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Cyclic Voltammetric Determination of Glutamic-pyruvic transaminase Activity based on the transdeamination

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Glutamic-pyruvic transaminase (GPT) is one of the most important enzymes in human liver and has a valuable clinical reference for the diagnosis of many liver diseases. Here a method for the determination of the GPT activity based on the transdeamination was presented. In this method, a three electrode setup was used for the cyclic voltammetric determination of enzyme. With the electrochemical detection of reduced nicotinamide adenine dinucleotide (NADH) produced from transdeamination, the GPT activity was characterized under optimal circumstance. Firstly, to verify the response for the NADH of the electrode, a series of NADH concentrations varying from 39 µM to 2.5 mM were calibrated with cyclic voltammetry (CV). And a linear relationship between the NADH concentration and the peak current with R² 0.9999 was obtained. Then the concentration of α-ketoglutarate (α-KG) which can exert great influence on the transdeamination was also optimized and the most sensitive response was achieved at the point of 0.75 mM α-KG. At last, the GPT activity was determined using both unmodified screen-printed carbon electrode (SPCE) and electrode modified with CNT. The results showed that the relationship between the GPT activity and the peak current of the CV curve was linear between 60 U/L and 300 U/L and the modified electrode exhibited a slightly better linear relationship than the unmodified electrode. This work proposes a new enzymatic reactive system based on the transdeamination for the electrochemical detection of GPT activity and combines the electrochemical detection of NADH with determination of GPT activity.

Introduction

GPT is one of most important enzymes in liver, heart, muscle and other organs. When a body tissue or an organ such as the liver or heart is diseased or damaged, additional GPT is released into the bloodstream, causing levels of the enzyme to rise. Therefore, the GPT activity in the serum has a valuable clinical reference for the diagnosis of many diseases such as biliary tract disease, cardiovascular disease, and hepatopathy. Typically, for human live, GPT mainly exists in hepatic cell plasma where the GPT activity is about 3000 times more than that in serum. Once 1% hepatic cells are damaged, the concentration of GPT in serum would be twice higher than normal concentration. Now the serum GPT activity has been regarded as a reliable and sensitive marker of liver disease.

A positive correlation between serum GPT and degree of hepatic disease has been proved. For example, GPT levels 5 times greater than the normal range indicate severe acute liver cell injury. So the GPT concentration is commonly used for the assessment of liver disease. Moreover, GPT has been discovered to play a role as a predictor of mortality which does not originate from liver disease. GPT activity may be important not only as a marker of liver disease but also as an indicator of general health. Therefore, a rapid and high sensitive method to detect the serum GPT activity is beneficial to diagnosis of those diseases. But methodologies for determination of GPT activity, routinely performed in clinical laboratories, are costly, requiring complex reagents and trained operators, such as spectrophotometric measurement, chemiluminescence, chromatography, fluorescence and UV absorbance.

Recently, the electrochemical detection of GPT has drawn increasing attentions because of high sensitivity, adjustable selectivity, low sample consumption and low cost. The assay of GPT activity is based on the electrochemical detection of electroactive analytes produced from enzyme reaction system because GPT acts as a biological catalyst in human body. For example, He and Chen detected the GPT in the human serum through measuring the production rate of β-nicotinamide adenine dinucleotide (NAD⁺) with the method of differential pulse voltammetry. And a linear range of GPT activity from 0-200 U/L with the detection limit of 0.3 U/L and sensitivity of 0.0567 nA-min/J-U·L⁻¹ were obtained. Paraíso et
used a graphite electrode modified with poly(4-aminophenol) and 4-aminoantipyrine as electrochemical indicator for the detection of H$_2$O$_2$, which showed good performance for detection of GPT with a linear range of $3.0 \times 10^{-5}$ to 3.0 U/L and a detection limit of $2.68 \times 10^{-5}$ U/L. Generally speaking, the NAD$^+$ electrochemical detection has a low current response, which adds to the measuring difficulties. The H$_2$O$_2$ electrochemical detection is widely used but the generation of H$_2$O$_2$ is susceptible to the amount of oxygen and the instability of H$_2$O$_2$ has an adverse impact on the detection of GPT.18

More recently, the electroactive analyte NADH acts as an essential cofactor in hundreds of dehydrogenase enzymes, which are widely applicable in electrochemical biosensor for biochemical detection.19 For example, Gholizadeh et al.20 fabricated an electrochemical glutamate biosensor by employing the high density vertically aligned CNT nanoelectrode array through detecting the NADH, which showed a wide detection range of 0.01-300 μM and a low detection limit of 10 nM for glutamate. In general, the generation of NADH is immune from the concentration of oxygen in the reaction system which is an outstanding advantage in the electrochemical detection. Besides, the dehydrogenase that this NADH electrochemical detection relies on is generally more specific for the substrates compared with the oxidase that is crucial for the H$_2$O$_2$ electrochemical detection.18 As far as we know, there are few papers about the NADH electrochemical detection published for the detection of GPT activity. Fonong and Barber21 measured the rate of consumption of NADH amperometrically which related to the activity of the GPT based on the enzymatic reactions.

