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Label-Free Microfluidic Characterization of Temperature-Dependent Biomolecular Binding

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Abstract—**We present a microfluidic approach to characterizing temperature dependent biomolecular binding. A ligand and its affinity receptor, one of which is surface-immobilized and the other remaining in solution, are allowed to achieve equilibrium binding in a microchip at a series of selected temperatures. The unbound molecules in solution are subsequently collected and analyzed with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), yielding temperature dependent equilibrium binding profiles of the ligand-receptor system. As examples, the thermally dependent properties of equilibrium binding of adenosine monophosphate, platelet-derived growth factor and arginine vasopressin with their affinity nucleic acid aptamers were investigated. The binding profile obtained for each biomolecular system revealed highly temperature dependent zones in which affinity binding or dissociation occurs. These results demonstrate that our microfluidic approach, which does not use any molecular labeling groups, can potentially be used to characterize receptor-ligand binding for applications in biosensing, biomolecular purification, and drug development.**

I. Introduction

BIOMOLECULAR binding phenomena are fundamentally present in vast biological systems. For example, the structural and chemical complementarity between ligands and their biomolecular counterparts, receptors, may influence metabolic or signal-transduction pathways, and thus initiate physiological effects on the cellular level. Naturally, biomolecular binding can be influenced by a number of environmental factors, such as electromagnetic radiation, pH, ionic strength, and in particular, temperature [1]. As temperature may change physiologically due to thermoregulation, understanding the mechanisms governing temperature dependent biomolecular binding is important for developing potential applications in biosensing, drug development, and therapeutics. For instance in physiological conditions, a protein can experience fluctuating thermal stimulation (depending on the present physiological state) which can alter its native structure, thereby affecting its interaction with, or recognition of, a drug molecule. Gaining insight into the nature of this stimulation can facilitate the determination of the efficacy of the drug in question. Therapeutically, knowledge of the temperature dependent relationship between ligand and receptor interactions may facilitate techniques which identify receptor dysfunction leading to failures in cellular regulation as possible disease pathways. Additionally, elucidation of temperature dependent binding of ligand-receptor systems provides fundamental insight for developing biomolecular sensing and manipulation methods requiring tailored control of binding properties (e.g., generating specific receptor-ligand complexes such as synthetic oligonucleotides aptamers by evolutionary selection).

Conventionally, temperature-dependant receptor-ligand binding can be characterized using methods such as scintillation, fluorescence resonance energy transfer (FRET), electrophoretic mobility shift assays, and differential calorimetry. FRET and scintillation techniques use fluorescent or radioactive molecular labels to indicate biomolecular binding and dissociation. Unfortunately, molecular labels are time-consuming and labor intensive to develop, may interfere with the binding under study, and can be themselves temperature-dependant. Electrophoretic assays utilize mobility shifts induced by biomolecular binding, and are limited to small-molecule ligands. Differential calorimetry directly measures the thermal power evolved in biomolecular binding events, but is typically limited to large volumes of relatively high-concentration samples. To address the limitations of existing methods, we present a label-free microfluidic approach to characterizing the temperature dependant nature of receptor-analyte interactions. This is accomplished using an integrated microchip coupled to label-free detection with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Our method is demonstrated by characterizing temperature dependent binding of aptamers with target ligand molecules. Aptamers are oligomers of ribonucleic (RNA) or deoxyribonucleic (DNA) acids that recognize a broad class of target ligands, such as small molecules, peptides, amino acids, and proteins via specific and reversible affinity interaction. The strong temperature dependence of aptamer-ligand interaction is demonstrated by a preliminary investigation of three example ligands: adenosine monophosphate (AMP), platelet derived growth factor (PDGF), and arginine vasopressin (AVP).

II. Experimental Methods

A. Principle

Microfluidic characterization of temperature-dependent receptor-analyte binding can followed in Fig. 1. Considering the spiegelmer-vasopressin system for instance, the method begins by introducing a solution of spiegelmer and standard nucleic acid sequence (P18) to vasopressin-functionalized

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microbeads at specified temperature setpoints (Fig. 1a, b). The spiegelmer binds to vasopressin while P18 does not. Spiegelmer molecules remaining in solution along with the standard are collected at the device's spotting outlet 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. 1 c, d).

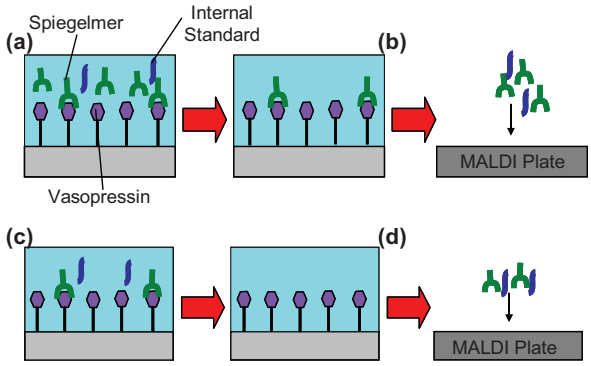

Fig. 1. (a) Label-free characterization of temperature-dependant binding: spiegelmer and standard introduced to vasopressin at specified temperature. **(b)** Carry volume removes unbound spiegelmers, and then is deposited onto a MALDI plate. Beginning with bound spiegelmer **(c)**, the temperature on the device is changed to release and deposit spiegelmer molecules **(d)**.

B. Device design

The label-free characterization of temperature-dependent binding 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 was fabricated from PDMS on glass using soft lithography techniques. Details of the device design and fabrication process are described elsewhere [2].

