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Short communication

Human β-defensin HBD3 binds to immobilized Bla g2 from the German cockroach (*Blattella germanica*)☆

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ABSTRACT

Human β-defensin 3 (HBD3) is a small, well-characterized peptide in mucosal secretions with broad antimicrobial activities and diverse innate immune functions. Among these functions is the ability of HBD3 to bind to antigens. In this study, we hypothesize that HBD3 binds to the allergen Bla g2 from the German cockroach (*Blattella germanica*). The ability of HBD1 (used as a control β-defensin) and HBD3 to bind to Bla g2 and human serum albumin (HSA, used as a control ligand) was assessed using the SensíQ Pioneer surface plasmon resonance (SPR) spectroscopy biosensor system. HBD1 was observed to bind weakly to Bla g2, while HBD3 demonstrated a stronger affinity for the allergen. HBD3 was assessed under two buffer conditions using 0.15 M and 0.3 M NaCl to control the electrostatic attraction of the peptide to the chip surface. The apparent K_D of HBD3 binding Bla g2 was 5.9 ± 2.1 μM and for binding HSA was 4.2 ± 0.7 μM, respectively. Thus, HBD3, found in mucosal secretions has the ability to bind to allergens like Bla g2 possibly by electrostatic interaction, and may alter the ability of Bla g2 to induce localized allergic and/or inflammatory mucosal responses.

1. Introduction

Allergens are exogenous antigens from a variety of environmental sources capable of inducing allergic responses, hypersensitivities, and asthma in children upon topical, mucosal, or systemic exposure. These include airborne indoor allergens from pets, house dust mites, and cockroaches [1,6]. Bla g2 from the German cockroach (*Blattella germanica*) is among these allergens and thought to be an important risk factor in the development and/or exacerbation of atopic asthma [6,9]. Bla g2 is detected in 87–93% of air samples in the bedroom and kitchen often in concentrations of Bla g2 that approach the threshold for sensitization in homes of children with asthma [6]. Exposure to 10^{-1} to 10^{-4} μg/ml Bla g2 induces positive reactions on cockroach-allergic patients [19]. Furthermore, simultaneous exposure to cockroach allergen and other environmental antigens, like endotoxin, can exacerbate the pulmonary response [14].

While many of the underlying mechanisms of Bla g2-induced allergy and hypersensitivity are becoming clearer [9], little is known about the interaction between innate immune molecules, like human β-defensins (HBDs), and Bla g2. HBDs are present in oral and respiratory tissues and secretions [4,10,11] and thus are ideally positioned to interact with allergens. HBDs are small, host-derived peptides with innate and adaptive immune functions [8,15,20,23]. They have direct antimicrobial activity against bacteria, fungi, and some enveloped viruses; chemoattract phagocytic and mast cells; induce inflammatory mediators; regulate the functions of phagocytes and the complement system; and enhance adaptive immune responses to co-administered antigens. HBDs bind to microbial antigens, which may facilitate receptor-mediated internalization of the antigen to immature dendritic cells [23] or attenuate antigen-induced pro-inflammatory cytokine responses [12,18].

In our recent work, we have observed that HBD1, HBD3, and histatin 5 specifically bind to immobilized adhesins from the periodontal pathogen *Porphyromonas gingivalis* [2,5,18]. This led us to hypothesize that HBD1 and HBD3 will also bind similarly to

