MEASURING RAPID BINDING KINETICS BY MICRO ION-SELECTIVE ELECTRODES IN DROPLET-BASED MICROFLUIDIC DEVICES

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ABSTRACT
Rapid kinetics was measured by potentiometric method in the paper. Droplet-based microfluidic chips with ion-selective microelectrodes and pneumatic valves were used to measure the binding kinetics of RNA with Mg\(^{2+}\) and DNA with K\(^+\). Phase separation channels were added to remove the oil before the aqueous droplets reached the electrodes. Phase separation channels were required to obtain stable signals by the electrodes. The total consumption of oligonucleotides was less than 20 \(\mu\)L for a single experiment. The reaction time measured was from 0.05 to 25 s. The microfluidic system developed here is applicable to the biochemical reactions involving electrochemically active reactants or products.

KEYWORDS: Ion-selective microelectrode, potentiometric method, phase separation channels, rapid kinetics, droplets

INTRODUCTION
Rapid kinetics measurement is important to understanding many biological processes, such as protein and RNA folding, protein-protein interaction, and enzymatic mechanism. Stopped-flow and quenched-flow are two commonly used methods. Several microfluidic platforms have been developed to minimize reagent consumption for bioanalysis. However, the fluorescence detection method has several drawbacks, such as necessity of labeling, photo-bleaching and interference of the fluorescence tag with the reaction of interest. Previously we applied amperometric method in droplet-based microfluidic systems for studying enzyme kinetics. This method is sensitive and label-free. However, the method is applicable to a limited group of molecules in biochemical reactions and lacks specificity. Herein, potentiometric method based on ion-selective electrode was applied to droplet-based microfluidics. To illustrate the application of the integrated microfluidic system, rapid binding kinetics of RNA with Mg\(^{2+}\) and DNA with K\(^+\) were studied.

THEORY
The nucleic acid of interest bound with Mg\(^{2+}\) or K\(^+\) and folded rapidly in the aqueous droplets. The binding kinetics was measured by detecting the decrease of the metal ions in the droplets at different reaction time. Ion-selective microelectrodes were used to detect the potentiometric signal. To solve the problem of the signal noise caused by the oil interference, phase separation channels were added to separate the oil phase from the aqueous phase.

EXPERIMENT
The PDMS chip contained fluid layer and control layer, both of which were fabricated by soft lithography. The solid-contact Mg\(^{2+}\) and K\(^+\) ion-selective microelectrodes were prepared by using Pt wires coated with poly(3-octylthiophene-2,5-diyi) solution and Mg\(^{2+}\) and K\(^+\) ionophore cocktail, respectively. The ion-selective microelectrode and Ag/AgCl microelectrode were then inserted into the microchannels in the fluid layer and sealed with epoxy glue (Figure 1a and 1b). Droplets containing the mixture of the nucleic acid and metal ion were produced when the two aqueous streams were jointly injected into an oil flow (Figure 1b). Pneumatic valves controlled the moving route and moving distance of the droplets, thus controlling the reaction time. The direct measurement of droplets by ion-selective electrode was unfeasible, because the oil interfered with the potentiometric signals and caused large noise. To solve the problem, a group of ten parallel 50 \(\mu\)m-wide microchannels were added perpendicularly at the end of the microchannel to separate the oil phase and to form a continuous aqueous phase (Figure 1c and 1d). By combining phase separation with the droplet-based microfluidic technique, we were able to overcome the intrinsic time limit of the ion-selective electrodes to measure rapid kinetics.
RESULTS AND DISCUSSION

In the first experiment, the binding kinetics of the RNA, P4-P6 domain of the *Tetrahymena* ribozyme constructs, and Mg$^{2+}$ was studied. The decrease of the Mg$^{2+}$ concentration was monitored by the Mg$^{2+}$ selective microelectrode (Figure 2). The fast binding rate constant $k$ increased from 1.96 s$^{-1}$ to 3.56 s$^{-1}$, when the concentration of Mg$^{2+}$ increased by 10 folds. The two-exponential model significantly improved the fitting quality over the single-exponential model, indicating that two Mg$^{2+}$ ions were associated with one RNA molecule in a two-step mechanism. Previous crystallographic study and Hill coefficient analysis indicated that five Mg$^{2+}$ ions are involved during the folding of each P4-P6 domain. Lately it was suggested that each P4-P6 domain uptakes two Mg$^{2+}$ ions upon metal ion core folding while the other Mg$^{2+}$ ions diffuse near the RNA molecule without specific binding. The results from the current work agree with the latter model of specific and diffusive binding, as the specific binding reduced the Mg$^{2+}$ concentration measured by the ion-selective electrode, while the diffusive
binding did not.

In the second experiment, the binding kinetics of a DNA molecule with K⁺ was studied. The DNA was called T30695, a homolog for the thrombin binding aptamer. The single-exponential model presented good fitting quality. The rate constant was 8.02 s⁻¹ when the DNA concentration was 0.25 mM and K⁺ concentration 1 mM. The decrease of the K⁺ concentration indicated that each DNA molecule bound with three K⁺, which was consistent with previous observation.⁷

CONCLUSION

We successfully applied potentiometric method to measuring rapid kinetics in microdroplets. Phase separation micro-channels were implemented to facilitate potentiometric measurement of the droplets. The system overcomes the intrinsic diffusion time limit of ion-selective electrodes. The dead time of the system was less than 0.1 s, and the total consumption of the reagents was less than 20 μL for a single experiment. The electrodes integrated microfluidic system is complementary to the fluorescence-based microfluidic system for studying biochemical processes with the advantages of high sensitivity, inexpensive fabrication and being label-free. We envision the system will find broad applications in studying biochemical reactions that involve small ionic species.

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REFERENCES


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