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Differential regulation of chemical reactions in a microchannel reaction system†

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Microchannel reactors have continued to attract attention in the fields of analytical and synthetic chemistry. An important feature of microchannel systems is their superior controllability of fluidic systems, which cannot be created in the batchwise reaction system with larger reaction apparatus. This study is designed to examine whether the superior controllability of microfluidics allows the use of microchannel reactors as a novel reaction control apparatus. Microfluidic analysis of ceramic microreactors using a solvatochromic dye demonstrated that the mixing of two solvents was strongly affected by the secondary flow that occurs at the corners of a microchannel. We examined the effects of such a microfluidic system on chemical reactions using amino acid substitution as a model reaction. Substitution of phenylalanine in a microreaction system using separate solutions was more efficient than in a batchwise reaction or in microchannel reaction using a homogeneous solution. Substitution of other amino acids showed that this enhancement is caused by localization of hydrophobic amino acids at the DMF–H_2O interface. Using the rapid Michael addition reaction of the SH group of cysteine to a maleimide group, we also demonstrated that such a reaction involving hydrophilic amino acids was diminished in this microreaction system. These results show that the microreaction system is a novel apparatus for regulating chemical reactions, depending on the structure of the reactant molecules, by controlling the mixing of two different solutions.

Introduction

Microchannel reactors have attracted attention in the fields of analytical and synthetic chemistry.[1–4] Several reaction devices have been developed, including solvent extraction systems, catalytic reaction and organic reaction devices. These devices have yielded increased efficiency, higher reaction yields, and shorter reaction times by taking advantage of microchannel system features such as rapid mass transfer and large surface and interface areas. Rapid mixing of different solutions of reactants is considered to be important in chemical synthesis. Therefore, several micromixing devices have been developed and utilized.

Another important feature of microchannel systems is their superior controllability of fluidic systems, which cannot be obtained in a larger reaction apparatus. The fluid forms a laminar flow because of the small channel dimensions. Taking advantage of the laminar flow system, Whitesides *et al.* developed several devices to control fluidic systems and apply them in chemical and biotechnological fields.[5–8] Such superior controllability of fluidics might be useful to create novel reaction devices to facilitate regulation of chemical reactions that cannot be achieved in batchwise systems. Thus, efforts to use the superior controllability of microfluidics in the chemical and biochemical fields are attracting attention.

Secondary flow, which occurs depending on the flow rate and microchannel structure, can take place at the turn structure of the microchannel.[9,10] When secondary flow occurs, the interface shape curves. The mixing of two different streams is enhanced, depending on the magnitude of the secondary flow. These findings inspired us to use such secondary flow for controlling the mixing of two different streams to consequently control the chemical reaction. The present study is designed to examine whether controlled mixing of two different flows by laminar and secondary flow of microfluidics can allow regulation of a chemical reaction. This study achieves such a regulation of the reaction rate of amino acid substitution in microfluidic systems by controlled the mixing of water and DMF solutions, depending on the amino acid structure and solution flow rates.

Experimental

General

A glass ceramic plate was chosen as the material for microreactor preparation because it is chemically inert. Fabrication of this plate can be achieved by convenient micromachining. The plate was obtained from Mitsui Mining Material Co. (Tokyo, Japan). Other materials for microreactor fabrication were commercially available. Amino acid derivatives were

† Electronic supplementary information (ESI) available: color of various DMF–water solutions of Reichardt's dye. See http://www.rsc.org/suppdata/nj/b4/b407019d/

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purchased from Calbiochem-Novabiochem AG (Laufelfingen, Switzerland) and Wako Pure Chemical Ind., Ltd (Osaka, Japan). Reagents for organic reactions were purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan) and Sigma–Aldrich Corp. (St. Louis, MO, USA). Water was freshly prepared using a Milli-Q system (Millipore Corp., Billerica, MA, USA); DMF was distilled prior to use. The entire solution was filtered using a PTFE filter (0.45 μm) prior to use, and charged into a 1 ml Hamilton gastight syringe (Hamilton Co., Reno, NV, USA). Solutions were supplied to the microreactor using a KDS 230 syringe pump with a parallel syringe holder (KD Scientific, Inc., New Hope, PA, USA). Thereby, two solutions were charged at equal volume per time. The flow rates indicated in the figures were calculated from the total of the two solutions. HPLC analysis was performed using a Wakosil C18AR column (3.0 × 250 mm, Wako Pure Chemical Ind., Ltd) with a linear gradient of 0.05% TFA–acetonitrile over 60 min at a flow rate of 0.5 ml min^{-1} at 30 °C. The identity of each compound was confirmed with LC-MS (ABI Mariner ESI-TOF system).

