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Capillary Zone Electrophoresis Investigation of Interactions between Granulocyte-colony Stimulating Factor and Dextran Sulfate / Carrageenan Oligosaccharide

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Abstract: The interactions between granulocyte-colony stimulating factor (G-CSF) and dextran sulfate / κ-carrageenan oligosaccharide were studied by capillary zone electrophoresis. Dextran sulfate could strongly interact with G-CSF and the complex was detected. The binding constant and stoichiometry were determined to be 1.2×10^6 $(mol/L)^{-1}$ and 3:1, respectively. However, the interaction between κ-carrageenan oligosaccharide and G-CSF was not found.

Keywords: Capillary zone electrophoresis, granulocyte-colony stimulating factor, dextran sulfate, κ-carrageenan oligosaccharide, interaction

Granulocyte-colony stimulating factor (G-CSF) is an *O*-glycosylated glycoprotein. G-CSF stimulates the proliferation and differentiation of hematopoietic progenitor cells committed to the neutrophil/granulocyte lineage in a dose-dependent manner. The most important application of G-CSF is in the treatment of transient phases of leukopenia following chemotherapy and/or radiotherapy. Using capillary zone electrophoresis (CZE), former studies showed that G-CSF could interact with polysulfated saccharides, heparin and low molecular weight heparin (LMWH)[1]. Sulfate groups of saccharides often take great effect in protein-saccharide interactions. To identify whether G-CSF can bind to any other polysulfated saccharides, the investigation of the interaction between G-CSF and other polysulfated saccharides is necessary.

Dextran sulfate and κ-carrageenan oligosaccharide, like heparin, are also polysulfated saccharides. Studies showed that dextran sulfate, as well as heparin, had antiviral effect on human immunodeficiency virus (HIV) *in vitro* [2] and had only about one eighth anticoagulant activity of heparin[3]. Carrageenan is a family of sulfated polysaccharides isolated from red algae. These polysaccharides used as additives to improve food texture, gelation, stability, and viscosity are generally regarded to be safe by the Food and Drug Administration in the USA[4]. Three types of carrageenans, ι, κ and λ, were found to be the most potent bFGF, PDGF and TGFβ1 antagonists, respectively[5]. Therefore, these carrageenans are potentially useful drug candidates.

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In this study, the interactions between G-CSF and dextran sulfate /κ-carrageenan oligosaccharide were investigated by CZE. The results showed that the κ-carrageenan oligosaccharide could not interact with G-CSF under the experimental conditions, while the dextran sulfate strongly interacted with G-CSF with the binding constant of 1.2×10^6 $(mol/L)^{-1}$, the binding stoichiometry of 3:1, and the complex was detected.

Experimental

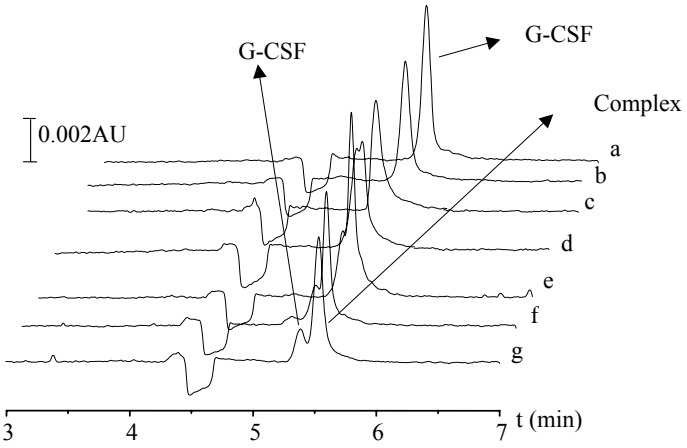
rhG-CSF (MW: 18,987, 360 μg/mL in 10 mmol/L NaAc-HAc, 5% mannitol buffer, pH 4.0) was kindly provided by Qilu Pharmaceutical Factory (Jinan, Shandong). Dextran sulfate sodium salt (MW: 500 kDa) was obtained from Pharmacia. Mannitol was purchased from Beijing Jingke Company. κ-carrageenan was purchased from Sigma (USA) and κ-carrageenan oligosaccharide was the degradation product of κ-carrageenan by acid. Other chemicals were all analytical grade. Redistilled water was used throughout this work. All the samples were diluted by NaAc-HAc buffers (10 mmol/L NaAc, 5% mannitol, pH adjusted to 4.0 by acetic acid) to various concentrations. A 50 mmol/L phosphate buffer (pH 7.0) was used as running buffer. The instrumentation consists of a P/ACE MDQ system (Beckman, Fullerton, CA, USA) with a photodiode array (PDA) detector and an uncoated fused silica capillary (Reafine Chromatography Equipment Co., Ltd., Hebei, China) with an internal diameter of 50 μm. The total and effective lengths of the capillary were 31.2 cm and 21 cm, respectively. The applied voltage was 8 kV and the detection wavelength was 210 nm. The temperatures of the cartridge and sample were kept at 25℃, 20℃, respectively. Before each run, the capillary was rinsed with blank buffer for 3.0 min at 137.895 kPa. Samples were injected at the anodic end using pressure injection mode with 3.447 kPa for 4 s and detected at the cathodic end. After each run, the capillary was flushed consecutively with 1 mol/L HCl for 2.0 min, water for 3.0 min, 1 mol/L NaOH for 2.0 min, and finally with water again for 3.0 min at 137.895 kPa.

