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Measuring hydrogen peroxide due to water radiolysis using a modified horseradish peroxidase based biosensor as an alternative dosimetry method

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ABSTRACT

H_2O_2 generated during water radiolysis was measured electrochemically as an alternative dosimetry method. A biosensor was fabricated by immobilising modified horseradish peroxidase (HRP) on a glassy carbon electrode (GCE) followed by evaluation of its analytical parameters. Anthraquinone 2-carboxylic acid was used to modify HRP. To assess sensor performance, phosphate buffer solutions were irradiated with 0.510 Gy of gamma ray emitted from ^{60}Co. The results showed that this sensor can detect low quantities of hydrogen peroxide in water radiolysis. Sensitivity, detection limit and linear range of the biosensor were 260 nA/Gy, 0.392 Gy and 0.5–5 Gy, respectively. Long term stability studies showed that sensor responses were stable for at least a month. The cathodic peak current, as biosensor response, subsequently decreased to 20% of its initial value.

1. Introduction

There are a range of techniques, methods and devices that have been developed for radiation detection and dosimetry. An appropriate dosimeter should have properties such as coverage of a range of radiation energies, online responses, high resolution, efficiency, adequate count rate and acceptable cost. Ionising radiation is absorbed by an appropriate substance. Generated pulses, because of these interactions, are proportional to the total deposited energy of the incident radiation [1,2]. Aqueous solutions are typically used as the detection medium. Different chemical reactions occur during water radiolysis, and various types of free radicals are produced which may then act as either oxidising or reducing agents. The free hydroxyl radical ($OH^{\bullet}$) is one of the most important products of water radiolysis. From water radiolysis hydrogen peroxide is produced by a combination of two OH radicals [3–5]. H_2O_2 as an electroactive species could thus provide a measure of water radiolysis and therefore have application in dosimetry. In this study, a biosensor was made using modified HRP to detect H_2O_2. The characteristics of this sensor were determined in both low concentrations of hydrogen peroxide and irradiated buffer phosphate solutions.

There are many methods to measure H_2O_2, such as spectrophotometry [6], fluorimetry [7], electrochemistry [8], chemiluminescence [9], electron spin resonance (ESR) [10] and colorimetry [11]. In this study spectrophotometry and electrochemical methods were employed. Cobalt 60 was used as a source of ionising radiation. Co-60 is a beta-emitter and the energy of the excited Ni-60 generated is emitted as gamma radiation. This radioisotope is widely used for diagnosis, radiotherapy and sterilisation. It is also used in engineering for a range of applications, e.g., the checking of welding seams to detect flaws, a labelling gauge and in food and agricultural product irradiation.

2. Materials and methods

2.1. Chemicals

Hydroquinone, anilinium sulphate, ammonium molybdate, 4-aminoantipyrine 98% (4-AAP), 1-(3-dimethylaminopropyl) -and 3-ethyl carbodiimide hydrochloride 98% (DEC) were obtained from Merck Chemical Co. 3,3″-Bis [bis (carboxymethyl) aminoethyl] cresol sulfone phtrane sodium salt (xylenol orange tetrasodium), ammonium ferrous sulphate hexahydrate, sulphuric acid, 3,5-di-tert-4-butylhydroxytoluene (BHT), H_2O_2 30% (w/w) and anthraquinone 2-carboxylic acid 98% (AQ) were obtained from Aldrich, USA. Horseradish peroxidase (HRP; EC 1.11.1.7), sodium 4-(2-hydroxyethyl) -1-piperazine ethansulfonate

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(Na-HEPES) and, superfine Sephadex G-25 were obtained from Sigma in St. Louis, MO.

2.2. Horseradish peroxidase modification

To modify horseradish peroxidase, anthraquinone 2-carboxylic acid (AQ) was covalently attached to HRP. To achieve this, 20 mg AQ was dissolved in 3 mL of a Na-HEPES solution (0.15 M, pH 7.2), followed by ultrasonication for 5 min at room temperature (Elmasonic, S30H, Germany). After that, 25 mg of DEC was added to the turbid solution. Then 6 mg of enzyme was added into AQ-Na-HEPES-DEC solution. This mixture was stirred with a magnetic stirrer (Velp, U.S.A.) at 4 °C. After 22 h, this solution was centrifuged and supernatant liquid was used for further purification. The modified enzyme (AQ-HRP) was divided from un-reacted AQ molecules using a column (2 cm diameter and 15 cm height) by gel filtration. The column was filled with superfine Sephadex G-25 and equilibrated with potassium phosphate buffer (0.2 M, pH 7.0) [12]. After gel filtration, the concentration of modified HRP (AQ-HRP) was determined by the Bradford method [13].

2.3. Production of the AQ-HRP/GCE

Modified HRP (AQ-HRP) was immobilised on a glassy carbon electrode (GCE) surface. The following steps were taken in GCE preparation. First, the GCE surface was mechanically polished using Alumina powder, 10 and 0.3 μm diameter. Second, to remove alumina pieces and other contaminants from the electrode surface, the GCE was ultrasonicated for 5 min in water and ethanol. Third, to activate the electrode surface, the GCE was electrochemically treated between −1 to 0.5 V (vs Ag/AgCl) at a 100 mV/s scan rate in 0.2 M sulphuric acid for 10 min. Fourth, the electrode was immersed in 0.2 M potassium phosphate buffer (pH 7.0) at + 1.70 V (vs Ag/AgCl) for 10 min. Finally, to immobilise AQ-HRP on the GCE surface, the electrode was immersed in the oxygen free AQ-HRP solution for one hour. Cyclic voltammetry was carried out using 0–0.7 V (vs Ag/AgCl) at a 100 mV/s scan rate during the immobilisation process. Now the modified GCE was ready for the measurement of H_2O_2. The biosensor is subsequently specified as AQ-HRP/GCE. The effectiveness of this method for H_2O_2 measurement was electrochemically evaluated. Cyclic voltammetry and chronoamperometry was carried out using a potentiostat/galvanostat (DropSens, Spain and Autolab, Holland). A three electrode system which consisted of a platinum rod, Ag/AgCl and the AQ-HRP/GCE was used, respectively, as auxiliary, reference and working electrodes.