Fabrication of the microreactor

A square microchannel structure (200 μm width × 200 μm depth × 50 cm length) was fabricated on a ceramic (3 cm × 7 cm, 1 mm thick) using a Robodrill α-T21iDs (Fanuc, Ltd, Yamanashi, Japan) equipped with a carbide end mill (Hitachi Koki Co., Ltd, Tokyo, Japan). The microchannel structure was confirmed using laser microscope analysis (VK-8510; Keyence Co., Osaka, Japan). The ceramic plate was covered with a glass plate (3 cm × 7 cm, 1 mm thick) by heating; then inlet and outlet tubes were connected. The microreactor structure is shown in Fig. 1.

Analysis of the microfluidics

Aqueous and DMF solutions of a solvatochromic dye (Reichardt's dye; 1.5 mg ml^{-1}; Sigma–Aldrich) were prepared separately and filtered using a 0.45 μm PTFE filter. The color changes from green to orange as the content of water in DMF is raised [see Electronic supplementary information (ESI)]. These solutions were loaded into the microreactor from each inlet using syringe pumping, as shown in Fig. 1. The color of the solutions was observed using a CCD digital microscope (High-scope system; Hirox Co., Ltd, Tokyo, Japan), as described in the literature.[9]

Reaction of amino acids with *N*-(4-maleimidobutyloxy)succinimide (GMBS)

One millimolar solutions of GMBS in DMF and of amino acids, as well as triethylamine, in water were prepared separately. The solutions were filtered and degassed prior to use. Each solution was loaded into the microchannel using syringe pumping, as shown in Fig. 1. The reaction was terminated by adding ammonium hydroxide solution at the end of the channel. The recovered solution was subjected immediately to HPLC analysis. Reaction yields were calculated from the peak area calibrated with a standard compound prepared on large scale. For the reaction using a homogeneous solution, each solution was mixed and charged from both inlets. Batchwise reactions were performed in a 50 : 50 (v/v) mixture of DMF and water at 25 °C, with constant vortex mixing (3000 rpm).

Reaction of cysteine derivatives with *N*-(4-maleimidobutyloxy)phenylalanine

The maleimide derivative, *N*-(4-maleimidobutyloxy)phenylalanine, was prepared by reaction of GMBS with phenylalanine and purified by preparative HPLC. One millimolar solutions of *N*-(4-maleimidobutyloxy)phenylalanine in DMF and of cysteine derivatives (or triethylamine) in water were prepared separately. The solutions were filtered and degassed prior to use. Each solution was charged into the microreactor by syringe pumping, as shown in Fig. 1. The reaction was terminated by adding ammonium hydroxide solution at the end of the channel; the recovered solution was subjected to HPLC analysis. The reaction yields were calculated from the peak area calibrated with the standard compound prepared on large scale.

Results

A ceramic microchannel reactor, fabricated as described above, was used for the experiments. Although the secondary flow that occurred at the turns of the microchannel was demonstrated to disrupt the interface in a water–water system, the effects of such secondary flow in a DMF–water system are not clear. Therefore, we analyzed the microfluidics using Reichardt's dye before performing the reactions.[11] Reichardt's dye changes color depending on the environment: in this case by changing the water content (see ESI). Fig. 1 shows that the solution forms a blue color even at the inlet part at a lower flow

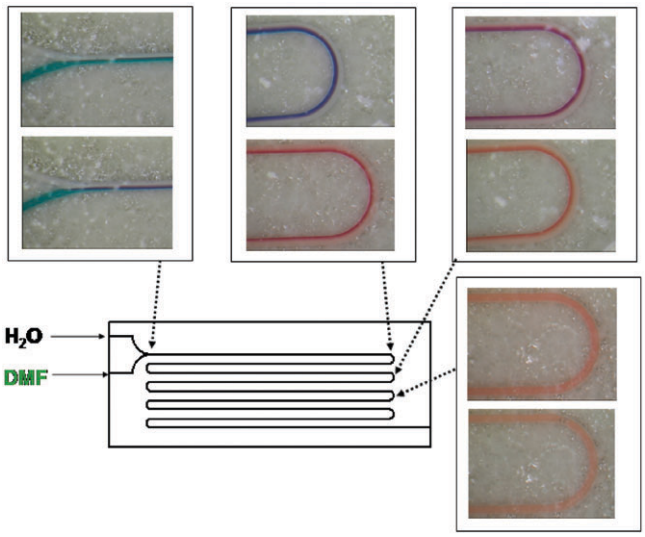
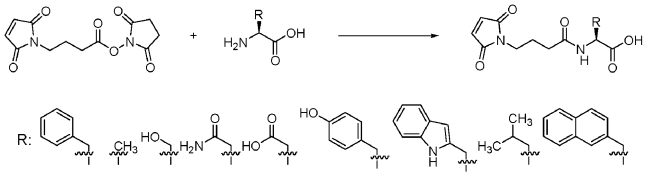


