Hydrogen peroxide biosensor based on carbon nanotube/horseradish peroxidase bionanocomposite

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Introduction

Carbon nanotubes (CNTs) have unique properties, as good conductivity, high chemical stability and it is possible to bring the nanotubes close to the redox centers of the proteins.

Peroxidases are heme containing enzymes in plant tissues which can oxidize different hydrogen atoms and for example xenobiotics in the presence of H_2O_2. Hydrogen peroxide is a strong oxidant so it is important to determine its exact concentration.

Horseradish peroxidase (HRP, Figure 1) has long been a representative enzyme in understanding the biological behavior of catalyzed oxidation of the substrates H_2O_2, so it is the most commonly used enzyme in H_2O_2 detection.

Figure 1. Three dimensional view of horseradish peroxidase. The heme group is located in the center (black) with the iron atom (in red).

In order to define the enzyme activity of HRP we used the chemical decomposition procedure of H_2O_2. To follow this process we chose guaiacol, which agent can be simply oxidized and its oxidation results colored product (tetraguaiacol). This product's cumulation then can be easily detected by absorption or emission (fluorescence) spectroscopy.

$$4\ H_2O_2 + 1\ \text{guaiacol} \rightarrow \text{enzyme} \rightarrow 4\ H_2O + \text{tetraguaiacol}$$

Aims

- Binding the HRP to the surface of the carboxyl-functionalized MWNT
- Measuring the HRP activity of the bio-nanocomposite
- Using fluorescence technology on the bio-nanocomposite
- Optimizing the parameters, reproducibility and more sensitive H_2O_2 detection

Experimental method

Bio-nanocomposite preparation

Carboxyl-functionalized multiwalled carbon nanotube (MWNT) was activated by the addition of crosslinkers NHS (N-hydroxysuccinimide) and EDC (1-[3-dimethylaminopropyl]-3-ethyl-carbodiimide). After the activation procedure the mixture was centrifuged for 15 minutes and the deposit was suspended in phosphate buffer (0.1 M, pH 6.0). Then the HRP enzyme solution (1 μM) was added to the activated MWNT and it was mixed at 4°C overnight. Finally the sample was separated by ultracentrifuge.

Absorption kinetics

Absorption change was measured at 470 nm by a single-beam kinetic spectrophotometer of local design. The enzyme activity can be determined from the absorption kinetic measurements by using the known absorption coefficient.

Fluorescence measurements

Fluorescence was measured by a fluorimeter (Perkin Elmer MPF44A). Due to the excitation peaks of tetraguaiacol the excitation and the emission wavelength were 295 nm and 350 nm, respectively. The reaction was initiated by addition of H_2O_2 to our bio-nanocomposite sample and guaiacol transformed to tetraguaiacol. The decomposition of the colored product was followed and then the amount of H_2O_2 was calculated.

Enzyme activity

The enzyme activity was determined from the absorption kinetics and fluorescence. The measurement of absorption change gives a chance to assign the concentration of guaiacol, so the concentration change of H_2O_2 can be determined in absolute value, because we know the molar extinction coefficient of the guaiacol ($\varepsilon(\lambda) = 26611\ M^{-1}cm^{-1}$). Applying the Beer-Lambert law the amount of the oxidized H_2O_2 can be calculated.

$$\Delta E(t) = \log \frac{I_0}{I_0 - 0.01 \cdot \Delta I(t)}$$

$$[H_2O_2](t) = \frac{E(t)}{4 \cdot \varepsilon(\lambda) \cdot l}$$

In view of used enzyme quantity the enzyme activity can be determined in $M_{H_2O_2}/(M_{enzyme}*s)$ unit. The initial slope of $[H_2O_2]$ vs. t diagram shows the initial rate of the enzyme reaction.

$$v_{initial} = \frac{d[H_2O_2]}{dt}$$

In the case of the bio-nanocomposite we measured the fluorescence of tetraguaiacol.

Results

Determination of the initial rate of H_2O_2 decomposition by measuring absorption change

To assign the enzyme activity the initial rate of H_2O_2 decomposition of the solutions with different enzyme concentrations were plotted as a function of the concentration. By means of the fitted line the increase of the initial rate of H_2O_2 decomposition can be seen as a function of the HRP enzyme concentration. The slope of the curve shows the enzyme activity with the unit of $\mu M_{H_2O_2}/(nM_{enzyme}*s)$, in our sample this value is 19.4 $\mu M_{H_2O_2}/(nM_{enzyme}*s)$.

