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Non-contact photothermal control of enzyme reactions on a microchip by using a compact diode laser

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Abstract

Photothermal temperature control of an enzyme-catalyzed reaction in a microchip using a diode laser was demonstrated. A laser beam with energy of 10 mW was used to irradiate an absorbing target placed on top of the microchip cover plate. Theoretical calculations have shown that temperature in the microchannel can be locally increased by 5–7°C during short time intervals, due to heat released by the target. The rate of the enzyme reaction, which was initially inhibited due to cooling of the chip to low temperature, was increased when the target was irradiated. The products were detected by a thermal lens microscope. The product concentration was shown to depend on irradiation time, laser intensity and substrate concentration. Reaction characteristics (rate constant of the reaction) were then derived from these dependencies. The reaction volume and absolute quantity of the reaction product were estimated as 10 nl and 100 fmol, respectively. It was also demonstrated that a direct solvent heating method using infrared radiation could control the reaction in the microchannel.

Keywords: Microchips; Microchannels; Thermal lens detection; Instrumentation; Diaminobenzidine tetrahydrochloride; Peroxidase; Enzymes; Hydrogen peroxide

1. Introduction

Integration of chemical reaction systems has advantages of efficiency, speed and volumetric reduction of resources and wastes. These advantages have been shown in many applications, such as flow injection [1–3], liquid–liquid extraction [4,5] immunoassay [6,7], organic synthesis [8] and polymerization [2]. Recent achievements in biochemical and biological fields have stimulated much interest in integrated ''labs-on-a-chip'' applications. In order to integrate certain bioorganic synthetic and analytical reactions on a microchip, it is necessary to provide temperature control of reaction media. The polymerase chain reaction (PCR) using conventional-sized temperature control systems has been intensively investigated in the field of genetic analysis [9]. Swerdlow et al. [10] demonstrated rapid PCR, purification and analysis within 20 min by using an air thermocycler that allowed efficient heat transfer to the sample during the cycling processes. Attempts have been made to apply contact-based heaters to control temperature in a microchip [11–13]. Eijkel et al. [14] demonstrated the PCR by using a heating

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resister array. But the implementation of rapid temperature control in similar contact devices is limited by heat capacity of the whole system and the heat transfer rate. In order to make the most of microspace features, non-contact heating is preferable for fast switching of temperature and reactions. This approach was demonstrated for conventional-sized systems by Oda et al. [15], who studied a non-contact thermocycling PCR system with non-coherent IR radiation source, and reported the reaction volume and time were 5 μl and 17 s, respectively. It should be noted, however, that even smaller volumes and faster heating rates are desired for microchip-based reactions. No reports have been made concerning applications of a similar system to microchips.

In this report, we have demonstrated a non-contact heating method for control of a chemical reaction in a microchip by utilizing the photothermal effect produced by a diode laser. Diode lasers operated in the red and IR wavelength regions are widely used and considered to be relatively cheap, convenient, compact and powerful light sources. A light absorbing target on top of a microchip above a microchannel through which reactants flowed was utilized to heat the reaction media. The enzyme-catalyzed reaction was controlled with this technique by using the compact diode laser. The dependencies of enzyme reaction yield on laser intensity, irradiation time and substrate concentration were measured and discussed. In addition, preliminary experiments of a reaction control method on a microchip by using an infrared laser were also demonstrated.

2. Experimental

2.1. Chemicals

Biochemistry grade 3,3′-diaminobenzidine tetrahydrochloride (DAB) was purchased from Dojindo Labs. (Kumamoto, Japan). Peroxidase from horseradish (HRP, biochemistry grade) and 35% hydrogen peroxide solution (electronic industry grade) were obtained from Wako (Osaka, Japan). All the reagents were used without further purification. Stock solutions of enzyme and DAB were prepared with phosphate-buffered saline (PBS) solution, pH 7.4. Before experiments, these stock solutions were properly diluted with PBS, and concentrations of DAB and HRP were adjusted to 50 μ*M* and 2 unit/ml, respectively. The hydrogen peroxide solution was diluted with ultra pure water to 50 μ*M*. Ultra pure water was obtained from a water purification apparatus (Nomura Micro Science, TW-600RU).

