MULTIPLEXED "DETECTORLESS" ELECTROPHORESIS

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ABSTRACT

A new microfluidic electrophoresis device and technique are described that were specifically designed for multiplexed, high throughput operation. The device consists of an array of short microfluidic channels connecting individual sample reservoirs to a common buffer reservoir. Each channel in the array functions as both a separation channel and as a conductivity-based detector. The system is extremely simple and can be easily and inexpensively scaled up to perform large numbers of simultaneous analyses. As a first demonstration, a 16 channel array device is used for high-throughput, time-series measurements of enzyme activity and inhibition.

KEYWORDS: conductivity, enzyme, gradient elution moving boundary electrophoresis, multiplexed

INTRODUCTION

The ability to acquire large amounts of high quality data very rapidly is increasingly important for progress in drug discovery and systems biology. Capillary and microchip electrophoresis are generally recognized as providing very high quality data, [1, 2] but current systems for high-throughput electrophoresis are expensive and complicated – with multiple optical detection points and the apparatus required for defining injections (extra channels and reservoirs in chip-based systems). [1-3] Therefore, we have set out to create a simple device for high quality, multiplexed electrophoresis.

The new technique is based upon the recently described gradient elution moving boundary electrophoresis (GEMBE). [4] The microfluidic device for GEMBE is simple – a channel connecting two reservoirs (Figure 1). A high voltage drives electrophoresis of the sample analytes through the channel. At the start of a separation, the bulk counterflow of buffer through the channel is high, and no analytes of interest can enter the channel. The counterflow is then gradually reduced (using a precision pressure controller) until each analyte, in turn, is able to enter the channel where it is detected as a moving boundary or step.

EXPERIMENTAL

The buffer used was 100 mmol/L bis-tris, 100 mmol/L HEPES, pH 7.1, the applied voltage was 2000V (6667 V/cm field strength). During each separation, the pressure applied to the buffer reservoir was varied from 32,000 Pa to -24,000 Pa at a rate of -200 Pa/s. The enzyme reactions solutions each contained 200 μmol/L ATP, 200 μmol/L kemptide, 2 mmol/L magnesium chloride, and 10 % (v/v) dimethylsulfoxide. For the data shown in Figure 2(A), each sample also contained a variable amount of protein kinase A (PKA) ranging from 25 U/μL to zero. For the

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data shown in Figure 2(B), each sample also contained 8 U/μL PKA, and a variable concentration of the inhibitor H-89 dihydrochloride ranging from zero to 1 mmol/L.

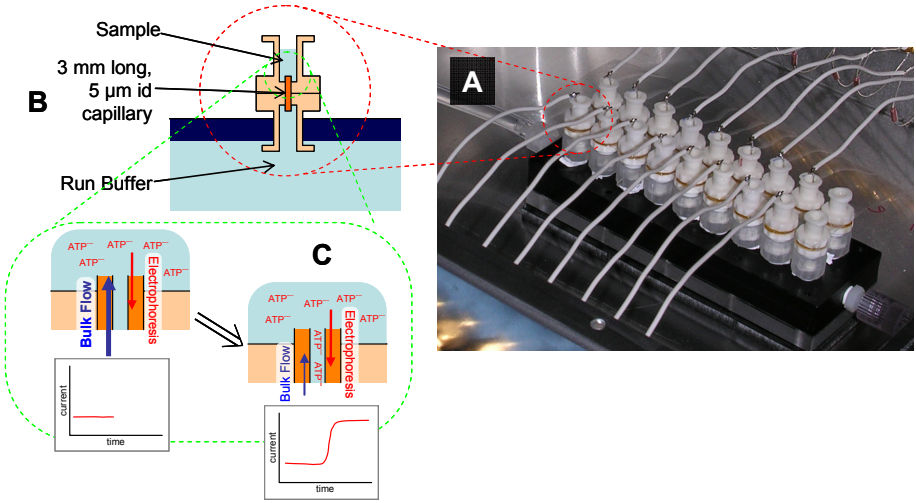

Figure 1. A) Photograph of 18-channel array electrophoresis device. B) Individual separation channels were fabricated into Luer lock fittings for ease of handling. C) At the beginning of a separation, the large bulk counterflow prevents analyte ions from entering the channel. As the counterflow is reduced, the ions eventually enter the channel and are recorded as a step change in the channel current.

RESULTS AND DISCUSSION

The advance presented here is the use of an array of very short channels with GEMBE, so that only one step at a time is present in each channel. The electric current through the channels can then be used as the detection signal – without any extra detector hardware. The current vs. time signal for each channel is then smoothed and differentiated to produce a set of simultaneous electropherograms.

In addition to its simplicity, a major advantage of the new technique is that the counterflow of buffer through the separation channels prevents problematic sample constituents such as proteins or enzymes from entering the channels. Consequently, enzyme reaction samples can be analyzed with no need for surface coatings or other additives to prevent surface fouling [5]. In addition, because all of the samples in the array are analyzed at the same time, no reaction-stop reagent is needed. Enzyme activity or other reaction rate data can be taken in a time series after loading the device once – with the assay solutions incubating in the sample reservoirs. Figures 2(A-B) show two examples: for enzyme activity measured at different enzyme concentrations and for inhibition of enzyme activity for a series of different inhibitor concentrations.

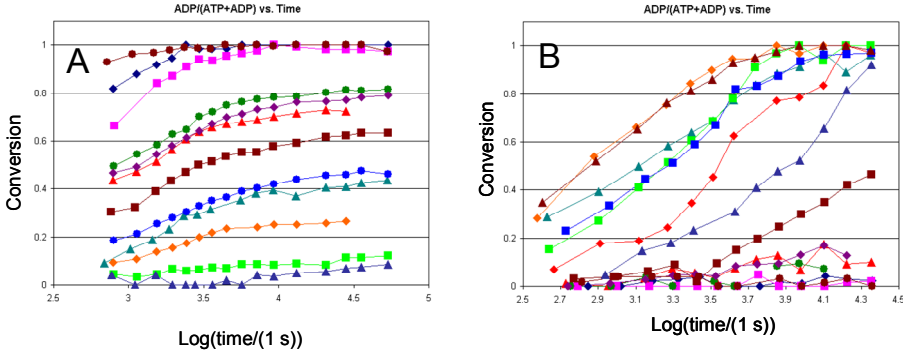

Figure 2. Enzyme activity time series data from 16-channel array device. The enzyme reaction: kemptide + ATP -> phosphokemptide + ADP was monitored by measuring the conversion of ATP to ADP. A) Reaction mixtures with different concentrations of enzyme (protein kinase A) were incubated (at room temperature, 23 °C) in each of the 16 sample reservoirs, and repeated measurements were made. Each trace is time series data for a different enzyme concentration. Traces for very low enzyme concentrations (showing no conversion) not shown. B) The enzyme concentration was the same for each sample, but a variable concentration of an inhibitor (H-89 dihydrochloride) was added to each sample.

CONCLUSIONS

The device presented here is a simple microfluidic device for performing high resolution electrophoretic separation and detection. Consequently, the cost to scale-up this technique to perform 96, or 384 simultaneous separations is expected to be much less than the scale-up cost of more conventional multiplexed separation techniques.

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