Fig. 1 Mixing of DMF and water in a microchannel. Fluid at the indicated part of the microchannel (200 μm × 200 μm × 50 cm) was observed using a microscope at two different flow rates. DMF and aqueous solution of Reichardt's dye were loaded from both inlets as shown at the same flow rates. Upper pictures in the boxes were taken at a total flow rate of 200 μl min^{-1}; lower pictures were at 20 μl min^{-1}.



Scheme 1 Reaction of amino acids with active ester.

rate, indicating that the two solutions are mixed. The color changes to orange with a higher flow rate, but complete mixing was observed at the 5th turn. In contrast, the solution within the microchannel was colorless and green near the inlet at a higher flow rate. The solution became colorless and blue in the flowing straight part of the microchannel. Subsequently, its color changed to colorless and then red, finally becoming uniformly orange near the fifth turn. This result implies that complete mixing was achieved at the 5th turn in both cases, although the flow rate was varied by a factor of 10 in our microreactor: a higher flow rate yielded a shorter mixing time. This result agrees with our previous finding for a water–water system.[9,10] Secondary flow occurs at the turn structure of the microreactor, possibly induced by inertial forces in a water–water system. Such secondary flow disrupts the solution interface and enhances mixing of the two solutions. Such effects also exist in DMF–water systems: they enable efficient mixing at higher flow rates. With a lower flow rate, diffusion mainly affects mixing. For this reason, we can control mixing modes of DMF and water from active mixing by secondary flow to diffusion-based mixing simply by changing the flow rate in this microreactor.

We performed a substitution reaction of amino acids using this microfluidic system. First, we executed a simple condensation reaction of the amino group with an active ester (Scheme 1), using a DMF solution of GMBS and an aqueous solution of phenylalanine. Fig. 2(a) shows that the reaction did not proceed at higher flow rates (<1 min, >50 μl min^{-1}). However, the reaction yield increased at slower flow rate and even exceeded 90% in a 10 min reaction time. This result indicates that the reaction rate was improved dramatically over that of a batchwise reaction, which gave 80% yield after 12 h [Fig. 2(a)]. We performed the reaction using a premixed homogeneous reaction mixture using phenylalanine to explore whether enhancement was promoted only by rapid mass transfer in the microchannel. As shown in Fig. 2(b), even at lower flow rates (<5 μl min^{-1}, reaction times were >3 min), the reaction did not proceed. We also performed the same reaction in a microcapillary system and obtained similar result (data not shown). These results show clearly that formation of an interface between the water and DMF solutions in a microchannel affects the reaction.

What produces such a great difference? We speculate that a difference in the solvent preference of each amino acid is the reason. Most amino acids show decreased solubility when adding DMF to a water solution, meaning that the solvation structure is disrupted. However, amino acids with a bulky side chain, such as phenylalanine, have a slightly higher solubility in aqueous DMF than in water alone. Addition of a small amount of DMF to aqueous solutions of these amino acids might change the solvation structure and stabilize it to improve solubility. Therefore, hydrophobic amino acids prefer aqueous DMF and these molecules can access a mixed DMF and water region in the microchannel. Such amino acids might localize into the DMF-containing area within a the microchannel flow because the reaction is improved over that occurring in a premixed solution [Fig. 2(b)]. At a higher flow rate, the reaction time was too short for conversion. In addition, strong secondary flow at the corner disrupts the interface. Therefore, the conversion rate was low. The reaction yield improved, especially at a lower flow rate, because disruption of the interface by secondary flow was weakened and the residence time was sufficient to complete the reaction. On the other hand, hydrophilic amino acids might localize in regions that do not contain DMF; therefore, the reaction gave a low yield, even at lower flow rates.