In the present work, a new enzymatic reaction system based on the transdeamination is proposed for GPT detection through examining the NADH voltammetrically. The NADH generating from the transdeamination can be oxidized on the electrode. The oxidation current has certain correlation with the GPT activity. This method combines the electrochemical oxidation of NADH with the detection of GPT activity and is of significance for the application of the dehydrogenase-based electrochemical biosensor to the detection of transaminase.

**EXPERIMENTAL**

**Materials**

Glutamic-pyruvic transaminase (GPT, from porcine heart, 200 UN), glutamic dehydrogenase (GLDH, from bovine liver Type II as aqueous glycerol solution, ≥35 units/mg protein), L-alanine (≥99.0%, NT), α-ketoglutarate, sodium salt (α-KG, ≥98%, enzymatic), β-Nicotinamide adenine dinucleotide, sodium salt (NAD$^+$, from Saccharomyces cerevisiae), pyridoxal 5'-phosphate hydrate (PLP, ≥98%) were purchased from Sigma/Aldrich. The reduced nicotinamide adenine dinucleotide (NADH) was purchased from Roche. All solutions were freshly prepared in phosphate buffer solution (PBS, pH 7.4, 0.1 M).

**Instruments and Measurement procedures**

The experimental device and the electrode are demonstrated in Fig. 1. Electrochemical measurements were carried out with a CHI 650E Electrochemical Work Station (CH Instrument Co. of Shanghai, China) in a three electrode setup using two kinds of screen-printed carbon electrode (SPCE) strips (the unmodified electrode, the electrode modified with CNT). The electrode strips consist of screen-printed carbon electrode as working electrode, silver as reference electrode, carbon as counter electrode purchased from Dropens in Asturias, Spain. All the experiments were performed at 25 °C in a total reaction bottle of 6 ml.

**Detection Principle**

The detection principle is based on the transdeamination which is one of most important ways for the ammonia release.
metabolism in human body. The transdeamination of alanine is illustrated in Fig. 2. NADH is generated through transamination catalyzed by GPT with the help of pyridoxal 5'-phosphate (PLP) and deamination catalyzed by glutamic dehydrogenase (GLDH) which has absolute specificity for the L-glutamate. The generated NADH can be oxidized to NAD$^+$ and release two electrons on the surface of electrode. The amount of electrons is linear with the concentration of NADH. And the concentration of NADH has a certain positive relationship with GPT activity. So the GPT activity can be detected through CV.

RESULTS AND DISCUSSION

Calibration for NADH

The direct oxidation of NADH on a bare electrode is highly irreversible and needs a considerable overpotential (typically more than 1.0 V on carbon electrodes) owing to the slow kinetics of the electron transfer.$^{22,23}$ To verify the response for the NADH of the unmodified SPCE strip, a series of concentrations of NADH varying from 39 µM to 2.5 mM were electrochemically tested through CV at the scan rate of 50 mV/s. The results are illustrated in Fig. 3.

From the cyclic voltammograms in Fig. 3A, the unmodified SPCE has a low oxidative potential (0.56 V) for NADH and response with a wide detection range even for the low concentration of NADH (1.57 µA for 0.039 mM NADH). Fig. 3B exhibits the relationship between the concentration of NADH and the peak current. As illustrated, the peak current proportionally increases as the concentration of NADH increases and a linear relationship with $R^2 = 0.9999$ between them can be obtained. The wide detection range, low oxidative potential and a good linear relationship for NADH are beneficial to the determination of GPT.

$\alpha$-KG Concentration Optimization

From the mechanism of transaminitation in Fig. 2, the concentration of $\alpha$-KG remains unchanged before and after reactions. It works as a carrier of amino. So it can be regarded as one kind of catalyst. The transamination can be simplified to the reaction (1) below. Previous report showed that the transamination for L-amino acid in the human tissue was slow-moving. However the transamination could be enhanced significantly if a small amount of $\alpha$-KG is added.$^{24}$

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\text{Cyclic voltammograms of different concentrations of NADH taken at the scan rate of 50mV/s, (B) Calibration curve between different concentrations of NADH and the peak currents of cyclic voltammograms.}
\]
The performance of this new reaction system for detecting the GPT activity was explored with CV. Different concentrations of GPT were prepared with PBS containing 83.3 mM L-alanine, 0.75 mM α-KG, 0.1 mM PLP, 7.5 mM NAD⁺ and 0.1 mg/ml GLDH. After the solutions reacting thoroughly for 2.5 h at 25 °C, the electrochemical measures were undertaken at the scan rate of 50 mV/s.

