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Study of Oxidation of Glutathione Treated with Hypochlorous Acid by Capillary Electrophoresis

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Abstract: Capillary electrophoresis (CE) method was developed for the separation and quantification of reduced glutathione (GSH), oxidized glutathione (GSSG) and glutathione sulphonic acid (GSO_3H). Baseline separation was obtained within five minutes. The effects of reaction time and molar ratio of hypochlorous acid (HOCl) to GSH on the oxidation of GSH were investigated.

Keywords: Glutathione, oxidation, capillary electrophoresis.

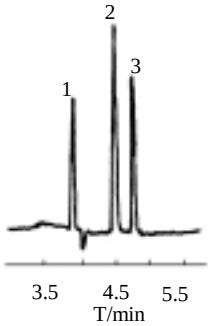
Glutathione (GSH) protects human and animal's cells by the exchange with reversible oxidized glutathione (GSSG) when cells meet with oxidants, such as hydrogen peroxide (H_2O_2) and lipid peroxide. But when GSH meets with hypochlorous acid (HOCl), *via* the myeloperoxidase-catalysed oxidation of chloride by H_2O_2, the amount of GSSG formed does not account for all the GSH lost, which is quite different from that we has investigated in the reaction of GSH with H_2O_2[1]. Prutz proposed the formation of some other oxidation products[2]. In this paper, we report a capillary electrophoresis (CE) method suitable for the separation and quantification of GSH, GSSG and glutathione sulphonic acid (GSO_3H), and apply this method to study the oxidative status of GSH treated with HOCl. Analysis was performed using a Waters Quanta 4000 CE system. A 50 μm I.D.×60 cm (52 cm to the detector) capillary was used with detection at 185 nm.

CE separation and quantification of GSH, GSSG and GSO_3H

A 20 mmol/L phosphate buffer was applied. 0.5 mmol/L tetradecyl trimethyl ammonium bromide (TTAB) was used in this buffer as electroosmotic flow (EOF) modifier[3]. The effects of different pH (7.0~8.0) run buffers on analysis were investigated. Under selected conditions, a perfect baseline separation was obtained within five minutes, as shown in **Figure 1**. Calibration curves of GSH, GSSG and GSO_3H showed excellent linearity covering the ranges of tested concentration (up to 100 mg/L) with correlation coefficients of 0.9997, 0.9993 and 0.9971 respectively. The limits of detection (signal/noise=3) were 1 mg/L for the three. All relative standard deviations in migration time and peak area were less than 1% and 4% (n=8)

respectively.

Figure 1 Capillary electropherogram of GSH, GSSG and GSO_3H

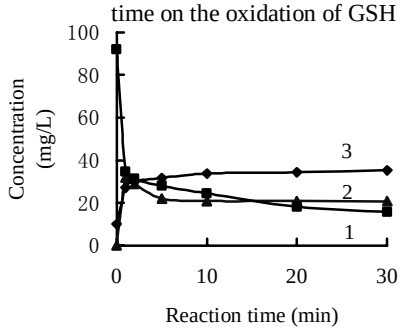


Operation conditions: applied voltage, -20 kV; temperature, 28 ℃; 20 mmol/L phosphates-0.5 mmol/L TTAB buffer, pH 7.8.
Peaks: 1, GSO_3H; 2, GSSG; 3, GSH

Study of oxidation of GSH treated with HOCl

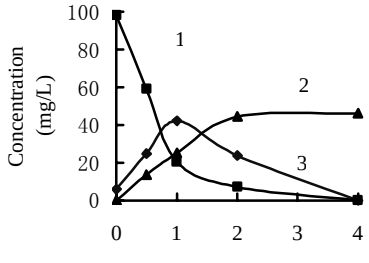
The oxidation products of GSH by HOCl were investigated by the described CE method. HOCl was added to GSH in a phosphate buffer (pH=7.4) with rapid stirring at 37°C. In the study of the effects of reaction time, HOCl was added with 1:1 molar ratio to GSH and the samples of the oxidation products were analyzed at different times. The major oxidation products were not only GSSG, but also GSO_3H, as shown in **Figure 2**. The effects of molar ratio of HOCl to GSH on the oxidation were also investigated with the reaction time set at 20 minutes. As shown in **Figure 3**, the concentrations of these two products changed differently.

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



1,GSH; 2, GSO_3H; 3, GSSG

Figure 3 The effect of molar ratio of HOCl to GSH on the oxidation of GSH



1,GSH; 2, GSO_3H; 3, GSSG

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