Chip Laboratories

DOI: 10.1002/anie.200504205

Enantioselective Catalysis and Analysis on a Chip**

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The application of microsystems technology to chemistry enables miniaturization of chemical processes on so-called microfluidic chips, where reactions are performed in micro channels and cavities rather than in flasks and tubes. The ambitious aim is to shrink chemical laboratories to so called lab-on-a-chip systems. Besides the advantages of miniaturization, such as improved portability, safety, and reduced reagent consumption, one of the most promising features of integrated palmtop laboratories is the accelerated speed of reaction and analysis,[1] with corresponding potential for high-throughput screening. While the introduction of classical high-throughput screening systems based on robotics and massive parallelization has revolutionized chemical synthesis, miniaturization and integration on chip size would constitute a quantum leap.

Currently, significant progress is being made in microfluidics and its application for down-sizing chemical reactors.[2] One of the most successful applications of microfluidics in analytical chemistry is microchip electrophoresis (MCE), which can be regarded as a miniaturized version of classical capillary electrophoresis. One of the most prominent features of MCE is the extraordinary high speed of analysis, which could also be successfully applied to the separation of enantiomers by us[3] and others.[4] By applying MCE to the enantioselective separations of amino acids in less than a second we have already reported the fastest chiral separation.[5] In previous work we laid the foundation for the general applicability of chip electrophoresis to chiral high-throughput screening of enantioselective catalysts.[6]

The ultimate potential of chip technology in chemistry can only be exploited fully by the integration of chemical synthesis and analysis. While complex integrated lab-on-a-chip systems have recently been described for biochemical applications, such as genetic analysis of the influenza virus,[7] similar achievements remain to be made in synthetic chemistry. Recently, a miniaturized T-reactor was impressively applied in asymmetric synthesis, chiral analysis of the reaction products was, however, performed offline utilizing traditional GC/MS.[8]

We have now implemented a novel integrated approach which combines a microfluidic reactor and analysis unit on a single device. We have utilized this reaction/analysis chip for testing the enantioselectivity of enzyme mutants created by directed evolution of the enzymes.[9] As a model system, the epoxide hydrolase catalyzed hydrolytic kinetic resolution of glycidyl phenyl ether[10] was integrated with chiral electrophoresis on a chip.

The working principle of the chip we developed for this purpose is shown in Figure 1. Substrate and catalyst are placed in respective microvials on the chip. Upon applying pressure or electrical fields, enzyme and substrate solutions are merged through microfluidic channels, the mixing and reaction are aided by means of a meandering channel. The reaction products are then guided to the separation channel by applying a voltage-controlled pinched injection.[11] The actual electrophoretic separation of products and educts occurs in an electrical field in the separation channel.

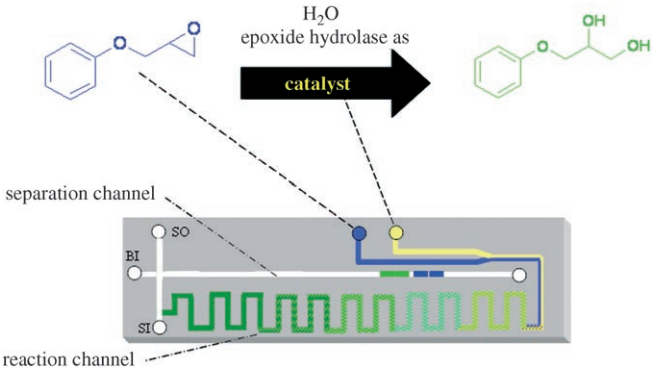

Figure 1. Schematic drawing of the working principle of the integrated catalysis/analysis chip. SO: sample outlet, SI: alternative sample inlet, BI: buffer outlet.

A photo of such a microfluidic chip made from fused-silica[12] together with a typical example of a separation of (+/−) substrate and (+/−) product is shown in Figure 2. This prototype contains four microvials for dispensing different substrates or catalysts, enabling, for example, three catalysts acting on one substrate to be tested in a single set of experiments.

For separation of the compounds a 50 mmol L^{-1} borate buffer at pH 8.5 containing 15 mmol L^{-1} heptakis-6-sulfato-β-cyclodextrin as a chiral selector was utilized. Under these conditions product and educt can simultaneously be separated into the respective enantiomers in less than 90 seconds (Figure 2). For the detection of the analytes we applied native fluorescence detection, using a deep-UV laser (Nd:YAG, $\lambda =$ 266 nm); a technique that we only recently introduced in chip analysis[13] and which requires UV-transparent fused-silica chips, rather than the commonly applied borofloat glass as chip material.