2.2. On-chip laser photothermal temperature control setup

The glass chip used for the photothermal reaction control is shown in Fig. 1. Details of its fabrication have been published previously [3]. Width and depth of the microchannels were 250 and 100 μm, respectively. The enzyme solution and substrate solution with hydrogen peroxide were introduced through the two inlets. The solutions were diffusely mixed after the Y-intersection. The ambient temperature of the microchip was controlled by a Peltier cooling stage (Linkam, TH-99) to repress the background enzyme reaction rate. In order to increase temperature locally in the microchannel, a 10-mW diode laser (Coherent, LabLasers Model 31-0334) operated at λ=635 nm was used to heat an absorbing target, which was placed on top of the microchip cover plate at the Y-intersection. The target was a black ink point, produced by felt tip pen marker (Zebra, Japan). After

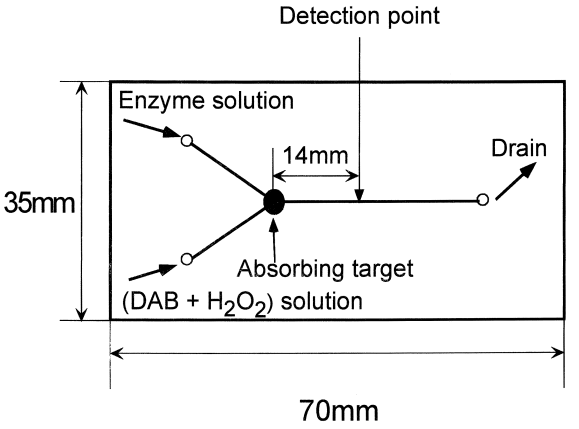

Fig. 1. Layout and dimensions of the microchip with a Y-shaped microchannel network. The channels were 100 μm deep and 250 μm wide. The microchip had a 170-μm thick top plate and 500-μm thick bottom plate. The Peltier cooling stage was used to adjust equilibrium temperature of the microchip.

drying, the uniformity of ink distribution across the target area was visually examined under the microscope. The procedure was easily repeated, if necessary, after removal of the target with acetone. Light energy absorbed by the target was converted to heat due to photothermal process. Heat flux was conducted though the glass cover plate and the temperature of the mixed solution in the main microchannel rose to the equilibrium temperature. The 1064-nm emission line of a YAG laser (Coherent, Compass 1064-10W) was also used as direct heating light source (i.e., without target). In both heating modes, the reaction media were heated in a stopped flow mode and then transported 15-mm downstream channel to the detection point just after the heating.

2.3. Thermal lens microscope

The colored product of the HRP–H_2O_2–DAB reaction was detected with a thermal lens microscope (TLM), described in detail elsewhere [16]. Since the reactants have little absorption at 514.5 nm, but this product has a strong absorption at this wavelength[1], an Ar^+ ion laser (Lexel Model-95, 514.5 nm, 200 mW) was used as an excitation laser. A He–Ne laser (Melles Griot, 632.8 nm, 15 mW) was used as a probe laser. The excitation laser beam was mechanically chopped by a light chopper (NF Electronic Instruments, 5584A) at 1025 Hz. Both excitation and probe laser beams passed through some prisms and beam expanders and were introduced into the optical microscope (Nikon, special design). Beams were coaxially aligned by a dichroic mirror and a mirror in the bodytube of the microscope and were focused by an objective lens (Nikon, ×20, NA 0.46). Transient divergence of the probe beam was detected as a change in the light intensity. The probe laser beam was collected by a condenser lens and passed through a glass long-pass filter (Melles Griot, 03FCG089, λ_{50}=580 nm) and an interference bandpass filter (Melles Griot, 03FIL024, λ=634.80 nm) to cut off excitation light. The probe beam intensity was monitored with a photodiode (Electro-Optics Technology, ET-2030), a low-noise preamplifier (NF Electronic Instruments, LI-75A), a lock-in amplifier (NF Electronic Instruments, LI-575) and a chart recorder. A CCD video camera (Victor, KY-F55B) was mounted on the microscope and it was used to display a picture image of microchannels.

3. Results and discussion

When the enzyme and substrate solutions were mixed, H_2O_2 was catalytically reduced to water and DAB was oxidized yielding a colored product. The TLM measured the change in absorbance at 514.5 nm. Before controlling the reaction by the lasers, ambient temperature dependence of the enzyme reaction was examined in the microchannel. Fig. 2 shows the dependence of reaction product concentration as the signal intensity on the equilibrium temperature of the microchip. The TLM signal was measured at 10 s after mixing the substrate and enzyme solutions in the microchannel. The ambient temperature of the microchip was controlled by the Peltier cooling stage and measured by a contact thermometer. As shown in Fig. 2, the enzyme reaction did not proceed at 2°C and it was sufficiently sensitive to temperature to use the described method. Although substrate and enzyme solutions

