesis of CAR-CBA

The synthetic route of CAR-CBA is illustrated in Fig. 1 The synthetic reactant selection and process optimization are described in details in another paper [16]. Firstly, an important intermediate CAR-Pbf was synthesized. Briefly, guanidine hydrochloride (1.00 g) was dissolved in water (10 ml) and added to a three necked flask. After the solution was cooled to 0–5 °C, Pbf-Cl (3.02 g, dissolved in 10 ml acetone) was added dropwise into the flask at 0–5 °C. After the addition was completed, the reaction mixture was stirred at room temperature for three hours. During the reaction process, the pH of the system was maintained at 11–12 with NaOH solution (4 M). Finally, white precipitation of CAR-Pbf was obtained after suction filtration. The reaction ratio of CAR and Pbf-Cl was analyzed by a mass spectrometer (micrOTOF_Q, Bruker Co., Ltd., USA).

The reducible CBA-CAR-Pbf-Cl was synthesized by Michael addition polymerization between CBA and Pbf-protected carbamidine (CAR-Pbf). In brief, CBA (0.10 g) and CAR-Pbf (0.24 g) were dissolved in DMF (5 ml) and added to a round-bottom flask. The reaction mixture was stirred in dark under nitrogen atmosphere at 60 °C for 7 days. Subsequently, 10% of excess CAR-Pbf (0.03 g) was added and stirring was continued for 2 days at 60 °C. Next, a mixture of TFA:H_2O:TIS (94:3:3, 10 ml) was added and stirred at room temperature for additional 3 hours. The reaction mixture was then diluted with water, alkalized to pH 7 with NaOH solution, and purified with an ultrafiltration membrane (MWCO 1000). Lastly, the solution was lyophilized to obtain the final product of CAR-CBA. The chemical structure of CAR-CBA was analyzed by proton nuclear magnetic resonance (Ascend 600, Bruker, USA).

2.3. Determination of molecular weight by GPC

The molecular weight and polydispersity index (PDI) of the synthesized CAR-CBA were determined by a GPC method using a Waters 1515 HPLC system (Waters Co., Ltd., USA) equipped with a Waters 2414 refractive index detector (RID). Three kinds of columns, namely Styragel columns (Waters Co., Ltd., USA), CLM 1031 column (Malvern Instruments Co., Ltd., UK), and Ultrahydrogel columns (Waters Co., Ltd., USA) were tested. The synthesized CAR-CBA and PEG were dissolved in three kinds of eluents, i.e. water, DMF and HAc–NaAc buffer solution, which correspond to the columns used. The prepared CAR-CBA and PEG solutions (2 mg/ml) were filtered with 0.22 μm syringe filters (Dikma Technologies Inc., China). The eluents were degassed prior to each analysis. The columns and detector were thermostated at 35 °C. The samples were analyzed at a flow rate of 0.7 ml/min and the injection volume was set at 20 μl. Both data collection and analysis were carried out using a Breeze 2 (Waters Co., Ltd., USA) software.