LABEL-FREE MICROFLUIDIC CHARACTERIZATION OF TEMPERATURE DEPENDENT BIOMOLECULAR BINDING BY MALDI-TOF MASS SPECTROMETRY

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ABSTRACT

We present a microfluidic approach to characterizing temperature-dependant binding of biomolecules. The method includes a microfabricated chip with biomolecule functionalized surfaces coupled to a matrix-assisted laser desorption/ionization mass spectrometer (MALDI-MS). Using an integrated microfluidic device, we observe the temperature-dependent characteristics of binding vasopressin to an RNA aptamer receptor. Our results reveal two binding temperature zones (34-45 ^{o}C and 70-75 ^{o}C) separating three melting temperature zones. This system can be useful for screening therapeutic and receptor ligands.

KEYWORDS: Affinity binding, aptamer, mass spectrometry, microfluidics, temperature dependence

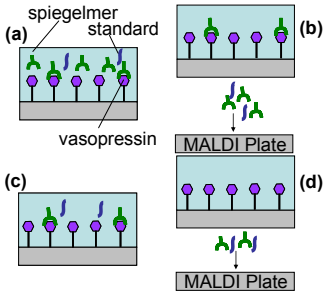
INTRODUCTION

Biomolecular binding (e.g., between a ligand and receptor) is generally temperature-dependant. Understanding such phenomena is important for applications such as biosensing and therapeutics [1]. Conventionally, temperature-dependant binding can be characterized by fluorescence scanners or magnetic resonance imaging. However, such methods require molecular labels that are time-consuming to develop, may interfere with the binding under study, and can be themselves, temperature-dependant. Here, we present a novel, label-free approach to characterizing temperature-dependant biomolecular binding in a microfluidic device. This is demonstrated with the affinity binding of vasopressin to a spiegelmer, which is an RNA aptamer modified for improved biostability. Using an integrated microfluidic device, an internal standard, and MALDI-MS, we determine optimal binding temperature intervals (34-45 ^{o}C and 70-75 ^{o}C) which separate three melting temperature zones. This demonstrates the potential of our approach for screening receptor molecules or therapeutic agents.

EXPERIMENTAL METHODS

In our approach (Fig. 1a-b), a solution of spiegelmer and standard nucleic acid sequence (P18) is introduced to vasopressin-functionalized microbeads at specified temperature setpoints. The spiegelmer binds to vasopressin while P18 does not. Spiegelmer molecules remaining in solution along with the standard are collected and measured by mass spectrometry. The temperature-dependence of spiegelmer-vasopressin binding is reflected by the temperature-dependent ratio of spiegelmer to standard spectral peaks (i.e., normalized peak). Also, initially bound molecules can

be released by modifying the surface temperature above or below a binding temperature (Fig. 1c-d). This approach is realized with a microchip, consisting of a microchamber packed with vasopressin-functionalized microbeads, a microheater and temperature sensor for temperature control, and microchannels and valves for transferring solution-borne spiegelmer onto a target plate subsequently used for MALDI-MS (Fig. 2). The chip is fabricated from PDMS on glass [2].



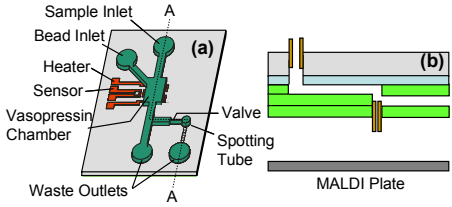


Fig. 2. Device schematics. (a): oblique view; (b): A-A cross-sectional view. Chip dimensions: 2 × 2 × 0.16 cm (l × w × h).

Fig. 1. (a) Introduction of spiegelmer/standard sample to vasopressin at set temperature. (b) Unbound spiegelmers deposited onto a MALDI plate. Beginning with bound spiegelmer (c), temperature changed to release and deposit spiegelmer molecules (d).

RESULTS

We first investigate the suitability of MALDI-MS for quantifying spiegelmer samples in the presence of P18 (Fig. 3). The spiegelmer (12.8 kDa) and standard (8.6 kDa) are spotted with pipetters in concentrations proportional to each other (1:0.1 and 0.1:1 spiegelmer:P18). In both instances, mass peaks for spiegelmer and P18 are clearly distinguishable and correctly proportional, justifying this approach.

