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Studies on Human Immunoglobulin G from GBS Patient (III) —The Determination of Molecular Weight of Human Immunoglobulin G by Capillary SDS Gel Electrophoresis

Qin Hua RU, Yi Ming WANG, Guo An LUO*

Department of Chemistry, School of Life Science and Engineering,
Tsinghua University, Beijing, 100084

Abstract: Guillain-Barre syndrome (GBS) is considered to be an autoimmune disorder of peripheral nervous system. In this paper, capillary SDS gel electrophoresis was performed on neutral coated fused-silica capillary to determine the molecular weight of purified IgG samples from GBS patient.

Keywords: Immunoglobulin G, Guillain-Barre syndrome, Capillary SDS gel electrophoresis, Molecular weight of protein.

Introduction

Guillain-Barre syndrome (GBS) is considered to be an autoimmune disorder of peripheral nervous system[1]. The balance of sympathetic nerves and parasympathetic nerves is disturbed, which leads to complete paralysis[1, 2, 3]. Most patients show the typical symptoms after being infected for one or two weeks, but a few shows the symptoms after four weeks of infection. About eighty percent of patients have no sequel[1]. When the immune system has been disturbed, the structure of immunoglobulin G may have some changes in the changeable region of T cell. Capillary SDS gel electrophoresis (CGE) has been shown to be a powerful tool for size separation of protein molecules[4]. This technique is based on the phenomenon that SDS binds to proteins in a constant ratio of 1: 1.4, protein to SDS. The constant mass-to-charge property of the SDS-bound proteins allows separation according to the difference in protein molecular weight.

Materials and Methods

2-Mercaptoethanol was obtained from Fluka (Ronkonkoma, NY, USA). eCAP^{TM} SDS 14-200 Kit was purchased from Beckman (Beckman Instruments, Fullerton, CA, USA). Other analytically pure reagents were obtained from Beijing Reagent Factory. All solutions were prepared with distilled water and filtered through 0.45 μm membrane.

Capillary (100μm I.D.× 47cm) was obtained from eCAP^{TM} SDS 14-200 Kit. Rapid purification of antibodies was carried out on the *Biosys^{TM}2000 Workstation* by the

combined use of Protein A-HyperD and S-HyperD chromatography media. (Beckman Instruments, Fullerton, CA, USA). CGE was carried out on a Beckman P/ACE System 5500 (Beckman Instruments, Fullerton, CA, USA).

Human serum sample was first precipitated with saturated ammonium sulfate solution for three times, the precipitate was diluted (v/v, 1/1) with physiological saline and used as the crude immunoglobulin G sample. The Protein A-HyperD column (4.6mm I.D. × 50mm length, 0.8mL) was used to capture the IgG from crude sample. The Protein S-HyperD column (4.6mm I. D.×100mm length, 1.7mL) was finally used to capture the contaminants from the isolated IgG samples. The effluent was dialyzed at 4°C for 48 hours with distilled water, then frozen and dried. The sample powder was dissolved with 100μl distilled water and stored promptly at -20°C as the purified IgG sample.

The purified IgG sample (containing 0.45mg protein) was treated as the instruction from the eCAP^{TM} SDS 14-200 Kit. The sample was sonicated for 5 to 10 seconds to remove air bubbles. Prior to each run, the capillary was rinsed with 1mol/L HCl for 2 minutes and gel buffer for 5 minutes. The sample was injected by high pressure for 30 seconds. The separation was carried out at 14.1kV for 35 minutes and monitored at 214nm, with the inlet as the cathode and the outlet as the anode. The temperature was maintained at 20°C.

Results and Discussion

1 Purification of Immunoglobulin G

Protein A is a chromatographic column for the direct capture of IgG. The selectivity as well as purity after Protein A column treatment is good. The IgG was eluted from the column by increasing the acidity and decreasing the salinity. Protein S is an anion-exchanger column, which is used to remove the occasional coprecipitation of IgG in a flow through mode. **Figure 1** shows that there are nearly no impurities existing in the IgG sample eluted from Protein S column.

