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Study of Oxidation of Glutathione by Capillary Electrophoresis

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Abstract: A capillary electrophoresis method for the separation and quantification of reduced glutathione (GSH) and oxidized glutathione (GSSG) was developed. A baseline separation was achieved within five minutes. The effects of time and the concentrations of hydrogen peroxide (H_2O_2) on the oxidation of GSH were investigated.

Keywords: Glutathione, oxidation, capillary electrophoresis.

Glutathione (GSH) occurs widely in animal and human's tissues, and protects cells by changing into reversible oxidized glutathione (GSSG) when cells meet with oxidants, such as hydrogen peroxide (H_2O_2) and lipid peroxide. They are of great importance in a variety of diseases, which possess an oxidative etiology. The conversion of GSH to GSSG is widely recognized as a reliable index of oxidative stress[1]. There are some reports about determination of GSH and GSSG by high performance liquid chromatography[2,3]. In this paper, we report a capillary electrophoresis (CE) method suitable for separation and quantification of GSH and GSSG, and apply this method to study the oxidative status of GSH treated with H_2O_2.

Analysis was performed using a Bio-Focus^{TM} 2000 capillary electrophoresis system. GSH and GSSG were from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical grade. Water for all applications was supplied by a Milli-QII system. The analysis was carried on a 50 μm I.D. × 40 cm (35 cm to the detector) uncoated fused-silica capillary by pressure injection (5 psi*sec), with detection at 200 nm.

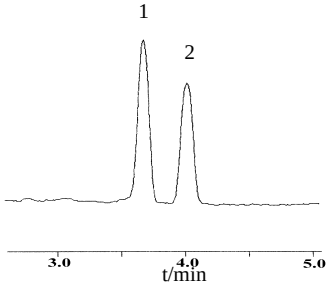
CE separation and quantification of GSH and GSSG

A 50 mM phosphate buffer was applied. The effects of different pH (1.8 ~ 9.0) run buffers on analysis were investigated. Complete or partial separations could be obtained in the range of low pH (1.8 ~ 3.0) and high pH (6.0 ~ 9.0). A low pH (1.8) run buffer was used for two reasons. First, thiol/disulfide exchanges is quenched at low pH due to protonation of the thiolate species. Second, migration time is shorter. The effects of temperature and potential on analysis were also investigated. With the increase of temperature (15°C to 30°C), migration time decreased slightly. Considering the stability of GSH, a 20°C temperature was selected. In the range of 8 kv to 20 kv, the analysis time was decreased obviously with the increase of potential. Thus, a 20 kv potential was used. Under these conditions, GSH and GSSG obtained a perfect baseline separation within five minutes, as shown in **Figure 1**.

Calibration curves of GSH and GSSG showed excellent linearity covering the ranges of tested concentration (up to 200 mg/L) with correlation coefficients of 0.9999

and 0.9979 respectively. The regression equations were A = 3577C + 602.3 for GSH and A = 3194C + 95.8 for GSSG (C: mg/L). The limits of quantification (signal/noise = 3) were 1 mg/L for both. Both relative standard deviations in migration time and peak area were less than 2% (n = 8).

Figure 1 Capillary electropherogram of GSH and GSSG

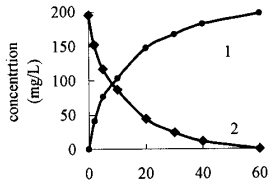


Operation conditions: applied voltage, +20 kv; temperature, 20°C; 50 mmol/L H_3PO_4, pH 1.8. Peaks: 1, GSSG; 2, GSH.

Study of oxidation of GSH

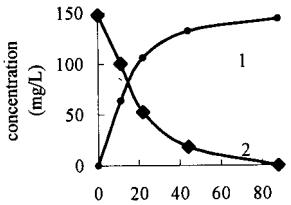
The oxidation products of GSH with H_2O_2 were investigated by the described CE method. H_2O_2 was added to GSH in a phosphate buffer (pH = 7.4) with rapid stirring at 37°C. The reaction was suspended by adding a great amount of L-methione and then analysis was carried on. In the study of the effects of the reaction time, H_2O_2 was added with 1:1 mole ratio to GSH and the samples of the oxidation products were analyzed at different time. Treatment of GSH with H_2O_2 produced only GSSG, with nearly exact stoichimetric conversion. As shown in **Figure 2**, GSSG increased with time on. The reaction was completed in 60 minutes. The effects of concentrations of H_2O_2 on the oxidation were also investigated, and reaction time was set at 10 minutes. As shown in **Figure 3**, the reaction was accelerated by the increase of H_2O_2, without other oxidation products except GSSG.

Figure 2 The effect of reaction time on the oxidation of GSH



Reaction time (min)
1. GSSG; 2.GSH

Figure 3 The effect of H_2O_2 concentration on the oxidation of GSH



Concentration of H_2O_2 (mg/L)
1. GSSG; 2.GSH

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