Following Fig. 1a-b, spiegelmer/vasopressin binding is characterized from room temperature to 75 ^{o}C (Fig. 4). A wide spiegelmer concentration range (0.01, 0.1, 1 and 10 μM) with equal concentrations of P18 standard is used. For example, with a 10 μM spiegelmer/standard sample, we observe optimal binding in two temperature zones, 34-45 ^{o}C and 70-75 ^{o}C. This is indicated by a very low normalized spiegelmer/standard peak. Melting (indicated by high normalized peak) occurs in three temperature zones: 15-30 ^{o}C; 50-65 ^{o}C; and above 75 ^{o}C. This is similar for all tested concentrations, indicating consistency over three orders of magnitude. Such complex binding profiles may be difficult to elucidate with a conventional approach, highlighting the advantage of our approach.

With certain biomolecular systems, interaction time can determine the level of complete binding between the two molecules, particularly in systems with high equilibrium dissociation constants (K_d) or diffusion limited setups. We discover that the spiegelmer/vasopressin interaction time has little effect on the level of unbound spiegelmer detected in mass spectra, as suggested by the identical spectrums obtained for 0.5 and 5 min incubation times (Fig. 5). This confirms both specific (spiegelmer/vasopressin $K_d \sim 1.7$ nM) and diffusion negligible binding.

Finally, utilizing the scheme presented in Fig. 1c-d, a single spiegelmer sample (1 and 10 μM) is cycled through binding at 34 ^{o}C and subsequent decoupling at 60 ^{o}C to test the efficiency of spiegelmer dissociation (Fig. 6). For instance, a 0.15

normalized peak is observed for a 10 μM spiegelmer/standard sample introduced at 34 °C, meaning 85 % of the spiegelmer sample formed a complex with the immobilized vasopressin. Upon heating to 60 °C, we notice a spiegelmer/standard normalized peak of 0.75, suggesting 88 % of the bound spiegelmer is released. This demonstrates the effectiveness of temperature modulations as a target release method in applications such as biomolecular purification [2].

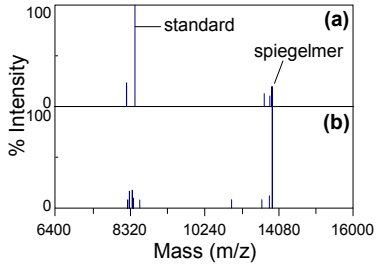


Fig. 3. Conventionally pipetted samples of spiegelmer; (a) 0.1:1 and (b) 1:0.1 μM spiegelmer:standard.

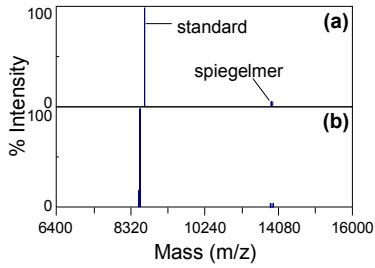


Fig. 5. (a) 30 sec; (b) 5 min interaction time for 10 μM spiegelmer sample (10 μM standard) binding vasopressin at 34 °C.

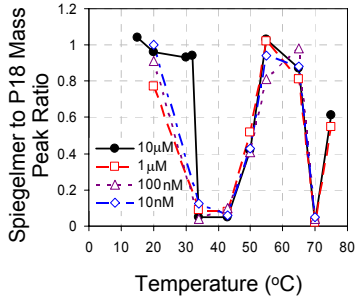


Fig. 4. Temperature-dependant binding for binding of spiegelmer-vasopressin.

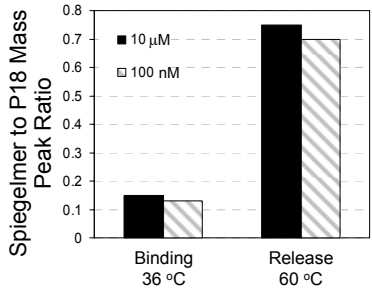


Fig. 6. Efficiency of spiegelmer release.

CONCLUSIONS

We have presented microfluidic approach to label-free characterization of temperature depending binding. Such studies of the binding of vasopressin with a spiegelmer reveal two binding temperature zones (34-45 °C and 70-75 °C) separating three melting temperature zones. This approach is useful for screening receptor molecules or therapeutic agents.

ACKNOWLEDGEMENTS

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REFERENCES

[1] P. D'Orazio, *Clin. Chim. Acta*, 334: 41-69, 2003.

[2] T. Nguyen, C. Qiu, R. Pei, et al., *(MEMS '08)*, pp. 196-199, Tucson, US, 2008.