This new system was successful in testing the enantioselectivity of new enzyme mutants created by directed evolu-

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[**] We acknowledge financial support from the Fonds der Chemischen Industrie and the DFG (SSP1170) and L.-W. Wang acknowledges support from the DAAD for a doctoral stipend.

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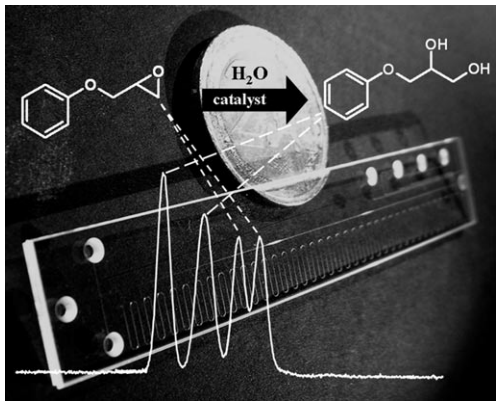

Figure 2. Photo of the microfluidic chip in comparison with a 2-Euro coin together with the reaction scheme (top) and the electrophoretic data (bottom) of the separated enantiomers of product and precursor.

tion. The data of on-chip electrophoretic separations after on-chip reaction of different epoxide hydrolase mutants[10b] from *Aspergillus niger*, as well as from the wild type,[10a] are shown in Figure 3. Reaction on-chip was possible using purified

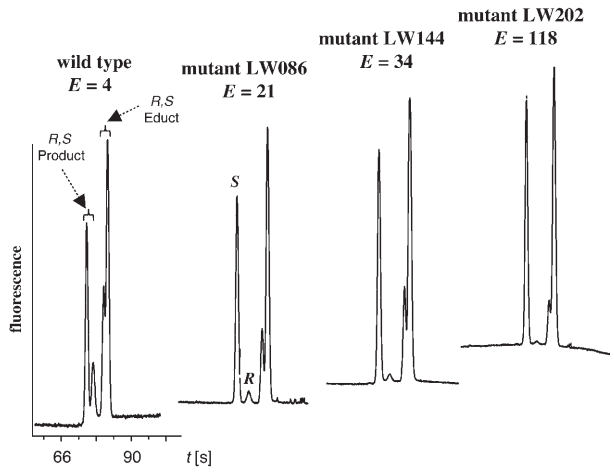

Figure 3. Application of the system for testing the wild type as well as different epoxide hydrolase mutants created by directed evolution. Electrophoretic separation of product and educt enantiomers after on-chip catalysis. The *E* values were calculated from the corresponding single measurements.

enzymes in the case of the wild type or cell lysates and with whole cells. No interfering signals for the enzymes or cell lysates or cells where obtained under the experimental conditions employed. As reaction medium a 10 millimolar phosphate buffer at pH 7.2 containing 7.5 % (v/v) acetonitrile was employed. With this approach it is possible to reliably differentiate the enzymes with respect to their selectivities, (Figure 3). While a selectivity factor[14] of $E = 4$ was obtained for the wild type, an *E* value of 118 was determined for the best mutant LW202 in the respective single measurements.

The results obtained with the miniaturized catalysis/analysis chip were compared with those determined by the traditional procedure, namely reaction on bench-scale format and subsequent analysis of the supernatant with conventional chiral HPLC (Table 1). The agreement is good, which shows in principle the applicability of our miniaturized integrated system. The data for the chip experiments in Table 1 are mean values of triplicate determinations. The relative standard

Table 1: Comparison of experimental results for determination of conversion, enantiomeric excess (*ee*), and *E* value obtained with the catalysis/analysis chip or with conventional reaction in bench-scale format and subsequent HPLC-Analysis.[a]

Enzyme	Method	Conv [%]	*ee* diol [%]	*E*
wild type	chip	38	49	4
	bench-scale/HPLC	18	58	4
LW080	chip	22	90	23
	bench-scale/HPLC	23	82	13
LW086	chip	43	84	21
	bench-scale/HPLC	40	84	20
LW144	chip	28	93	37
	bench-scale/HPLC	37	89	29
LW202	chip	41	95	101
	bench-scale/HPLC	48	95	115

[a] The data are mean values from triplicate experiments.

deviations (RSD) are in a quite acceptable range, especially in view of the fact that these are data from a prototype. For the *ee* value determinations we obtained RSDs of 1–3 % and for the *E* values RSD of 3–21 % were determined. Although the RSDs for the *E* values are somewhat high, unequivocal identification of the best mutants is still possible.

