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Utilization of magnetic nanobeads for analyzing haptoglobin in human plasma as a marker of Alzheimer's disease by capillary electrophoretic immunoassay with laser-induced fluorescence detection

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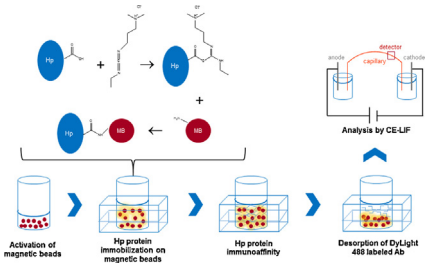
HIGHLIGHTS

- A novel immunoassay-CZE method has been developed for determining free form haptoglobin in human plasma.
- Compared with conventional method, the proposed immunoassay procedure is 55 min faster.
- Haptoglobin concentrations are significantly higher in the AD patients with severe symptoms.

GRAPHICAL ABSTRACT



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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder resulting from an impaired cholinergic function with loss of cognitive activity in the brain. Haptoglobin is a useful biomarker for AD analysis. Compared to the conventional enzyme-linked immunosorbent assay for haptoglobin analysis, the proposed immunoassay procedure reduces sample analysis time by approximately 55 min. Therefore, immunoassay was coupled with capillary electrophoresis (CE) to determine haptoglobin concentrations indirectly by using magnetic nanobeads (MBs) as a support and laser-induced fluorescence detection. In human plasma sample, the haptoglobin was immobilized on the MBs and reacted with the purified anti-haptoglobin antibody. The optimum separation time for the analyte was shorter than 6 min at 25 °C with a fused-silica capillary column of 40.2 cm × 50 μm ID (effective length 30 cm) and a run buffer containing 25 mM phosphate (pH 8.0) with 0.01% poly(ethylene oxide) (PEO). When using Atto 495 NHS ester as an internal standard (IS) (250.0 ng mL^{-1}), the linear range of the proposed method for indirect determination of haptoglobin was 0.2–3.0 mg mL^{-1}. The method was further used to monitor the course of AD in patients with behavioral and psychological symptoms of dementia (BPSD).

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia and is characterized pathologically by the successive intracellular accumulation of neurofibrillary tangles and extracellular deposition of plaques in the brain. The highest incidence of AD occurs in

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individuals aged 65 years and older, and the main presentation are progressive loss of memory and other cognitive functions. The disease may substantially impact not only on the patients, but also the family and society [1–3]. Behavioral and psychological symptoms of dementia (BPSD) are significant symptoms of AD and are very common [4]. The behavior and functional impairments caused by AD are well known, and AD is a leading cause of disability in older people living in developed countries [1]. Although many specific aspects of AD have been documented, the disease is difficult to diagnose in the early stage and the efficacy of current treatments is very limited [3]. Therefore, accurate diagnosis and control of pharmaceutical treatment are essential.

A clinical diagnosis of AD is based on the symptoms of the patient. Currently, the Clinical Dementia Rating (CDR) is widely used for diagnosing the dementia stage [5]. Statistical data indicate that the prevalence of BPSD increases as the CDR increases. Major psychotic symptoms may also occur. However, diagnosing AD based on early symptoms is difficult and error-prone. Therefore, biomarkers are needed for early diagnosis of AD and for monitoring its course.

Schizophrenia is accompanied by an activation of the inflammatory response system with signs of an acute phase response, such as increased plasma haptoglobin concentrations [6–8]. Haptoglobin is an acute phase protein capable of binding hemoglobin in plasma and a heterogeneous plasma protein mostly synthesized by the liver [9–11]. The haptoglobin monomer consists of two α-chains connected to two β-chains by a disulfide bond. The three major haptoglobin types are haptoglobin 1-1, 2-1 and 2-2 [6,8]. Studies indicate that mean haptoglobin concentrations are significantly higher in the schizophrenic individuals (>1.5 mg mL^{-1}) than in normal individuals (1.0 mg mL^{-1}) [6,8,12,13]. A search of the literature shows no studies that have linked haptoglobin concentration to AD. Statistical data indicate that the prevalence of BPSD increases as the CDR increases, and the psychotic symptoms are the same as those for schizophrenia [6,8,12,13]. Therefore, we developed this analytical method for determination of haptoglobin concentration in human plasma to monitor the course of AD. This study is the first to report the use of haptoglobin concentration for this purpose. Since haptoglobin concentrations in human plasma is a useful marker of the course of AD, a reliable analytical method of measuring haptoglobin concentrations in AD patients is needed.

