Improved Yield of Enzyme Reaction in Microchannel Reactor

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Trypsin-catalyzed hydrolysis of benzoylarginine-*p*-nitroanilide using a microchanel reactor showed higher yield than batchwise reaction at lower enzyme concentration. The rate of reaction seemed to be 20 times greater than that in the batchwise system.

The miniaturized chemical analysis and synthesis systems have been attracted interest.[1] The chemical analysis devices, which are commonly referred to as micro total analytical systems (μTAS), have been reported for a diverse range of applications. For example, an electrophoresis separation system on a glass chip was used for the laser induced fluorescent detection of a number of acetylcholine esterase inhibitors.[2] However, application of such microdevice as chemical reactor is deriving much interest. Several reaction devices have been reported to demonstrate the potential application of such devices.[3] These include highly exothermal reactions, the in situ generation of hazardous compound, and rapid energy transfer system. The catalytic reactions, which represent an important area in organic synthesis, have also been interested. Several efforts have been made to utilize microreaction systems for the catalytic reactions.[4,5] Still, there are many potential applications for miniaturized synthetic reactors able to utilize small amount of catalysts in conjunction with very limited volumes.

Enzyme-catalyzed chemical transformations are widely recognized as synthetic methods that satisfy stringent environmental constrains.[6] Several chemical processes using enzymatic reactions have been developed. However, most of the reactions require relatively high enzyme concentrations, and this causes difficulty to develop enzymatic chemical processes. If the microchannel reaction system have very rapid mass transfer, it is possible to improve enzymatic reaction rate at lower enzyme concentration. Here we report the effect of microchannel reaction for the enzymatic reaction using trypsin-catalyzed hydrolysis as a model.

The microreaction channel (200 μm × 200 μm × 40 cm) was mechanically fabricated on an acryl plate (3 cm × 3 cm × 5 mm) using Robodrill equipped with flat end mill (ϕ100 μm). The top plate assembly was achieved by baking (100 °C, 1h) under vacuum. The total systems, microchannel reactor and microsyringe pumps were assembled as shown in Figure 1. Trypsin and benzoylarginine-*p*-nitroanilide (BAPA) were separately dissolved in phosphate buffered saline (pH 7.4). Each solution was charged into the reactor by pumping, and the reaction was terminated by addition of 30% acetic acid solution at the end of microchannel. The reaction was evaluated as the amount of released *p*-nitroaniline calculated from absorption at 405 nm.[7]

The results were shown in Figure 2 and 3.[8] Comparing with the batchwise reaction, the microchannel reaction seemed no effect at high enzyme concentration (Figure 2). On the other hand, the reaction yield was strongly improved in microreactor at low enzyme concentration (Figure 3). The rate of reaction seemed to be enhanced than that in batchwise condition. This phenomenon was also observed in chymotrypsin-catalyzed hydrolysis of Bz-Tyr-pNA (data not shown). There are no specific effects, which affect enzyme reaction rate, exist for this simple system. The non-specific absorption of enzymes for the surface of the acryl channel is considerable. However, similar result was obtained for the horseradish peroxidase-catalyzed reaction in aqueous microfluidic system within a glass microchannel reactor,[9] and therefore the effect was not specific for acryl microreactor. These high yields might result from

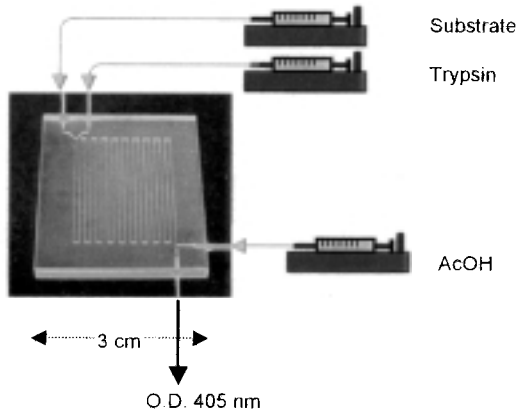

Figure 1. The microchannel reactor system used in this study

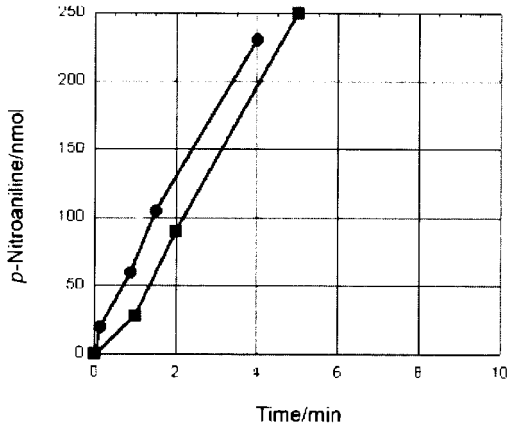

Figure 2. Hydrolysis of BAPA by high concentration of trypsin in batchwise (■) and microchannel (●) systems. Trypsin (53 μM), BAPA (1 mM), in Dulbecco's phosphate-buffered saline (pH 7.4), room temperature.

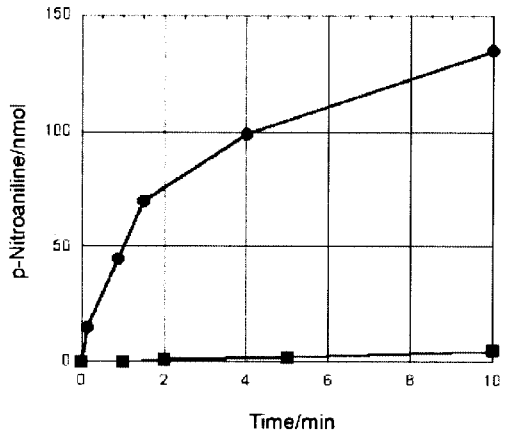

Figure 3. Hydrolysis of BAPA by low concentration of trypsin in batchwise (■) and microchannel (●) systems. Trypsin (0.65 μM), BAPA (1 mM), in Dulbecco's phosphate-buffered saline (pH 7.4), room temperature.

specific fluidic system within the microchannel. The solution forms laminar flow within the channel, and therefore flow velocity difference occurs at the in wall surface and central part of the channel. One speculation can be made that this velocity difference enables the enzyme localization within the narrow channel. Still, the mechanism of these results needs to be probed. Further studies to elucidate the mechanism of enzymatic reaction in the microchannel are in progress in our laboratory.

The enzymatic process prefers lower enzyme concentration, especially for enzymes that are expensive or difficult to obtain. Therefore, the microchannel reaction system might be a strong tool to develop the novel enzymatic chemical process.

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