2.4. Spectrophotometric measurement

Hydroquinone–aniline catalysed by molybdate as well as by ferrous oxidation–xylenol orange (FOX-2 method), were used for spectrophotometric measurements of H_2O_2 [14,15]. For the first method, 0.25 M hydroquinone, 0.125 M anilinium sulphate and ammonium molybdate (0.5% w/v) were individually prepared in doubly distilled water. 2.4 mL of hydroquinone, 1.6 mL of anilinium sulphate and 0.25 mL of ammonium molybdate were mixed in blank and spectrophotometer cuvettes test (CECIL, CE 2501 Model). The instrument was adjusted to zero at 550 nm. For measurement at each absorbed dose, 0.75 mL of unirradiated and irradiated potassium phosphate buffer (0.2 M, pH 7) was added to blank and test cuvettes, respectively. The absorbance was measured after 10 min.

To measure H_2O_2 concentration using the FOX-2 method, 1 mM xylenol orange and 2.5 mM ammonium ferrous sulphate were prepared in 250 mM H_2SO_4. This solution is defined as working solution I; working solution II (4.4 mM BHT) was prepared in methanol. 0.2 mL of working solution I and 1.8 mL of working solution II were mixed in the blank and into a test cuvettes. The final concentration of ammonium ferrous sulphate, xylenol orange, and H_2SO_4 in each cuvette was 250 μM, 100 μM and 25 mM, respectively. To measure the H_2O_2 generated, 3 mL of irradiated potassium phosphate buffer (0.2 M, pH 7) was added to test cuvette. After 10 min, absorbance was monitored at 590 nm. Appropriate correction coefficients and dilution factors were considered in both methods to measure precise amounts of H_2O_2.

2.5. Gamma irradiation

12 samples containing 15 mL of 0.2 M potassium phosphate buffer were prepared at pH 7 (25 °C). Samples received irradiation at the following doses using a Picker-V9-Co-60 unit (USA). Zero (as reference dose), 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 Gy. The dose rate of the gamma source was 0.8 Gy/min, therefore, the appropriate absorbed dose was adjusted by changing exposure time for each sample. Irradiation was performed at the Secondary Standard Dosimetry Laboratory (S.S.D.L.) in the Iran Atomic Energy Organisation (I.A.E.O.).

3. Results and discussion

3.1. Effect of HRP modification on the AQ-HRP/GCE performance

To discover the roles of native and modified HRP on biosensor performance, the electrochemical behaviour of the HRP/GCE and the AQ-HRP/GCE was evaluated in H_2O_2 at a constant concentration of AQ (0.2 mg/mL) in phosphate buffer. Cyclic voltammetry was carried out to determine biosensor response. Fig. 1 (cyclic voltammograms A, B and C) represents the responses of a bare GCE electrode (in the absence of HRP) and native (HRP/GCE) and modified HRP (AQ-HRP/GCE) electrodes, respectively. Voltammogram A shows that cathodic and anodic peak current is not produced in the absence of HRP, therefore it indicates that the surface of the GCE is free of any confounding materials. Sharp anodic and cathodic peak currents in voltammograms B and C provide evidence of excellent native HRP and AQ-HRP immobilisation on GCE surface. However, anodic and cathodic currents due to AQ-HRP/GCE immobilisation are considerably greater than those due to HRP/GCE immobilisation. So, use of AQ-HRP as biosensing agent leads to a significant improvement in AQ-HRP/GCE response. It appears that

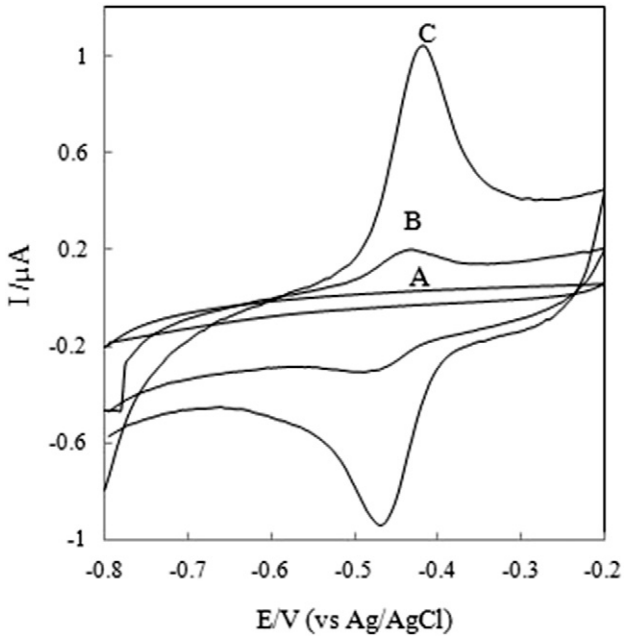

Fig. 1. Voltammograms A, B and C represent the biosensor responses. Voltammogram A is related to bare glassy carbon electrode. Voltammograms B and C are related to biosensor responses after immobilisation of native HRP (HRP/GC) and modified HRP (AQ-HRP/GCE) on glassy carbon electrode surface, respectively. The cyclic voltammetry experiments were carried out in N_2 saturated 0.2 M buffer phosphate solution at pH 7, 25 °C in 200 nM H_2O_2 solution at 50 mV/s scan rate.