To resolve this speculation, we performed the same reaction (Scheme 1) using other amino acids: Ala, Ser, Asn, Asp, Leu, Tyr, Trp, and naphthylalanine (Nal). The reaction rate was not different for each amino acid in the batchwise reaction. The reaction in the microchannel was performed as in the case of Phe. Amino acids having bulky side chains—Leu, Tyr, Trp, and Nal—reacted with GMBS in the microchannel in the order of hydrophobicity of their respective side chains (Fig. 3). In contrast, amino acids with smaller or hydrophilic side chains (Ala, Ser, Asn, and Asp) did not react in the microchannel. From these results we conclude that the amino acid's structure apparently influences the reaction rate strongly in this microfluidic system.

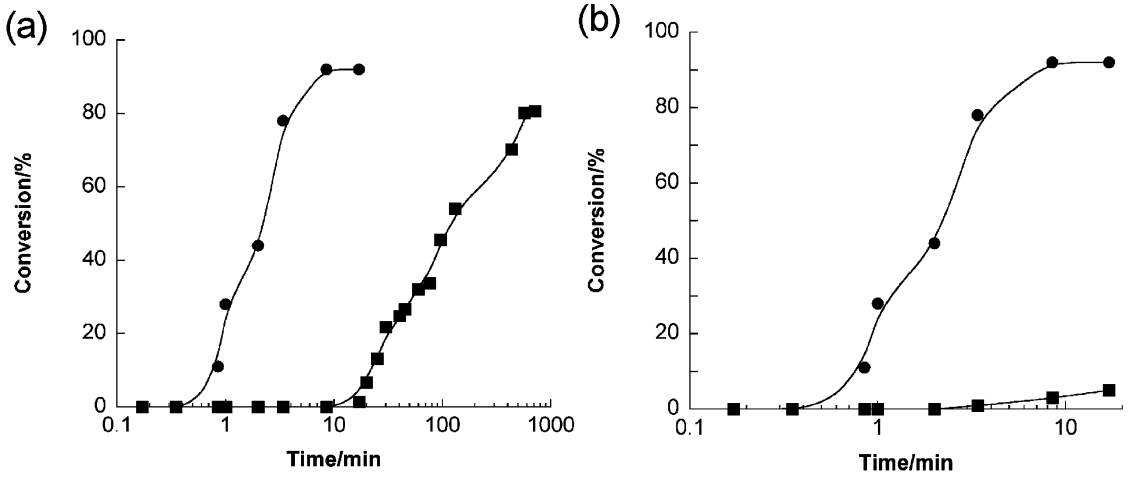


Fig. 2 Reaction profile of phenylalanine with GMBS in DMF. (a) Comparison of bulk (■) and microchannel (●) reactions. (b) Reaction yield in the microchannel reactor using separate solutions (●) and a premixed solution (■).

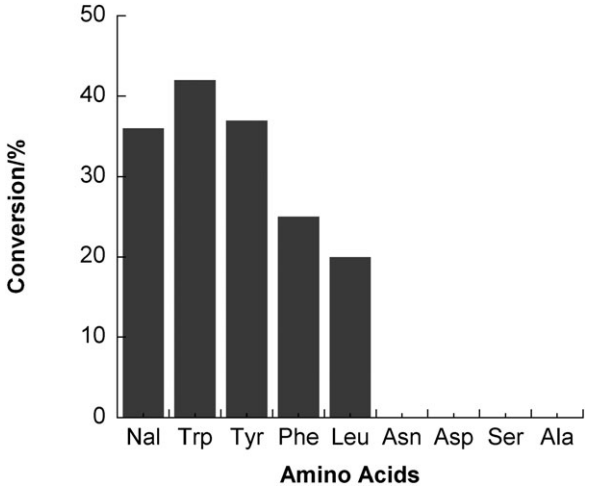


Fig. 3 Effect of amino acid side-chain structure on the reaction with GMBS. The reaction was performed in the same microreactor as in Fig. 2. Each bar shows the reaction yield at a flow rate of 15 μl min^{-1}; the interface was maintained and effects of secondary flow were minimized.

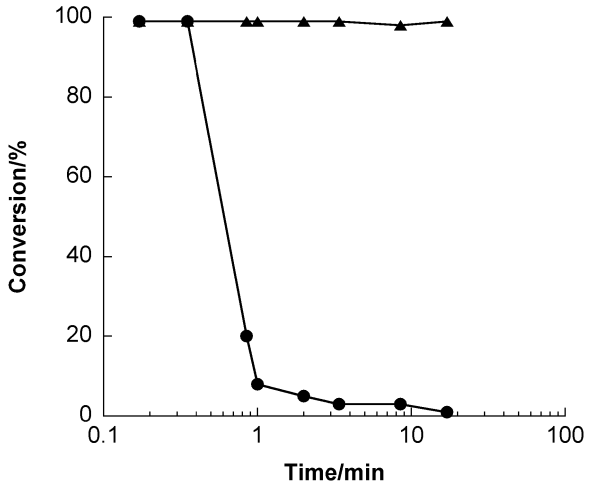


Fig. 4 Reaction profile of cysteine with *N*-(4-maleimidobutyloxy) phenylalanine, both batchwise (▲) and in a microreactor (●). The reaction was performed in the same microreactor as that used in Fig. 2.

