Analytical Biochemistry 378 (2008) 127–131

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Determination of uric acid in human urine and serum by capillary electrophoresis with chemiluminescence detection

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ARTICLE INFO

Article history:
Received 15 December 2007
Available online 10 April 2008

Keywords:
Capillary electrophoresis
Chemiluminescence detection
Uric acid
Human serum
Human urine

ABSTRACT

A simple and sensitive method based on capillary electrophoresis (CE) with chemiluminescence (CL) detection has been developed for the determination of uric acid (UA). The sensitive detection was based on the enhancement effect of UA on the CL reaction between luminol and potassium ferricyanide ($K_3[Fe(CN)_6]$) in alkaline solution. A laboratory-built reaction flow cell and a photon counter were deployed for the CL detection. Experimental conditions for CL detection were studied in detail to achieve a maximum assay sensitivity. Optimal conditions were found to be 1.0×10^{-4} M luminol added to the CE running buffer and 1.0×10^{-4} M $K_3[Fe(CN)_6]$ in 0.2 M NaOH solution introduced postcolumn. The proposed CE–CL assay showed good repeatability (relative standard deviation [RSD] = 3.5%, $n = 11$) and a detection limit of 3.5×10^{-7} M UA (signal/noise ratio [S/N] = 3). A linear calibration curve ranging from 6.0×10^{-7} to 3.0×10^{-5} M UA was obtained. The method was evaluated by quantifying UA in human urine and serum samples with satisfactory assay results.

Uric acid (UA,[1] 2,6,8-trihydroxypurine) is the end product of catabolism of the purine nucleosides [1]. Most of UA produced from the catabolism is reabsorbed into the blood circulation system after primary filtration and partial secretion by the kidney. UA levels in physiological fluids such as plasma and urine serve as valuable indicators for certain clinical conditions. For example, an elevated level of UA in blood is associated with gout, renal failure, leukemia, and lymphoma as well as other pathological conditions [2,3]. Therefore, a simple, sensitive, and accurate analytical method for the quantitation of UA would be useful for physiological investigations as well as disease diagnosis.

During recent years, analytical methods have been developed for quantitative analysis of UA in biological samples [4]. Methods based on colorimetry and flow injection analysis with the use of uricase enzyme have been widely used [5–8]. Although enzymatic assays were very selective, these methods still suffer from drawbacks, including the pronounced effects of temperature, the need for unstable and expensive reagents, and the need for relatively large volumes of samples. Assay methods based on electrochemical analysis were also developed [9–13]. However, determination of UA in biological samples by electrochemical procedures was found to be difficult due to problems associated with selectivity and sensitivity [14]. Therefore, a number of alternative methods have been developed. HPLC methods [15–21] have become routine clinical methods because high sample throughput and assay specificity better than that with the enzymatic methods can be obtained. Capillary electrophoresis (CE) has been investigated extensively during recent years for quantitative analysis of biological samples, especially in the clinical fields [22–24], because of its simplicity, high efficiency, mass sensitivity, minimum needs of solvent and sample, and relatively low cost. CE-based methods with UV [25–30] and electrochemical detection [31–33] were developed for UA determination. A major drawback of the CE method with UV detection was its poor sensitivity, which limited its application to the determination of trace UA in some biological samples. Chemiluminescence (CL) detection is one of the most sensitive detection schemes [34]. CL detection has become an attractive detection scheme in CE because of its high sensitivity, low cost, low-power demands, and high compatibility with micromachining technologies [35,36]. To the best of our knowledge, there have been no reports on UA determination by using a CE–CL method.

In the current study, the CL reaction between luminol and potassium ferricyanide ($K_3[Fe(CN)_6]$) in the presence of UA [37] was examined, leading to the development of a proposed CE–CL method for the determination of trace UA in biological samples. UA assay of human urine and serum by the current CE–CL method was demonstrated.

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[1] *Abbreviations used:* UA, uric acid; CE, capillary electrophoresis; CL, chemiluminescence; $K_3[Fe(CN)_6]$, potassium ferricyanide; PMT, photomultiplier tube; RSD, relative standard deviation; S/N, signal/noise ratio.

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doi:10.1016/j.ab.2008.04.014

Materials and methods

Chemicals

Luminol was purchased from Fluka (Buchs, Switzerland). UA was supplied by Sigma (St. Louis, MO, USA). All other chemicals and organic solvents used in this work were of analytical grade. Milli-Q water was used throughout. All solutions were filtered through a 0.45-μm membrane filter.

The 0.1 M sodium borate stock solution was prepared by dissolving 3.814 g $Na_2B_4O_7{\cdot}10\ H_2O$ in 100 ml of water. The 0.01 M luminol solution was prepared by dissolving 0.0177 g luminol in 2 ml of 0.1 M NaOH solution and diluting to 10 ml with water. The 0.01 M $K_3[Fe(CN)_6]$ stock solution was prepared in water. The running buffer solution was prepared by mixing 7.5 ml of 0.1 M sodium borate solution and 250 μl of 0.01 M luminol solution, adjusting the pH value to 9.2 with a 0.1 M NaOH solution, and then diluting to 25 ml with water. The oxidizer solution was prepared by dissolving 250 μl of 0.01 M $K_3[Fe(CN)_6]$ stock solution in 25 ml of 0.2 M NaOH solution. The UA standard stock solutions were prepared in 0.01 M NaOH solution to give a final concentration of 1 mg/ml. Working solutions were freshly prepared by diluting standard stock solution with water. All solutions were stored in a refrigerator at 4 °C.