2 The Molecular Weight Determination of Purified IgG Sample from GBS Patient

The SDS 14-200 Kit was first used to estimate a molecular weight standard curve generated from the migration times of protein standards. In the Kit, the protein standards included α-lactalbumin (14.2kDa), carbonic anhydrase (29kDa), ovalbumin (45kDa), bovine serum albumin (66kDa), phosphorylase b (97.4kDa), β-galactosidase (116kDa), and myosin (205kDa), and the internal standard was orange G. The standard curve was obtained by plotting the log of molecular weight vs RMT for each standard protein. RMT refereed to the ratio of internal standard migration time and standard protein migration time. The linear equation is as below: Log (mol. wt.) = -3.377 RMT + 6.833, r = 0.995. The CGE result of IgG samples is shown in **Figure 2**, the migration time of reference standard was 14.650 min, while the migration time of IgG sample was 29.927 min, RMT was calculated as 0.4895. The molecular weight of IgG sample was determined as 151.3kDa.The molecular weight of normal IgG was about 160.0kDa, the difference of

molecular weight between IgG samples and normal IgG indicated that a structural change had taken place

Figure 1. Chromatography on S-HyperD of IgG purified by affinity chromatography on Protein A-HyperD. IgG sample was eluted with 1% glacial acetic acid-300mmol/L NaCl from Protein A-HyperD column, which was then loaded onto an S-HyperD column (4.6 × 100mm). The purified IgG was eluted with 50mmol/L Tris- 1000mmol/L NaCl (pH 7.5). The detection wavelength was 280nm.

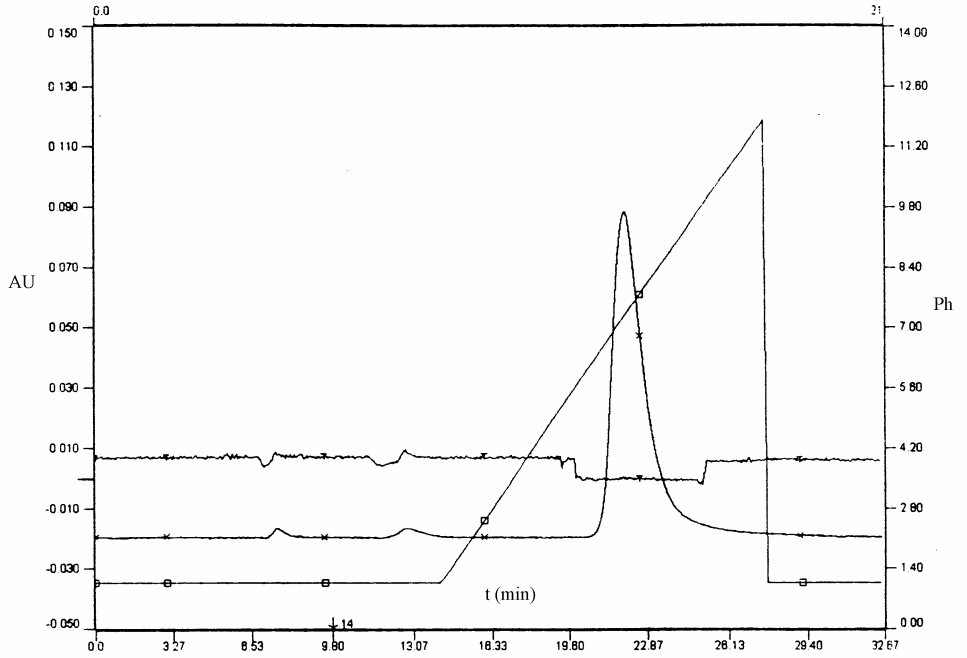
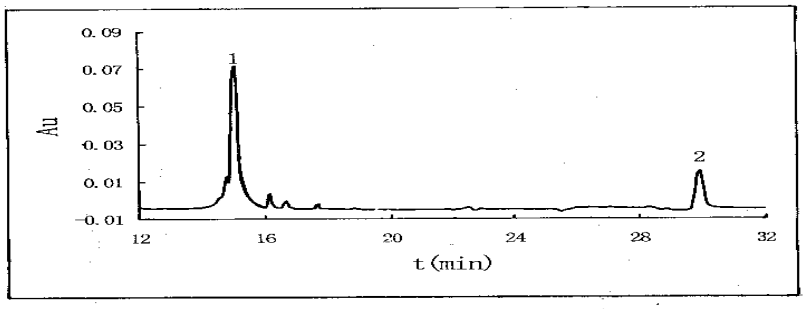


Figure 2. High-performance capillary SDS gel electropherogram of IgG sample from GBS patient. 1 = orange G, 2 = IgG sample. Capillary, 47cm × 100μm I.D. Running buffer was obtained from SDS 14-200 Kit. Sample injection, by high pressure for 30 seconds. Separation was carried out at 14.10kV for 35 minutes. The outlet, anodic side. The detection wavelength, 214nm. Temperature, 20°C.



Conclusion

In this paper, immunoglobulin G was first purified from GBS patient. With CGE, the molecular weight of IgG from GBS patient was determined as 151.3kDa, it was different from that of normal human The result indicated that structural changes had taken place in antibody protein, which led to GBS.

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