Label-Free Detection of Alanine Aminotransferase Using a Low Operation Voltage and Single Reaction Step of Graphene Field-Effect Biosensor

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Alanine aminotransferase (ALT) is an enzyme that catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate. It is released to the blood because of tissue injury; consequently, the level of ALT activity in the blood may be increased with acute damage to hepatic cells. Although not specific for liver disease, it can be employed in combination with other enzymes to diagnose the course of various liver disorders [1]. In addition, the blood of voluntary donation is carefully screened to protect patient safety in Taiwan. All donated blood was tested to be elevated in ALT levels by serological techniques [2]. The blood with high levels of ALT activity has to be discarded because they are unsuitable for the use in blood transfusion. Consequently, rapid and low cost devices for rapid blood screening of ALT activity in routine blood donation are needed. The concept of such an ISFET device for electrical detection of chemical and biological species has also been shown to work using novel nanomaterial devices, such as silicon nanowires [3], carbon nanotubes [4] and graphene [5]. Graphene field-effect transistor (GFET) has been proposed as a promising candidate for sensitive and label-free detection of chemical and biological species because of its high carrier mobility, high saturation velocity, and large current density [6]. In this study, we developed a single reaction step GFET biosensor for ALT detection.

In order to develop a rapid and simple biosensor for the ALT detection, the single-process biochemical reaction of a GFET biosensor was proposed. We prepared a GFET biosensor version of our device, using L-alanine and α-ketoglutarate as the biocomponent. Fig. 1 illustrates the hybrid configuration of our ALT GFET biosensor, which measured the ALT concentration by detecting the variation in pH caused by the generation of H^+ ions of the L-glutamate and pyruvate. According to the equation 1, the L-alanine with α-ketoglutarate is converted to L-glutamate and pyruvate by ALT.

$$\alpha - Ketoglutarate + L - Alanine \xrightarrow{ALT} L - Glutamate + Pyruvate \quad (1)$$

Briefly, the ALT concentration was proportional to the rate of pH change in the solution. The pH change is based on the fact that there is the difference in the deprotonation abilities between reactants (L-alanine: pK_{a1}^{a} =2.4; α-ketoglutarate: pK_{a1}^{k} =1.90) and products (L-glutamate: pK_{a1}^{g} =2.1; pyruvate: pK_{a}^{p} = 2.39) [7]. The hydrogen ion concentration ($[H^+]$) in solution could be demonstrated as:

$$[H^+] = \sqrt{C_a K_{a1}^a + C_k K_{a1}^k + C_p\left(K_{a1}^p + K_{a1}^g - K_{a1}^a - K_{a1}^k\right)}$$

$$pH = -\log[H^+] = \frac{1}{2}\log\left[\frac{1}{C_a K_{a1}^a + C_k K_{a1}^k + C_p\left(K_{a1}^p + K_{a1}^g - K_{a1}^a - K_{a1}^k\right)}\right] \quad (2)$$

where C_a is the L-alanine concentration, C_k is the α-ketoglutarate concentration, and C_p is the pyruvate concentration. According to the equation (2), a higher ALT concentration causes a more rapid production of L-glutamate and pyruvate, thus giving rise to the increase in pH. The deprotonation ablilities of products are less compared with the reactants. For the detection of ALT, we employed the entrapment method to immobilize the L-alanine and α-ketoglutarate—employing an alginate and $CaCl_2$ solution, which reacted to form calcium alginate—on a graphene film. This immobilization procedure preserved the native properties of the trapped enzyme. Fig. 2(a) presents the σ-V_{gs} curves of the ALT GFET biosensors for various ALT concentrations in buffer solutions. We found that the conductivity and Dirac point voltage increased upon increasing the ALT concentration. Fig. 2(b) depicts that a single biochemical reaction step of an ALT GFET biosensor exhibited a linear response (R^2 = 0.99) toward the ALT concentrations in the range from 10 to 100 U/L, with an evaluated detection sensitivity of 0.42 mV(U/L)$^{-1}$. It is expected that this biosensor can potentially serve as the diagnosis tool for general clinical examinations.

References

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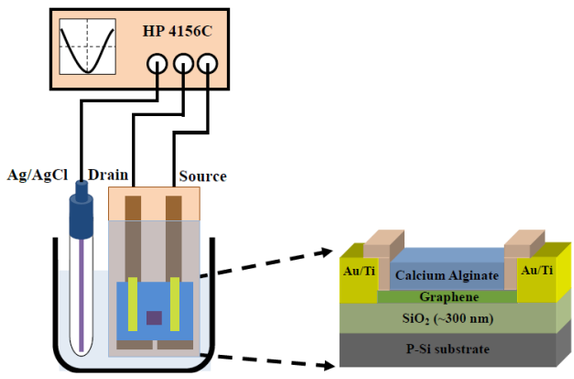

Fig. 1. Schematic representation of the preparation of a GFET ALT biosensor.

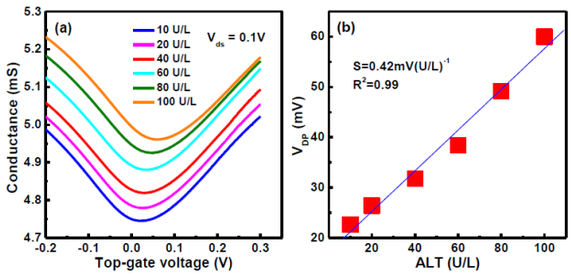

Fig. 2. (a) Conductivity versus gate potential plot of a GFET device for different ALT concentrations. (b) Dirac point voltage as a function of ALT concentrations for a GFET biosensor.