CE–CL apparatus

The basic design of the CE–CL system was described previously [38]. Briefly, a high-voltage supply (0–30 kV, Beijing Cailu Science Instrument, Beijing, China) was used to drive the electrophoresis. Uncoated fused silica capillaries (75 μm i.d. × 50 cm effective length, Hebei Optical Fiber, China) were used for the separation. The polyimide on the 2.5-cm end section of the capillary was burned and removed. After etching with hydrogen fluoride (HF) for 1 h, this end of capillary was inserted into the reaction capillary (320 μm i.d., Hebei Optical Fiber). A four-way Plexiglass joint held a separation capillary and a reaction capillary in place. The CL solution was siphoned into a tee. The grounding electrode was put in one joint of the tee. The CL solution flowed down to the detection window, which was made by burning 1 cm of the polyimide of the reaction capillary and was placed in front of the photomultiplier tube (PMT, R374 equipped with a C1556-50 DA-type socket assembly, Hamamatsu, Shizuoka, Japan). CL emission was collected by a PMT and was recorded and processed with a computer using a Chromatography Data System (Zhejiang University Star Information Technology, Hangzhou, China).

Human urine and serum sample preparation

A 1.0-ml fresh human urine sample was collected in a vial and stored for 2 h at 4 °C. Then the human urine sample was centrifuged for 10 min at 2000 rpm. The supernatant was transferred to a 100-ml flask, and the volume was completed with 30 mM borate buffer (pH 9.2) solution. The solution was homogenized and filtered through a 0.45-μm syringe filter and diluted 400-fold prior to introducing the separation capillary. The human serum sample was obtained from a healthy volunteer. A 0.5-ml serum sample was deproteinized by adding 1.0 ml of acetonitrile. After centrifugation at 12,000 rpm for 20 min, the supernatant was transferred into another 1.0-ml centrifuge tube and dried with an N_2 stream. The residue was dissolved in 0.5 ml of water. The solution was vortexed and kept at 4 °C. The sample solution was diluted 100-fold before injecting the solution into the CE system.

CE conditions

A new capillary was preconditioned by flushing with 1 M NaOH for 30 min before first use. Between two consecutive injections, the capillary was rinsed sequentially with 0.1 M NaOH, water, and running buffer for 3 min each. Sample solutions were injected into the capillary by hydrodynamic flow at a height differential of 20 cm for 10 s. The running voltage was 18 kV. Electrophoresis electrolyte was 1.0×10^{-4} M luminol in 30 mM borate buffer (pH 9.2). The oxidizer solution was 1.0×10^{-4} M $K_3[Fe(CN)_6]$ in 0.2 M NaOH solution.

Results and discussion

CL reaction between luminol and $K_3[Fe(CN)_6]$ in the presence of UA

It was found that UA could enhance the CL reaction between luminol and $K_3[Fe(CN)_6]$ as shown in Fig. 1. To maximize the sensitivity of CL detection, CL intensity was used to compare and examine the effects of luminol, $K_3[Fe(CN)_6]$, borate, and NaOH concentration on the UA determination. In these experiments, 6.0×10^{-6} M UA solution was injected into the CE–CL system, and the CL intensity (peak height or peak area) was recorded. Experimental conditions were selected based on the average of three determinations for each test when the relative standard deviation (RSD) of each test point was less than 5.0%.

Effect of luminol concentration

Influence of luminol concentration (from 1.0×10^{-5} M to 8.0×10^{-4} M) on the CL intensity was studied in a system keeping $K_3[Fe(CN)_6]$ concentration at 1.0×10^{-4} M, borate concentration at 30 mM, and NaOH concentration at 0.2 M. As can be seen from Fig. 2, the CL intensity increased with increasing luminol concentration in the range of 1.0×10^{-5} to 1.0×10^{-4} M, whereas it decreased with further increasing luminol concentration from 1.0×10^{-4} to 8.0×10^{-4} M. A concentration of 1.0×10^{-4} M luminol was used for further experiments.

Effect of $K_3[Fe(CN)_6]$ concentration

The effect of $K_3[Fe(CN)_6]$ concentration on CL intensity were studied in the range of 1.0×10^{-5} to 5.0×10^{-4} M. The results

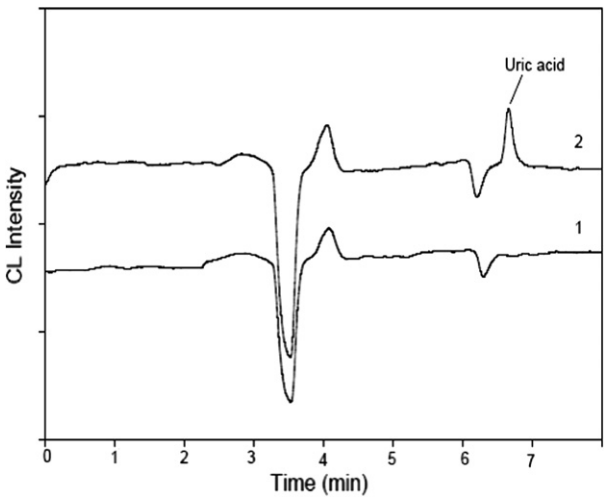

Fig. 1. Electropherograms obtained from a standard solution of UA at 6.0×10^{-6} M (trace 2) and a blank solution (trace 1). Electrophoresis electrolyte was 0.1 mM luminol in 30 mM borate buffer (pH 9.2). The oxidizer solution was 0.1 mM $K_3[Fe(CN)_6]$ in 0.2 M NaOH solution. The capillary was 75 μm i.d. × 50 cm effective length. The voltage applied was 18 kV.