MICROFLUIDIC SYSTEM FOR THE ANALYSIS OF GOT AND GPT ACTIVITIES

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Rapid and precise determination of the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in physiological fluids is critical in the diagnosis of diseases such as myocardial infarction and hepatitis. For that purpose, a micro analysis system which can conduct necessary procedures on a single chip will be very advantageous. Conventional methods for the GOT and GPT activities require incubation, relatively large amount of expensive reagents including enzymes and cofactors, and complicated procedures. To solve the problem, we used an L-glutamate sensor and mixing of reagents was done in a microfluid channel.

The system consists of a thin-film three-electrode system formed on a glass substrate and a PDMS micro flow channel (Fig.1). The working and auxiliary electrodes were made from platinum, whereas reference electrode was formed with Ag/AgCl. The active areas of the electrodes were delineated with a polyimide layer. The active area of the working electrode was 500 µm × 300 µm. L-glutamate oxidase was immobilized on the working electrode in a bovine serum albumin matrix crosslinked with glutaraldehyde. A Y-shaped micro flow channel was formed in a PDMS substrate using a template with thick-film photoresist structures. The width and depth of the micro flow channel were 500 µm and 110 µm, respectively. The substrate with electrodes and the substrate with the flow channels were carefully aligned and fixed as shown in Fig.1. The L-glutamate produced by the enzymatic reaction was determined with the on-chip L-glutamate sensor. The produced H_2O_2 as a result of the enzymatic reactions was measured on the working electrode.

Batch-style measurements were first conducted to characterize the basic performance of the L-glutamate sensor. The lower detection limit of the senor was 10 µM and a linear relationship was observed for concentrations < 0.5 mM, which was enough to measure the GOT and GPT activities of normal levels in physiological fluids. The sensor showed cross-sensitivity to L-aspartic acid and L-alanine, both of which are the substrates for GOT and GPT. However, the sensitivities to these amino acids were negligible (0.22 µA/M for L-aspartic acid, 0.05 µA/M for L-alanine) compared with that to L-glutamate (115 µA/M).

The determination of the GOT and GPT activities was conducted in the flow channel. In the present application, it is not necessary to flow solutions continuously. A sample solution and a substrate solution (L-aspartic acid and 2-oxoglutaric acid for GOT, L-alanine and 2-oxoglutaric acid for GPT) were injected from the two injection ports by using micro syringe pumps and the flows were stopped thereafter. Reagents in the two streams of solutions were mixed by free diffusion. An advantage of miniaturizing the flow system is that mixing caused by diffusion is very rapid because of its small dimensions. Actually, when the flows were stopped, changes were observed within seconds and a steady current increase was observed as time elapsed due to the increase of L-glutamate by the enzymatic reactions (Fig.2). A linear relationship was observed between the averaged slope of the response curve and the activity of the enzymes for lower activities of the enzymes (Fig.3).

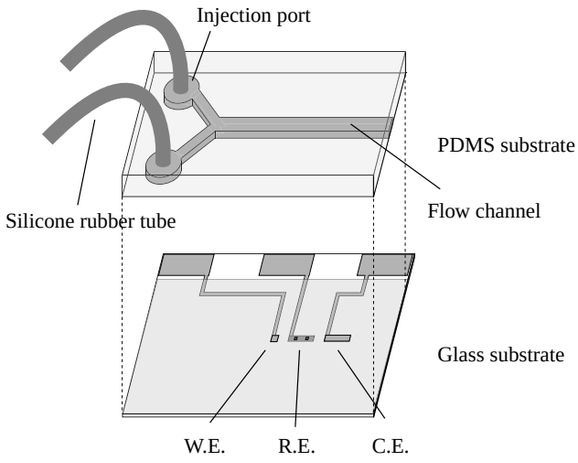

Fig.1 Decomposed structure of the system. The PDMS substrate with the micro flow channel was placed on the glass substrate with the three-electrode system. L-glutamate oxidase was immobilized on the working electrode (W.E.).

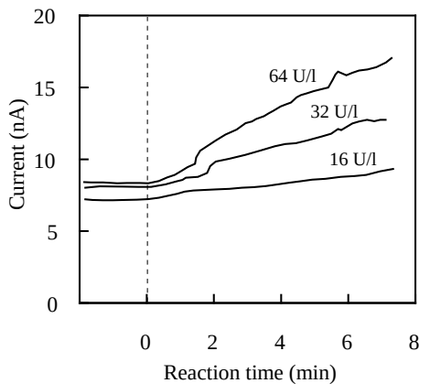

Fig.2 Variation of the current of the L-glutamate sensor after the flows were stopped. The three curves correspond to three different activities of GOT as indicated.

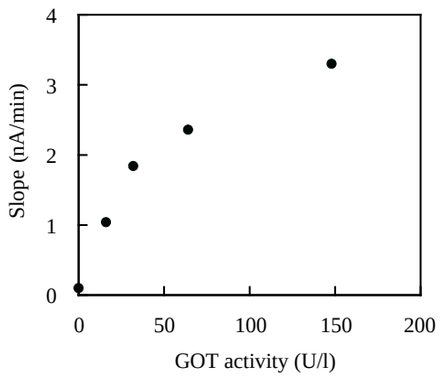

Fig.3 Dependence of the slope of the response curve of the L-glutamate sensor on the activity of GOT.