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Rapid Communications

Acceleration of an Enzymatic Reaction in a Microchip

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In recent years, integrated chemical systems, which are named micro total analysis systems (μ-TAS),[1] or labs-on-a-chip,[2] have become of major interest, especially to analytical chemists due to its desirable characteristics, such as reduction in reagent consumption, required space, and analysis time. Taking these advantages, we have demonstrated many applications, including flow-injection analysis,[3,4] solvent extraction,[5-7] immunoassay,[8,9] organic synthesis[10] and laser reaction control.[11] There have been, however, no intensive studies focused on chemical or biochemical reactions themselves in a liquid microspace so far. In this letter, we report on the acceleration of an enzymatic reaction in a microchip and preliminary kinetic studies of the reaction.

Experimental

The experiments were carried out based on procedures described in a previous paper[11] with small modifications. Briefly, the time course of an enzymatic reaction was monitored in a microchannel (100 μm in depth and 250 μm in width) fabricated in a quartz glass chip (3 × 7 cm). As a reaction system, horseradish peroxidase (HRP) with three substrates, *i.e.* sodium (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS; Dojindo Molecular Technologies), 4-aminoantipyrine (4AA), and H_2O_2, was selected. The reaction products had a strong purple color with an absorbance maximum at 555 nm ($\varepsilon = 3.92 \times 10^4$). The enzyme and substrate solution were mixed and introduced into the microchannel immediately. The colored products were monitored by a thermal lens microscope (TLM)[12,13] with an Ar^+ laser (Lexel Model-95, 514.5 nm, 200 mW) as an excitation laser and a He-Ne laser (Melles Griot, 632.8 nm, 15 mW) as a probe. To make a comparison with the reaction at a bulk scale, a standard cuvette (10 mm in depth and width and 40 mm in height) was used as a reaction vessel, and the reaction was monitored with a spectrophotometer (Shimadzu, UV1600PC) at 514.5 nm.

Results and Discussion

The time courses of the reaction in the microchip and the standard cuvette are shown in Fig. 1. The time to reach a plateau in the microchip was half of that in a bulk scale, while the absolute quantities of the final products were same. These results may suggest an acceleration of the enzymatic reaction in a microspace. To clarify the characteristics of the reaction in a microspace, the kinetic parameters of the enzyme in the microchip and cuvette were estimated. The initial reaction rate for various H_2O_2 concentrations in the 2 – 250 μM region are shown in a Lineweaver-Burke plot in Fig. 2. At all concentrations, the reaction in the microchip is approximately two-times faster than that in a bulk scale. From these plots, the maximum reaction rates (V_{max}) and Michaelis constant (K_m)

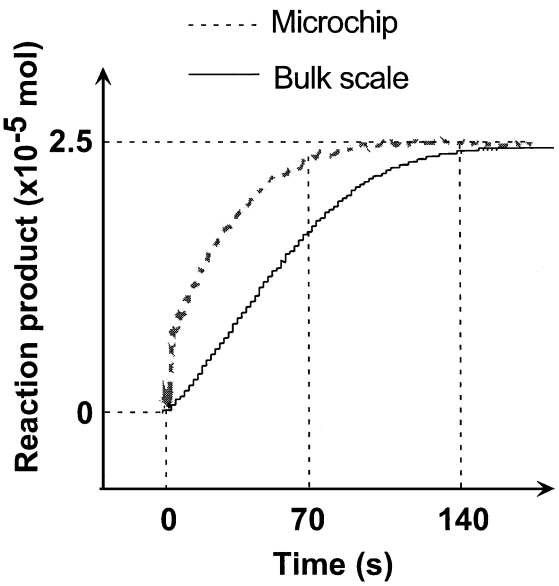

Fig. 1 Time courses of the reaction in the microchip and cuvett. The concentrations of HRP, TOOS, 4AA and H_2O_2 were 0.005 units/ml, 500 μM, 500 μM and 25 μM, respectively.

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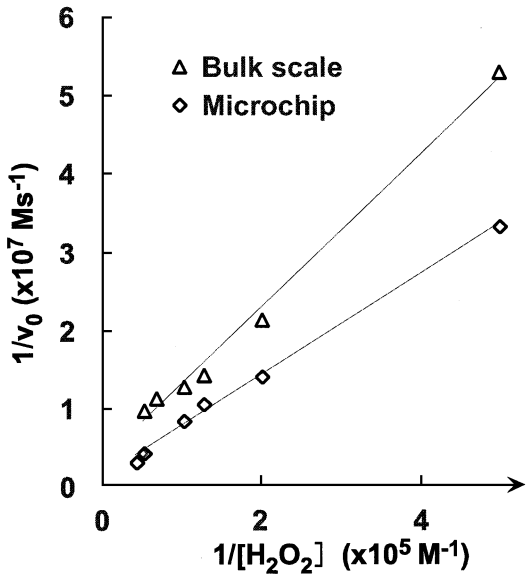

Fig. 2 Initial reaction rates for various H_2O_2 concentrations. The reaction conditions were the same as in Fig. 1.