Currently methods of measuring haptoglobin include immunonephelometry [8], gel electrophoresis [6,13], gel filtration [12], fluorimetric immunoassay [14], competitive immunoassay [15], enzyme-linked immunosorbent assay (ELISA) [16], high-performance liquid chromatography (HPLC) [17] and capillary zone electrophoresis (CZE) [10,18] for haptoglobin–hemoglobin complex analysis. Additionally, other analytical methods for haptoglobin phenotyping by capillary electrophoresis were published [19–22]. As noted above, proteins are usually quantified by conventional immunoassays based on the reaction of antibodies and antigens. However, these methods are susceptible to over-reporting (false positives) and under-reporting (false negatives) results, and they are difficult to couple with mass spectrometry for data corroboration [23,24]. Capillary electrophoresis (CE) is a powerful separation technique for determination of proteins. For haptoglobin analysis, a CZE method using dynamic coating has been developed based on the haptoglobin–hemoglobin complex [10,18]. The obtained haptoglobin concentration was based on total amount in plasma but not free haptoglobin. To date, no CE method has been developed for determining free haptoglobin in human plasma for the purpose of monitoring the course of AD.

Therefore, the aim of this study was to develop an immunoassay-CE with MBs as a support phase, a technique based on the immunocapture step and combine it with CE separation. A very useful characteristic of MBs is their easy manipulation by using external permanent magnets, independently of the microfluidic process. One interesting technique is the use of MBs as a solid support for immunoextraction [24–28]. In this study, haptoglobin was immobilized on particle surfaces. CE was combined with laser-induced fluorescence (LIF) detector to enable rapid and sensitive quantification of haptoglobin indirectly in human plasma. The proposed method was used to monitor the course of AD patients with BPSD. Moreover, haptoglobin concentrations in forty different AD patients with BPSD symptoms were compared with those in forty normal individuals. As the result shown, the mean haptoglobin concentrations were significantly higher in the AD patients with severe symptoms (CDR 2.0 and 3.0).

2. Experimental

2.1. Reagent and solutions

All reagents used in this study were analytical reagent grade. Haptoglobin protein standard, anti-haptoglobin antibody (mouse monoclonal to haptoglobin) and Donkey F(ab') 2 polyclonal secondary antibody to mouse IgG–H&L (DyLight 488®) (catalogue number: ab98766) were purchased from abcam® (Cambridge, England). Atto 495 NHS ester used as the internal standard (IS) (Fig. S1) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA) and ICN Biomedicals (Aurora, Ohio, USA), respectively. Sodium dihydrogen phosphate monohydrate, sodium hydroxide (NaOH), hydroxychloric acid (HCl), phosphoric acid (H_3PO_4), poly(ethylene oxide) (PEO) (MW: 8000000) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). The phosphate buffered saline (PBS) was purchased from UniRegion BioTech (Taiwan). Water treated with a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare the buffer and related aqueous solutions. The MBs 200 nm amino functionalized, Amino 1 Activation Buffer, Storage Buffer, magnetic devices, and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC) were purchased from Ademtech (Pessac, France).

2.2. Instrument and CE conditions

A Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a LIF detector (λ_{ex}: 488 nm; λ_{em}: 520 nm) and a liquid-cooling device was used. The CE was performed in a 40.2-cm uncoated fused-silica capillary with an effective length of 30 cm and an inner diameter of 50 μm (Polymicro Technologies, Phoenix, AZ, USA). The separation temperature was controlled at 25 °C by immersing the capillary in cooling liquid circulating in the cartridge, and the temperature of the sample tray was maintained at 25 °C. Before its initial use, the capillary was conditioned with MeOH for 10 min, 1 M HCl for 10 min, de-ionized water for 2 min, 1 M NaOH for 10 min and de-ionized water for 2 min. Between runs, the capillary was routinely washed with 100 mM SDS (5 min), de-ionized water (2 min), 1 M NaOH (5 min), de-ionized water (2 min) and rinsed buffer (2 min) under positive pressure applied at the injection end. The capillary was filled with background electrolyte (BGE), phosphate buffer (25 mM, pH 8.0) containing 0.01% PEO. After sample injection, a high conductivity buffer plug (HCB: 100 mM phosphate buffer (pH 8.0) containing 0.01% PEO) was introduced into the capillary at 1.0 psi for 30 s. Sample loading was achieved by electrokinetic injection at a positive voltage of 10 kV for 70 s. After HCB plug loading, the voltage was switched to reverse polarity (−7 kV) for 0.8 min to stack haptoglobin between the HCB and the sample zone. A constant voltage of +25 kV was applied throughout a run under 25 mM phosphate buffer (pH 8.0) with 0.01% PEO as BGE and at an average current of approximately 60 μA. The