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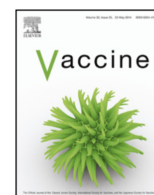

Molecular shape and immunogenicity of meningococcal polysaccharide group A conjugate vaccine

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ABSTRACT

Neisseria meningitidis is a leading cause of severe bacterial infections in infants and young children. As a major virulence factor, meningococcal capsular polysaccharide (PS) is poorly immunogenic and generally does not induce immunological memory. Conjugation of PS with a carrier protein can significantly increase the PS-specific immunogenicity and induce immunological memory. It is well known that the molecular shape/size of the conjugate vaccine is important for its immunogenicity. However, little is known about the molecular shape/size of the meningococcal conjugate vaccine. A meningococcal PS–ovalbumin (OVA) conjugate vaccine was prepared using cystamine as linker. Four components (P1–P4) with different molecular size were fractionated from the conjugate. Small angle X-ray scattering (SAXS) analysis revealed that the conjugate vaccine exhibited a rod-like shape similar to virus-like particles. PS-specific immunogenicity of the conjugate vaccine was related to its molecular shape and increased as a function of its molecular size. Thus, the present study provides a three-dimensional shape of the conjugate vaccine and helps to identify optimal design of a potent meningococcal conjugate vaccine.
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1. Introduction

Neisseria meningitidis is a leading cause of severe bacterial infections such as meningitis and other clinical manifestations in infants and young children [1]. As a major virulence factor, meningococcal polysaccharide (PS) capsule on the bacterial surface has been used to control outbreaks or epidemics of meningococcal diseases [2]. However, plain capsular PS is a T-cell independent antigen inducing short-term immunity and generally does not induce immunological memory [3]. In order to induce a satisfactory immunity, PS was conjugated with a carrier protein possessing T-helper cell epitopes. The resultant PS component becomes a T cell dependent antigen that undergoes class switching and induces long-term immunity and strong immunological memory in infants and young children [4]. Therefore, meningococcal conjugate vaccines have almost eliminated meningococcal disease incidence in areas where immunization using these vaccines is widespread [5].

Recently, the shape and size of nanoparticle over length scales were found to play an important role on cellular internalization and intracellular trafficking [6]. Similarly, internalization of the conjugate vaccine by the PS-specific B cells is required for the PS-specific antibody production [7]. Thus, it is interesting to establish the relationship of the molecular shape/size and the immunogenicity of the meningococcal conjugate vaccine. However, the information about the molecular shape of meningococcal conjugate vaccine is still inadequate. Small angle X-ray scattering (SAXS) is a powerful tool to investigate the three-dimensional shape of biological macromolecules in solution. In addition, the SAXS technique can provide a best fit model that represents the probable overall, time-averaged ensemble shape of a macromolecule in solution under nearly physiological conditions [8].

In the present study, meningococcal group A capsular PS was conjugated with ovalbumin (OVA), using cystamine as the linker. Four components (P1–P4) with different size were fractionated from the conjugate using the size exclusion chromatography (SEC). SAXS was used to investigate the three-dimension low resolution shape of the conjugate vaccine. The molecular shape and the PS-specific immunogenicity of the four components were measured. The present study is expected to provide a three-dimensional shape of the conjugate vaccine and helps to identify optimal design of a potent meningococcal conjugate vaccine.

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2. Material and methods

2.1. Materials

Cyanogen bromide (CNBr), 1-(3-dimethylaminopropyl)-3-ethylcarbodimide (EDC), cystamine dihydrochloride, 2,4,6-trinitrobenzene sulfonic acid (TNBS), and ovalbumin were purchased from Sigma (USA). Horse radish peroxidase (HRP)-conjugated goat anti-mouse IgG Fc antibody (HRP-IgG), IgG1 Fc antibody (HRP-IgG1), IgG2a Fc antibody (HRP-IgG2a) and IgM antibody (HRP-IgM) were purchased from Abcam (USA). Meningococcal group A capsular PS was kindly provided by Hualan Biological Engineering, Inc. (China).

2.2. Preparation of the conjugate

PS solution (4 ml, 5 mg/ml) was incubated with 20 mg CNBr (40 μl, 50% (w/v) in chloroform) in 0.9% (w/v) NaCl at room temperature for 15 min (Fig. 1). NaOH (0.2 M) was occasionally added to keep the solution at pH 10.5. Then, 1 ml of 60 mg/ml cystamine dihydrochloride was added and HCl (0.5 M) was occasionally added to keep the solution at pH 7.0. The reaction mixture was incubated at room temperature overnight, followed by removal of the unreacted cystamine through extensive dialysis against 0.15 M NaCl solution. The conjugate (PS–OVA) was obtained by incubation of the activated PS (20 mg) with 20 mg OVA in the presence of 60 mg EDC in 20 mM MES buffer (pH 6.0) at 4 °C overnight (Fig. 1).