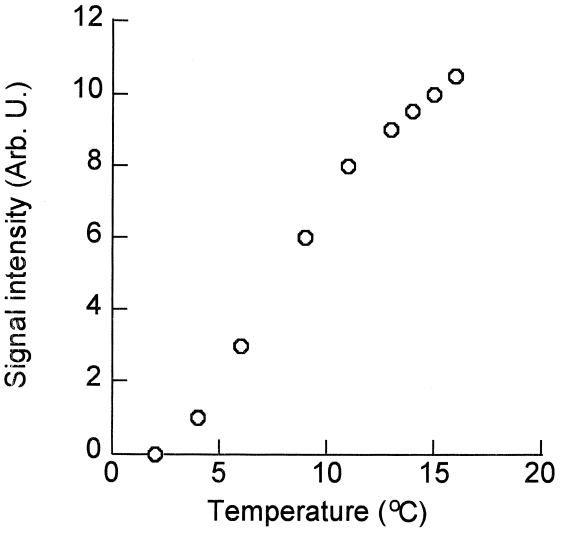

Fig. 2. Dependence of reaction product concentration as signal intensity on ambient temperature of the microchip. Reaction time was fixed at 10 s.

[1]This is a required condition in order that the thermal lens signal to be proportional only to product concentration, provided all other experimental conditions are constant [3,4].

with temperatures of 23°C were introduced through the inlets of the microchip, the solutions had cooled before mixing due to their relatively small heat capacity. This result indicates that the TLM signal directly corresponds to the product amount, without background, in the following reaction control experiments.

In the photothermal reaction control experiments, the absorbing target was placed on top of the microchip cover plate above the Y-intersection of the microchannels and the target was irradiated with the laser beam of 500 μm diameter. Taking into account the width and depth of these microchannels (250 and 100 μm, respectively), reaction volume was estimated to be 10 nl. Since heating was carried out in stopped-flow mode, the product plug length was approximated to be equal to irradiated area diameter, i.e., 500 μm. Under the present experimental conditions, the absolute quantity of product was estimated at fmol order based on the concentration and volume.

Typical responses of the HRP–H_2O_2–DAB reaction on photothermal heating by laser light are shown in Fig. 3. At TLM measurement 1, no laser beam have irradiated the target. Before measurement 2, the reaction control diode laser beam (8 mW) irradiated the target for 5 s. The induced temperature change was estimated to be 5.6°C from theoretical thermal conductivity calculations. The heated solution was transported to the measuring point by flow

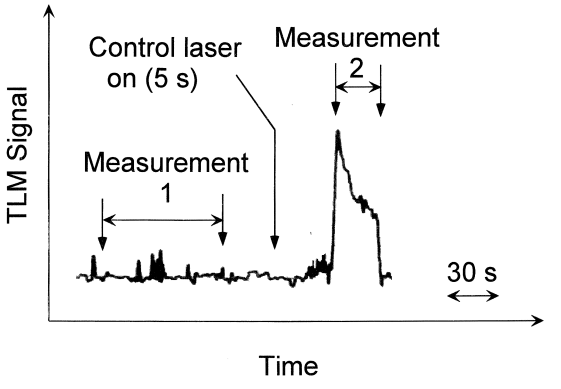

Fig. 3. Typical responses of the photothermal reaction control. Before the photothermal heating (measurement 1), no product was observed. After the 5-s heating, a significant increase of the product was measured (measurement 2).

created with syringe pump during 2 s (linear flow-rate was 7 mm/s). The flow was stopped when the product plug was located at the detection point (note the distance between heating and detection points of 14 mm indicated in Fig. 1). The reaction product formed during irradiation was determined with the TLM. Fig. 3 shows the enzyme reaction in the microchannel can be controlled by the thermal-conduction-assisted photothermal heating method using the small diode laser.