We also examined the reaction of maleimide with the thiol group of cysteine (Scheme 2) using a DMF and water system to support this speculation. This reaction proceeds rapidly, even in a batchwise system. Free cysteine shows higher solubility in H_2O alone than in the presence of DMF; also, it might localize in an area of the microreactor that does not contain DMF. Therefore, the reaction should give a lower yield when the active mixing effect is weakened at a lowered flow rate, although reaction time would increase. The reaction was performed using a H_2O solution of cysteine and a DMF solution of *N*-(4-maleimidobutyloxy) phenylalanine (Fig. 4). At high flow rates, the reaction proceeds rapidly, as in the batchwise system, in all cases. As expected, the reaction yield dramatically decreased at slower flow rates (>50 μl min^{-1}, reaction times were <0.5 min), which maintains a laminar flow system, in the case of free cysteine. This tendency was also observed by reactions using cysteamine and penicillamine (data not shown). Therefore, it can be concluded that the hydrophobicity of molecules allows both improved accessibility of amino acids to the DMF–water mixture and higher reaction yields. Amino acids with hydrophilic side chains do not prefer DMF and localize in areas that do not contain DMF. Therefore, the reactivity of such amino acids os decreased in the microfluidic system, giving lower yields than those of hydrophobic molecules.

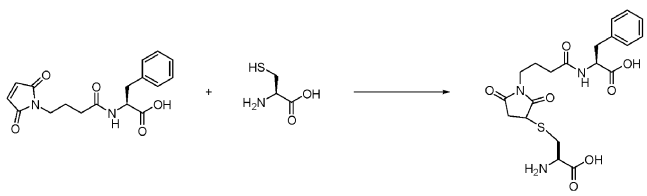
Discussion

Microchannel reactors have garnered interested as a novel reaction apparatus in synthetic chemistry. Several synthetic reactions have been performed in microreactors. Improved reaction efficiencies of Friedel–Crafts reactions and peptide syntheses were demonstrated.[12,13] However, in most cases, common guidelines for designing efficient microreaction system are not well-established. An understanding of microfluidics is essential to establish such guidelines.

Microchannel systems have specific fluidic systems that are different from those of a bulk solution. The flow is mainly laminar; therefore, it forms a large interface area between two different solutions. Such a system offers advantages for solvent extraction and phase-transfer reactions.[14–17] For example, Kitamori *et al.* demonstrated that the reaction efficiency of phase-transfer reactions can be improved in a microchannel reactor.[16,17] Not only immiscible solutions but also miscible solutions form different streams in the straight portions of the microchannels. We have shown that secondary flow occurs at the turns of the channel; this disrupts the laminar flow system to yield a change of interface shape and might therefore enhance mixing.[9,10] However, effects of such secondary flow on the reaction were not examined.

The present study used a solvatochromic dye to demonstrate that secondary flow enhances the mixing of water and DMF in a microchannel with several turns. We also demonstrated that such mixing can affect reaction rates, depending on the reactant molecule properties. We accelerated substitution of hydrophobic amino acids and diminished efficiency in the reaction of maleimide with the SH group of cysteine. These results emphasize the importance of microfluidics in the design of microreaction systems. It has also been demonstrated that computer simulation is advantageous to analyze microfluidic behavior.[10] Such simulations might be a useful tool for designing efficient microreactors.

The present study also demonstrated that substitution reactions of amphiphilic molecules, such as hydrophobic amino acids, are possibly accelerated by localization of such molecules at the interface of DMF and water. This effect might be advantageous for the substitution reactions of biopolymers. Most biopolymers, such as peptides, proteins and nucleic acids, have amphiphilic properties. Such molecules might localize at the interface of DMF and water, leading to a dramatic acceleration of the reactions in a microreactor. Applications of our microreaction system for substitution of biopolymers are in progress.



Scheme 2 Reaction of thiol group of cysteine with maleimide.

In conclusion, using amino acid substitution as a model reaction this study demonstrated that chemical reactions can be regulated differentially in a microchannel by controlling the mixing mode of two different solvents.

Acknowledgements

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