2.3. Purification of the conjugate

PS–OVA was purified from the reaction mixture by size exclusion chromatography (SEC) based on a Sephacryl S-400 column (2.6 cm × 60 cm, GE Healthcare, USA). The column was equilibrated and eluted by 20 mM sodium phosphate buffer (pH 7.2) at a flow rate of 3.0 ml/min. The elution peak corresponding to PS–OVA was divided by 4 equal components (P1, P2, P3 and P4). The four components were fractionated, respectively, followed by concentration and storage at −80 °C.

2.4. SEC analysis

The four components (P1, P2, P3 and P4) were analyzed by SEC using a Superose 6 column (1 cm × 30 cm, GE Healthcare, USA) at room temperature. The column was equilibrated and eluted by 20 mM sodium phosphate buffer (pH 7.2) at a flow rate of 0.5 ml/min. The effluent was detected at 280 nm.

2.5. Quantitative assay

PS content of the four components was measured by phosphorus method. Free PS content was measured based on the ethanol precipitation [9]. *O*-acetyl content of PS was measured by a colorimetric assay [10]. The degree of linked cystamine was measured by 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay [11], using cystamine as the standard. Bicinchoninic acid (BCA) protein assay kit (Vigorous Biotechnology, Beijing, China) was used to measure the protein concentrations, using bovine serum albumin as the standard.

2.6. Dynamic light scattering

The molecular radii of the four components were measured by dynamic light scattering using a DynaPro Titan TC instrument (Wyatt, Santa Barbara, CA, USA) at 25 °C. The samples were at a protein concentration of 1.0 mg/ml in 20 mM sodium phosphate buffer (pH 7.2).

2.7. Circular dichroism spectroscopy

Circular dichroism (CD) spectra of the four components were recorded on a Chirascan spectropolarimeter (Applied Photophysics, UK) between 260 and 190 nm, using a cuvette with 0.2 cm path length.

2.8. NMR spectroscopy

PS–OVA and PS were characterized by NMR at 600 MHz. Freeze-dried PS and PS–OVA were dissolved in deuterated water to a final PS concentration of 4 mg/ml. The ^{1}H NMR spectra were obtained on Bruker NMR Spectrometer Avance DRX 600 MHz, equipped with a 5 mm NMR probe (Bruker) at 25 ± 0.1 °C.

2.9. SAXS analysis

SAXS data were collected at beamline1W2A of the Beijing Synchrotron Radiation Facility (BSRF). Two-dimensional CCD detector was utilized to record the SAXS intensities. PS, P1–P4 were at the PS concentrations of 5.0, 3.0, 6.4, 4.5, and 6.0 mg/ml in PBS buffer (pH 7.4), respectively. The radius of gyration (R_g) was calculated by the Guinier plot. The distance distribution functions, $p(r)$ functions were calculated by the indirect Fourier transform program GNOM in a q-range of 0.1–2.6 nm^{-1} [12]. Based on $p(r)$ functions, R_g and the maximum dimension (D_{max}) of the scattering particles were obtained for PS and P1–P4 particles. The overall shapes of PS and P1–P4 in the solution were restored through their $p(r)$ functions, using the well-established reconstruction method DAMMIN [13].

2.10. Immunization

BALB/c female mice of weight 15–20 g were supplied by Animal Center of Peking University Health Science Center (Beijing,

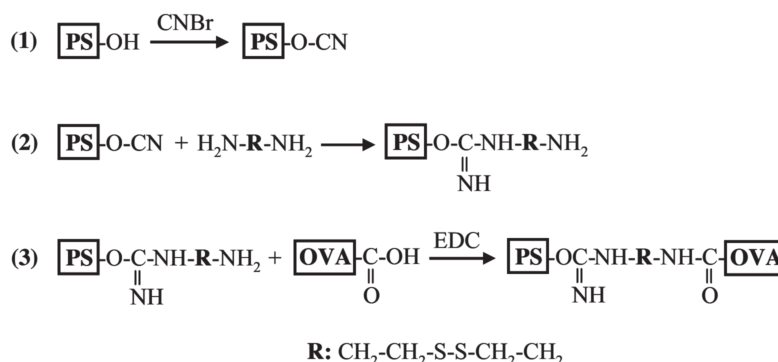

Fig. 1. Schematic representation of PS–OVA conjugate vaccine.

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