Laser power and irradiation time dependencies of the TLM signal are shown in Fig. 4a and b, respectively. In Fig. 4a, the control laser irradiated the target for 5 s. The numerical calculation provided information on the temperature dependence on the laser power, the size of the irradiated area and the size of microchannel. For present chip dimensions and laser powers of 2, 4, 7, 8 and 10 mW, the temperature change induced was estimated to be 1.9, 3.2, 5.0, 5.6 and 7°C, respectively. The product concentration shown in Fig. 4a product concentration clearly increased with power of the control laser. It should be noted, that during the 5s of irradiation, two processes occurred simultaneously: temperature equilibration and reaction kinetics. It is difficult to estimate their relative contributions to the product yield. However, the product concentration increase due to temperature rise seems to be reasonable explanation. In Fig. 4b, the laser power was fixed at 5 mW and irradiation time was varied from 5 to 60 s. Since heat capacity of the heated volume was much smaller than that of the Peltier cooling stage, temperature in this microchannel reached equilibrium within 1~2 s depending on solution flow-rate, as was estimated from thermal diffusion time taking into account real size of the channel and material properties [14]. Therefore, the experiment shown in Fig. 4b can be interpreted as measurements of reaction time dependence at fixed temperature. The estimated temperature change in this condition was 3.8°C. Since the concentration of enzyme is sufficiently high (2 unit/ml), the reaction can be regarded as a pseudo-first-order reaction for substrate. The pseudo-first-order reaction was observed for HRP–H_2O_2–DAB reaction in a 4-ml sample cell, when the product was monitored by absorption spectrometry at 488 nm even for lower concentration of enzyme. Similar kinetic behavior for the HRP–H_2O_2 system

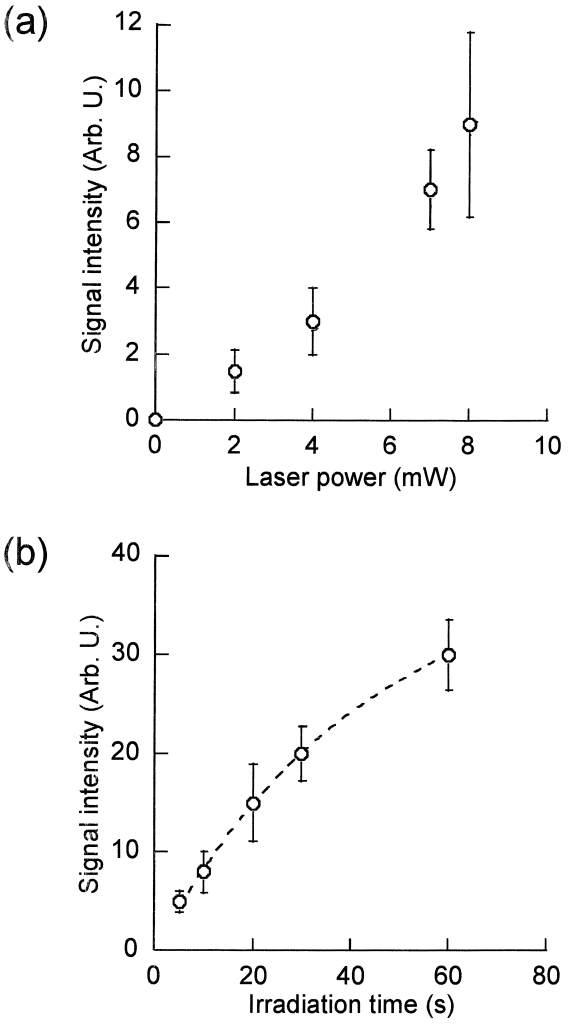

Fig. 4. (a) Laser power dependence of the TLM signal. Irradiation time was fixed at 5 s. (b) Irradiation time dependence of the TLM signal. Laser power was fixed at 5 mW. The dashed line corresponds to fitting result of the pseudo-first-order kinetics. The error bars indicate fluctuations of the TLM signal.

with different hydrogen donor compounds has been reported [17]. The dotted line in Fig. 4b indicates the fitted time course of the pseudo-first-order reaction, where the kinetic constant is fitted as 0.024 s^{-1}. Despite the high enzyme concentration the rate constant value is relatively small due to low temperature of the reaction media. As we have demonstrated here, the enzyme reaction can be reproducibly controlled by the diode laser with relative standard deviation being 5–7%. In addition, kinetic and thermodynamic parameters can be easily obtained by using the photothermal temperature control method.

Next, we applied the photothermal control of the enzyme reaction to determination of H_2O_2. Results for H_2O_2 concentrations from 0 to 100 μM are shown in Fig. 5. The laser power for reaction control was 7 mW and the irradiation time was 5 s. Since the reaction volume was estimated to be 10 nl, absolute quantity of reacted H_2O_2 was less than 100 fmol. A correlation coefficient for linear regression was 0.997 and determination limit estimated by the 2σ-criterion was 5 μM. Although the determination limit presented here is not so low, performance of this method may be improved by optimizing the experimental parameters of the photothermal heating such as the laser power, irradiation time and flow control.