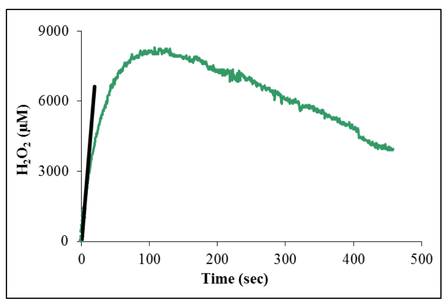

Figure 2. The amount of H_2O_2 oxidized by 16 nM solution of HRP as a function of the time. The figure was made by the absorption change of guaiacol.

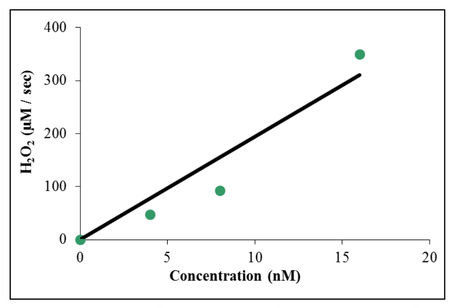

Figure 3. The initial rate of the decomposition of H_2O_2 as a function of the enzyme concentration.

Determination of the initial rate of H_2O_2 decomposition by fluorescence measurements

In order to determine the enzyme activity of the MWNT/HRP complex we need to calculate the amount of the transformed guaiacol by measuring the fluorescence of tetraguaiacol. Fluorescence measurement is proved to be more sensitive and more appropriate method for sample that scatters the light. Similarly to the absorption change measurements we plotted the fluorescence change kinetics with different enzyme concentrations and we assigned the initial slopes of the curves.

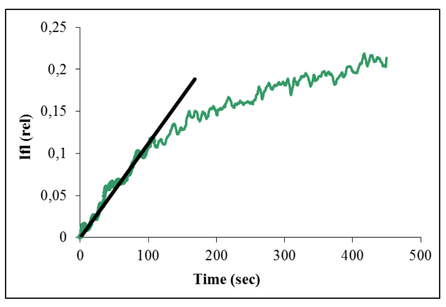

Figure 4. The fluorescence of tetraguaiacol as a function of time in the 16 nM HRP solution.

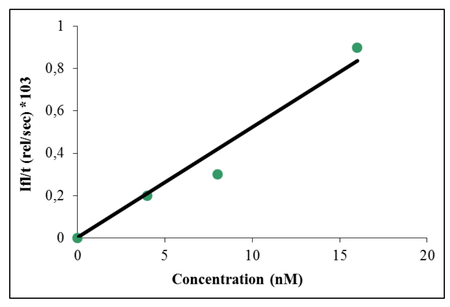

Figure 5. The initial rate of the fluorescence change as a function of the enzyme concentration.

After these a joint calibration line was made from the concentration-dependences belonging to the absorption and fluorescence kinetics. This line shows the degree of the H_2O_2 decomposition belonging to the different fluorescence changes.

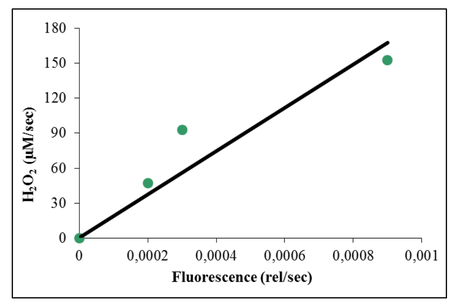

Figure 6. The amount of the dissolved H_2O_2 as a function of the initial slope of fluorescence change of tetraguaiacol.

Determination of the H_2O_2 decomposing ability of carboxyl-functionalized MWNT/HRP complex

Based on the calibrations showed earlier, from the slope of the line the amount of the H_2O_2 decomposition can be calculated. In the case of our sample this value arose 4 $\mu M_{H_2O_2}$/sec.

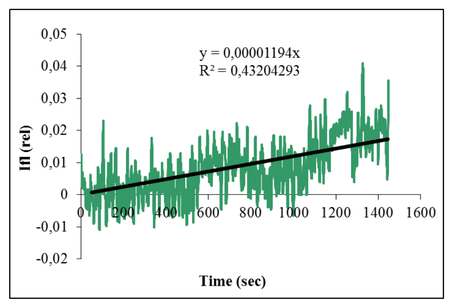

Figure 7. The fluorescence of tetraguaiacol in the case of HRP enzyme/CNT complex in PBS buffer as a function of time.

Conclusions

Our results indicate that we managed

- to determine the enzyme activity of horseradish peroxidase by absorption change measurements in a very sensitive way
- to create the conditions of definition of the enzyme activity by fluorescence measurements
- to bind HRP enzyme to carboxyl functionalized MWNTs
- determine the amount of decomposable H_2O_2 by our bio-nanocomposite in a concentration near to the LOD (limit of detection)
- our LOD result is 4 $\mu M_{H_2O_2}$/s which seems to be good compared to the sensitivity written in the literature

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