Fig. 5A and 5B show the cyclic voltammograms on the unmodified and modified SPCE respectively. Five concentrations of GPT including 60 U/L, 120 U/L, 180 U/L, 240 U/L and 300 U/L were prepared for the reaction system. From the cyclic voltammograms, the oxidative potential of five curves is in the range of 0.59 mV and 0.62 mV for the unmodified SPCE whereas 0.56 mV and 0.60 mV for the modified SPCE. The oxidation potential for NADH on the modified SPCE is slightly lower than that on the unmodified SPCE. From the calibration curves in Fig. 5C and 5D, the peak currents increase as the concentration of GPT varies from 60 U/L to 300 U/L. The results exhibit a linear relationship between the GPT activity and the peak current with R² 0.9722 on the unmodified SPCE and 0.9879 on the modified SPCE. A slightly better linear relationship can be obtained on the modified electrode which might contribute to the electrical and chemical characteristics of CNT. Additionally, this method possesses a sensitivity of 0.0103 μA/U·L⁻¹ on the unmodified SPCE and 0.0108 μA/U·L⁻¹ on the modified SPCE on the basis of the slope of the calibration curve. Similarly, the modified

\[ L - \text{alanine} + \text{NAD}^+ + H_2O \xrightarrow{\alpha-KG} \text{Pyruvate} + \text{NADH} + \text{NH}_4^+ \] (1)

Here the effect of α-KG concentration on the transdeamination is quantitatively investigated. All the solutions were freshly prepared with PBS. The concentration gradient of α-KG composed of 0.125 mM, 0.25 mM, 0.5 mM, 0.75 mM, 1 mM was prepared. The final concentrations of the other enzymes and the substrates except α-KG were 83.3 mM for L-alanine, 250 U/L for GPT, 0.1 mM for PLP, 7.5 mM for NAD⁺ and 0.1 mg/ml for GLDH. All the solutions above were mixed thoroughly in the detection bottles. CV curves were taken after the enzymatic reactions fully reacting at 25 °C and illustrated in Fig. 4. Both unmodified and modified SPCE strips were implemented for the electrochemical detection.

Fig. 4A and 4B demonstrate the cyclic voltammograms of enzymatic reaction systems containing different concentrations of α-KG on the unmodified and modified SPCE respectively. From the cyclic voltammograms in Fig. 4A and 4B, the peak current on the modified SPCE increases by 14.19 % at most and 5.37 % at least compared with response of the unmodified SPCE. More importantly, from the profile of the curves in Figure 4C, the peak current rises firstly and then descends with the increasing of the concentration of α-KG and has a maximum when the concentration of α-KG is 0.75 mM. According to the result, the effect of α-KG is coarsely consistent with the results reported by Hird and Marginson 24.

Determination of Enzyme Activity

\[
\begin{align*}
&\text{y} = 0.0103x + 1.1806 \\
&R^2 = 0.9722
\end{align*}
\]

\[
\begin{align*}
&\text{y} = 0.0108x + 1.1544 \\
&R^2 = 0.9879
\end{align*}
\]
electrode holds a slightly better sensitivity for the detection of GPT activity.

CONCLUSION

This work proposes a new enzymatic reactive system based on the transdeamination for the electrochemical detection of GPT activity. This reaction system combines the electrochemical detection of NADH with determination of GPT activity. Here a series of concentrations of NADH were calibrated with CV. A linear relationship between the concentration of NADH and the peak current with R² 0.9999 was obtained. Then the concentration of α-KG was optimized and the most sensitive response was achieved at the point of 0.75 mM α-KG. The responses of an unmodified SPCE strip and a SPCE strip modified with CNT were compared. The result showed that the response of the electrode modified with CNT increased by 14.19 % at most and 5.37 % at least. At last, different concentrations of GPT were detected based on this reaction system. The results showed that the relationship between the GPT activity and the peak current of the CV curve was linear between 60 U/L and 300 U/L. This makes it possible to apply the dehydrogenase-based electrochemical biosensor to the detection of GPT activity and has potential application for the detection of transaminase activity.

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We propose a new enzymatic reactive system based on transdeamination for the electrochemical detection of glutamic-pyruvic transaminase activity.