As an advanced photothermal laser reaction control method, we examined a system with direct solvent heating by an infrared laser. Utilizing the near infrared absorption of water, direct radiation heating without any effects of solute absorption may be realized. A 1064-nm radiation of a Nd–YAG laser was applied to the enzyme reaction control. Experimental procedures, except for the heating method, were the same as indicated for the diode laser with target. The beam irradiated the solution 5 mm

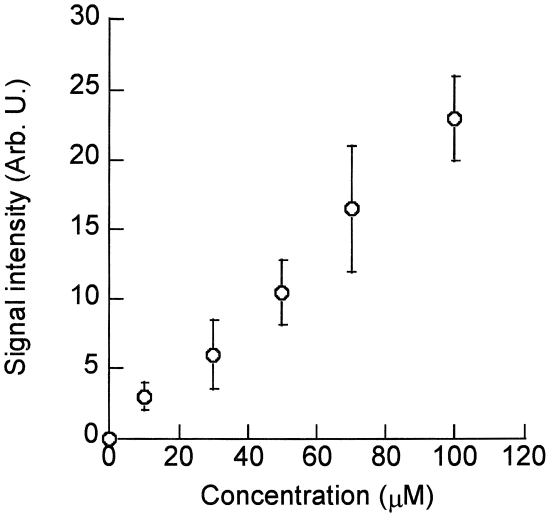

Fig. 5. TLM signal dependence on H_2O_2 concentration.

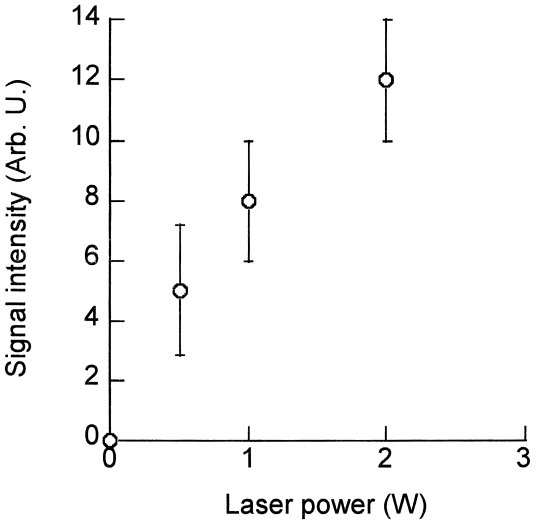

Fig. 6. Infrared laser power dependence of the TLM signal. Irradiation time was 2 min. The solvent in the microchannel was directly heated.

downstream from the mixing point and the reaction product was detected. Since light absorption of water at 1064 nm is weak, laser irradiation time was 2 min, and estimated temperature change at 2 W was about 7°C using model approximation [18]. Fig. 6 shows laser power dependence of the TLM signal corresponding to the product. The product quantity increased with the laser power. By applying the infrared laser energy to the solvent directly, the photothermal reaction control in the microchannel was demonstrated. Although there are several problems as to experimental procedure, such as discord between the absorption peak and the laser emission and long irradiation time, we think that direct solvent heating can be realized in the microchannel. Compact IR diode lasers with a more suitable wavelength for water heating are commercially available and modification of the set up is currently being done in our laboratory.

4. Conclusions

In summary, we have demonstrated fast photothermal reaction control in microchannels by using a small diode laser that locally heated an absorbing target. The reaction volume estimated from the cross section of the microchannel and the spot size of the heating laser was 10 nl and the absolute quantity of the reaction product was less than 100 fmol. This finding could provide the possibility for control of nanoscale reactions and precise synthesis of substance by using photothermal stimulation. The small heat capacity of the heated reaction space allowed the equilibrium temperature to be reached within 2 s. The temperature rise from the ambient microchip temperature of 2°C was controlled between 1.9 and 7°C by changing the laser power. Reproducible photothermal reaction control was established for reaction time from 5 to 60 s. In these experiments, we showed that a kinetic constant could be obtained. We applied our method to determination of 10 to 100 μM H_2O_2 and obtained a linear relationship. Photothermal reaction control by direct IR radiation solvent heating was also demonstrated. The direct radiation heating method has a great potential to achieve faster switching of temperature, which are required for polymerase chain reaction. We hope that this method will be a powerful tool not only for chemical and biological analyses but also for fundamental chemistry. From the viewpoint of microchip chemistry, this method can be easily connected to other techniques of integrated chemical systems and is a promising tool for physical, synthetic and analytical chemistry in microchips.

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