Results and Discussion

G-CSF with the concentration range of 0.033 – 0.133 g/L was injected into capillary column to get calibration plot. The peak height of each sample was proportional to its concentration and the equation of standard curve was H = 1600.6 C + 235.97 with a coefficient of 0.995. The free concentrations of G-CSF in subsequent experiments were calculated from this equation.

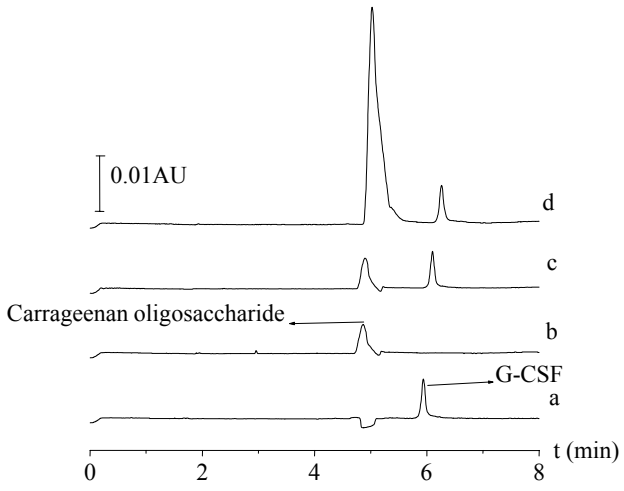
Figure 1 shows the electropherograms of 0.074 g/L G-CSF mixed with various concentrations of dextran sulfate. The peak height of G-CSF decreased with the increase of the added dextran sulfate. When the concentration of dextran sulfate reached to 0.25 g/L, a new peak appeared, after which the free G-CSF peak continued to decrease and the complex peak increased with the increase of dextran sulfate. From the peak heights of free G-CSF, the binding constant and stoichiometry were determined, by Scatchard plot, to be 1.2×10^6 $(mol/L)^{-1}$ and 3:1, respectively.

Figure 1 Electropherograms of 0.074 g/L G-CSF mixed with various concentrations of dextran sulfate.



a) 0 g/L, b) 0.05 g/L, c) 0.15 g/L, d) 0.25 g/L, e) 0.50 g/L, f) 0.78 g/L, f) 1.0 g/L. Injection: 0.5 psi for 4 s. Detection wavelength: 210 nm. Applied voltage: 8.0 kV. Uncoated capillary, 50 μm I.D., total length 31.2 cm, and effective length 21 cm. Running buffer: 50 mmol/L phosphate, pH 7.0.

Figure 2 Electropherograms of interaction between G-CSF and κ-carrageenan oligosaccharide.



a) 0.06 g/L G-CSF. b) 1.50 g/L κ-carrageenan oligosaccharide. c) 0.06 g/L G-CSF + 1.50 g/Lκ-carrageenan oligosaccharide. d) 0.06 g/L G-CSF + 10.00 g/L κ-carrageenan oligosaccharide. Experimental conditions were the same as conditions in **Figure 1**

Former studies showed that G-CSF could bind to heparin[1]. In this experiment, the results showed that G-CSF could also bind to another polysulfated saccharide, dextran sulfate. To identify whether G-CSF can bind to any other polysulfated saccharides, the interaction between G-CSF and the other polysulfated saccharide, κ- carrageenan

oligosaccharide was studied. As shown in **Figure 2,** the peak heights and areas of G-CSF and κ-carrageenan oligosaccharide keep constant when they were injected simultaneously. Even the concentration of κ-carrageenan oligosaccharide increased 10 times, the peak of G-CSF still kept constant. Therefore, the interaction between G-CSF and κ-carrageenan oligosaccharide was not found in this experiment.

Sulfate groups of saccharide are often important in protein-saccharide interactions. This study showed that G-CSF could strongly bind to dextran sulfate, but not to κ-carrageenan oligosaccharide, which may due to the shorter chain length of κ-carrageenan oligosaccharide. These results showed that G-CSF could selectively interact with polysulfated carbohydrates. Further studies of the effect of chain length in the G-CSF – polysulfated saccharide interaction are under going.

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