III. Results and Discussion

Using our microfluidic approach, we first examined the temperature-dependent interaction properties between AMP and its specific aptamer. However, in this system an anti-AMP aptamer was immobilized on microbeads while AMP in solution was introduced for binding, release, and subsequent MALDI-MS detection. Guanosine monophosphate (GMP) was utilized as a standard non-binding analyte. Using a single concentration of 10 µM AMP, binding was characterized from room temperature to 60 °C (Fig. 3). The AMP system experienced optimal binding within the temperature range of 25-35 °C, indicated by the low AMP to GMP mass peak ratio. Binding dissociation appeared to initiate in a temperature zone immediately higher than 35 °C, while becoming more considerable above 45 °C.

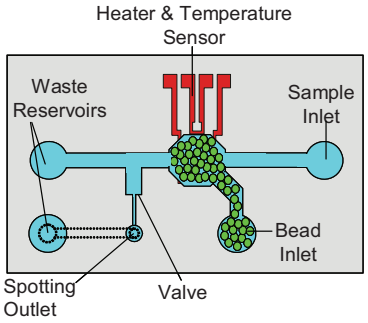

Fig. 2. Schematic of the microfluidic device used for MALDI-MS based characterization of temperature dependent aptamer-protein binding.

Following the same protocol as AMP and its specific aptamer, we additionally investigated the temperature dependent binding characteristics of PDGF and its correlating specific aptamer. However, in this system a PDGF specific aptamer is immobilized on microbeads while PDGF in solution was introduced for binding, release, and subsequent MALDI-MS detection. Furthermore, vascular endothelial growth factor (VEGF) was used as a non-binding standard. Using a single concentration of 10 µM PDGF, binding was characterized from room temperature to 60 °C (Fig. 4). In this case, we observed optimal binding in one temperature zone, 24-45 °C as indicated by a very low normalized PDGF/standard peak. Release of PDGF from its specific DNA aptamer occurred in only one observable temperature zone: 45-60 °C. It is interesting to note that the release temperature zone of the PDGF system (45-60 °C) is well below the melting temperature of PDGF itself [3]. While we are not aware of any reported melting temperature for vasopressin, it is reasonable that one of the observed release temperature zones (15-30 °C) for that system is well within the limits of vasopressin melting, considering vasopressin is active in human physiological conditions (~37 °C).

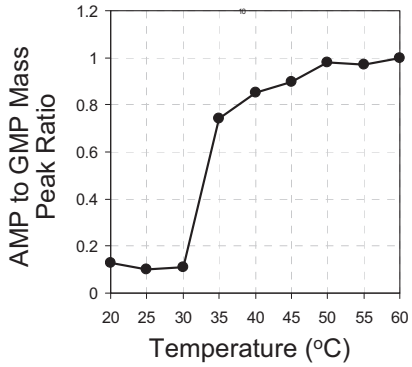

Fig. 3. Temperature dependent binding of AMP to anti-AMP aptamer. GMP standard is of equal concentration to AMP for each sample.

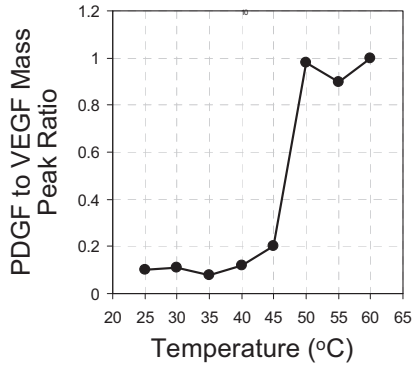

Fig. 4. Temperature dependent binding of PDGF to anti-PDGF aptamer obtained similarly to protocol used for AMP system. VEGF utilized as a standard.

We next characterized the polypeptide, vasopressin from room temperature to 75 °C (Fig. 5). A wide spiegelmer concentration range (0.01, 0.1, 1 and 10 µM) with equal concentrations of P18 standard was used. For example, with a 10 µM spiegelmer/standard sample, we observed optimal binding in two temperature zones, 34-45 °C and 70-75 °C. This is indicated by a very low normalized spiegelmer/standard peak. Release of spiegelmer from the vasopressin (indicated by a high normalized peak) occurs in three temperature zones: 15-30 °C; 50-65 °C; and above 75 °C. This is similar for all tested concentrations, indicating consistency over three orders of magnitude.

These results demonstrate that our method can be used as a powerful tool for label-free characterization of temperature dependent binding of biomolecular targets with aptamers. Such complex binding profiles may be difficult to elucidate with conventional approaches. Additionally, this work provides an ideal method for surface-based biosensor characterization.

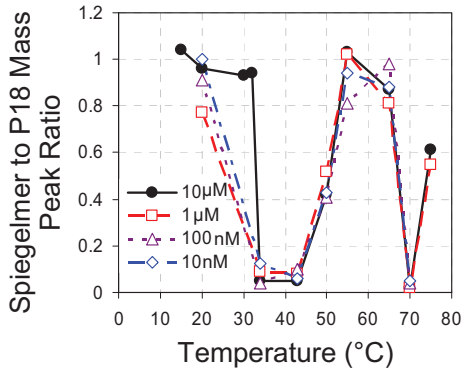

Fig. 5. Temperature dependent binding of spiegelmer-vasopressin. P18 standard is of equal concentration to the spiegelmer for each sample.

IV. Conclusions

We have presented microfluidic approach to label-free characterization of temperature-depending binding. Such studies of the binding of adenosine monophosphate, vasopressin, and platelet-derived growth factor with their respective aptamer receptors. A binding profile for each biomolecular pair revealed zones of either strong or weak interaction depending on the localized system temperature. This approach is useful for screening receptor molecules or therapeutic agents.

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