In the experiments described above the mixing process and reaction of substrates and enzymes occurred in microfluidic channels on a chip. The liquids were forced through the reaction channel by the application of vacuum; alternatively this was also carried out by applying an electrical field. Due to the long channel geometry the voltage driven reaction step was, however, more time consuming (about 25 min) than the vacuum transport (10 min). By integration of a more effective and faster micromixer it can be expected that the reaction and mixing times can be reduced considerably.[15]

Instead of merging substrate and enzyme in a microfluidic channel, mixing and reaction can be performed much faster and straightforward by pipetting the compounds together in a microvial. The reaction will then start instantly, which however may be disadvantageous for testing large libraries of catalysts because different conversions are then to be expected. On the other hand, this instant-start effect can be exploited to study the conversion and selectivity in real time by utilizing the fast and integrated analysis part of the chip. This approach can be put into practice by pipetting substrate and enzyme into the microvial labeled BI (Figure 1) and applying a so-called gated injection[16] by repetitive switching between defined electrical potentials. This results in sequential injections of reactants into the separation channel from a continuous sample flow. As a result stacked electropherograms are obtained which can be used to follow the kinetics of the reaction. (Figure 4).

The respective data together with the extracted values of conversion and *E* value are displayed in Figure 5. These experiments were carried out with 10 μL of a 0.02 mg mL^{-1}

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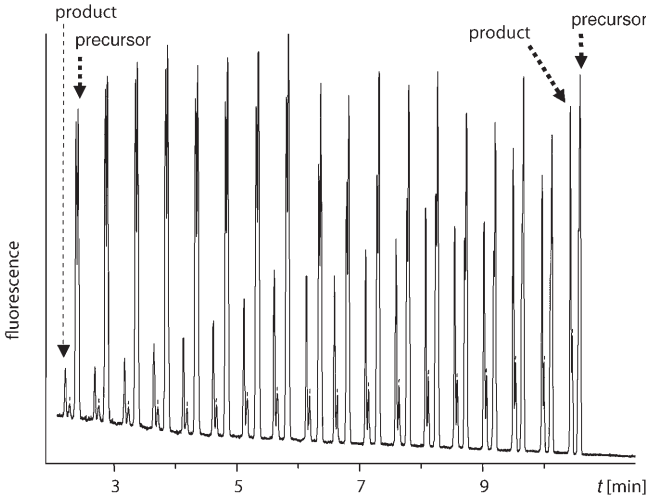

Figure 4. Monitoring reaction kinetics with stacked electropherograms. For this purpose the wild-type enzyme and substrate were pipetted together in a microvial on a chip and repetitive chiral separations were preformed.

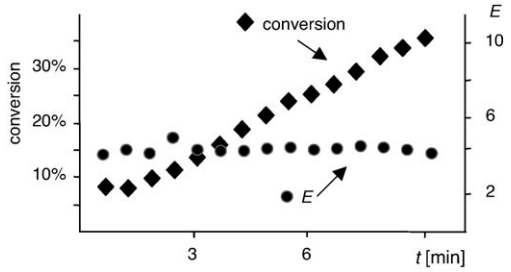

Figure 5. Slope for conversion and *E* value, derived from data of stacked electropherograms in Figure 4.

solution of epoxide hydrolase of the wild-type enzyme and with 10 μL of 0.75 mmol L^{-1} of epoxide solution.

Herein we have demonstrated the first example of an integrated chip-based system applied in synthetic chemistry and catalysis which integrates a microfluidic reactor with chemical analysis. In this initial study the device was successfully applied for testing enantioselective biocatalysts created by directed evolution of enzymes; the device also has great potential for high-throughput screening. Integration of chemical reaction and analysis on a chip results in reduced reagent consumption and allows the speed of the whole screening process to be increased considerably. Further improvement of the system, with regard to high-throughput appears possible by implementing faster and more effective micromixers, by parallelization of the microfluidic separation and reaction channels and/or by coupling with microdroplet devices as previously suggested.[17]

Received: November 25, 2005
Published online: January 27, 2006

Keywords: directed evolution · electrophoresis · enantiomer separation · high-throughput screening · microreactors

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