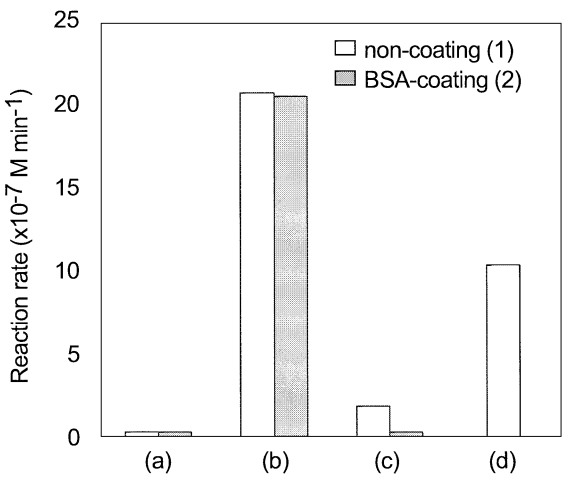

Fig. 3 Reaction rates in the microchip and bulk scale: (a) – (c) microchip and (d) bulk scale; (a) substrates only; (b) substrates and enzyme; (c) substrates only after (b). The reaction conditions were the same as in Fig. 1.

values were calculated to be 2.9×10^{-7} M s^{-1} and 2.83×10^{-5} M in the bulk scale and 6.5×10^{-7} M s^{-1} and 4.1×10^{-5} M in the microchip, respectively. These results clearly show that the reaction rate was increased in a microspace.

We considered that this acceleration of the reaction resulted from an accumulation of the enzyme molecules of the previous reaction by adsorption on the inside wall of the microchip. We therefore performed two series of experiments: first, the microchip was cleaned with an NaOH solution and rinsed with a phosphate buffer (PB); substrate solutions without any enzyme were then mixed and introduced into the microchip (measurement 1(a)). After measurement 1(a), the microchip was cleaned with only PB, and the enzyme and substrates solutions were mixed and introduced (measurement 1(b)). Then, after cleaning with PB, substrate solutions without enzyme were mixed and introduced (measurement 1(c)). Next, the microchannel was coated with a 1% BSA solution overnight after cleaning with an NaOH solution in order to estimate the effect of adsorption. After being rinsed with PB, the measurements were carried out in the same way as measurements 1 mentioned above (measurements 2(a) – 2(c)). The initial reaction rates of these measurements are shown in Fig. 3. Whereas the result of measurement 1(c) indicated that the adsorbed enzyme would affect the reaction rate, its reaction rate is 10% of that of measurement 1(b), not sufficient to accelerate the reaction two-times faster than the bulk scale (d). Moreover, measurement 2(b) also showed almost a two-times higher reaction rate than that of the bulk scale (d). The reaction rate of measurement 2(c) was the same as measurement (a). Therefore, there should be other effects that influence the reaction rate or mechanism in a microspace.

Although the reason for the acceleration is not clear, and further studies are necessary, the present results indicate that these features should be useful to develop biochemical sensors and reactors on the cm scale. Detailed discussions on the reaction in a microchip should appear in a succeeding paper.

References

1. D. J. Harrison and A. van den Berg, "*Micro Total Analysis Systems '98*", **1998**, Kluwer Academic Publishers, Dordrecht.
2. D. J. Graves, *Tre. Biotechnol.*, **1999**, *17*, 127.
3. K. Sato, M. Tokeshi, T. Kitamori, and T. Sawada, *Anal. Sci.*, **1999**, *15*, 641.
4. H. M. Sorouraddin, A. Hibara, M. A. Proskrunin, and T. Kitamori, *Anal. Sci.*, **2000**, *16*, 1033.
5. M. Tokeshi, T. Minagawa, and T. Kitamori, *Anal. Chem.*, **2000**, *72*, 1711.
6. M. Tokeshi, T. Minagawa, and T. Kitamori, *J. Chromatogr. A*, **2000**, *19*, 894.
7. H. Hisamoto, T. Horiuchi, M. Tokeshi, and T. Kitamori, *Anal. Chem.*, **2001**, *73*, 1382.
8. K. Sato, M. Tokeshi, T. Odake, H. Kimura, T. Ooi, M. Nakao, and T. Kitamori, *Anal. Chem.*, **2000**, *72*, 1144.
9. K. Sato, M. Tokeshi, H. Kimura, and T. Kitamori, *Anal. Chem.*, **2001**, *73*, 1213.
10. H. Hisamoto, T. Saito, and T. Kitamori, submitted.
11. Y. Tanaka, M. Slyadnev, A. Hibara, M. Tokeshi, and T. Kitamori, *J. Chromatogr. A*, **2000**, *894*, 45.
12. K. Uchiyama, H. Kimura, T. Sawada, and T. Kitamori, *Jpn. J. Appl. Phys.*, **2000**, *39*, 5316.
13. M. Tokeshi, M. Uchida, A. Hibara, T. Sawada, and T. Kitamori, *Anal. Chem.*, **2001**, *73*, 2112.