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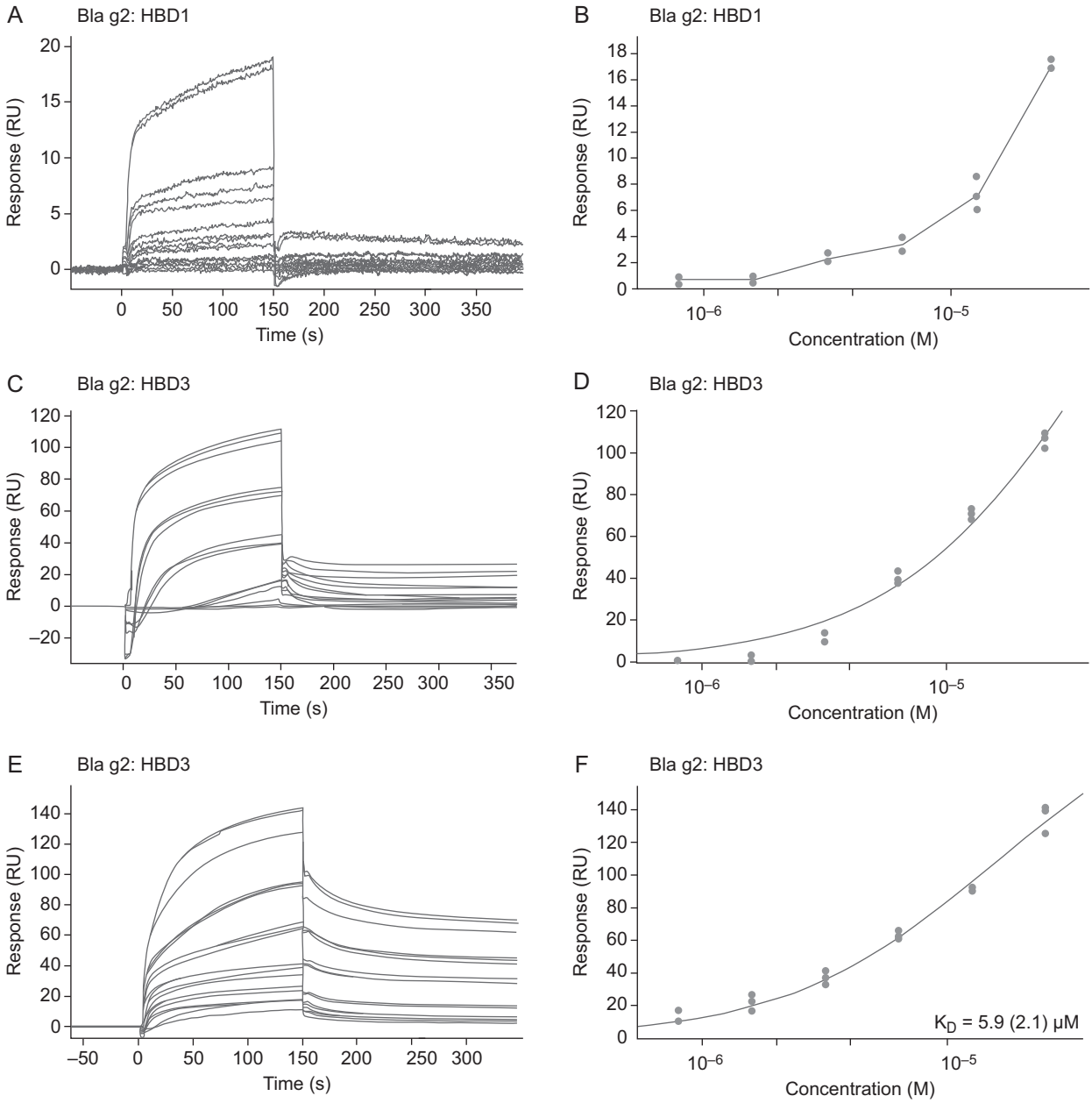

Fig. 1. SensíQ Pioneer Sensograms of HBD1 and HBD3 binding to immobilized Bla g2 from the German cockroach (*Blattella germanica*) showing (A) HBD1 binding to Bla g2 in 0.15 M NaCl and (B) the corresponding dose response; (C) HBD3 binding to Bla g2 in 0.15 M NaCl and (D) the corresponding dose response; and (E) HBD3 binding to Bla g2 in 0.3 M NaCl and (F) the corresponding dose response.

allergens like Bla g2. In this short communication, we report that HBD3 binds via electrostatic interactions, rather than specific binding domains, to immobilized Bla g2.

2. Methods

2.1. Reagents

Bla g2 (Indoor Biotechnologies, Charlottesville, VA, USA) was suspended in 10.0 mM sodium acetate, pH 5.0 (immobilization buffer) and used as the immobilized ligand in SPR spectroscopy.

HBD1 and HBD3 (PeproTech, Inc., Rocky Hill, NJ) were suspended in 10.0 mM HEPES, pH 7.4 containing 0.15 M NaCl, 3.0 mM EDTA, and 0.005% surfactant P20 (HBS-EP buffer) and used as the mobile analytes in SPR spectroscopy.

2.2. SPR spectroscopy

All SPR measurements were performed with the SensíQ Pioneer biosensor system at a controlled temperature of 25 °C as recently described [2]. Running buffer for experiments contained 10.0 mM HEPES, pH 7.4, 0.15 M NaCl, 3.4 mM EDTA, 0.005% (w/w) Tween-20, and 1.0 mg/mL CM-Dextran unless otherwise specified. A COOH2 sensor chip (SensíQ Technologies) was used for the peptide assays.

The COOH2 sensor chip was installed and conditioned according to manufacturer's protocol. Bla g2 and human serum albumin (HSA), used as a protein control, were each immobilized to different flow channels (FC) using a standard amine coupling method. The method involved activation by injecting 20.0 mM EDC with 5.0 mM NHS followed by an injection of 25.0 µg/mL protein solution in 10.0 mM sodium acetate buffer (pH 4.3 for Bla g2 and

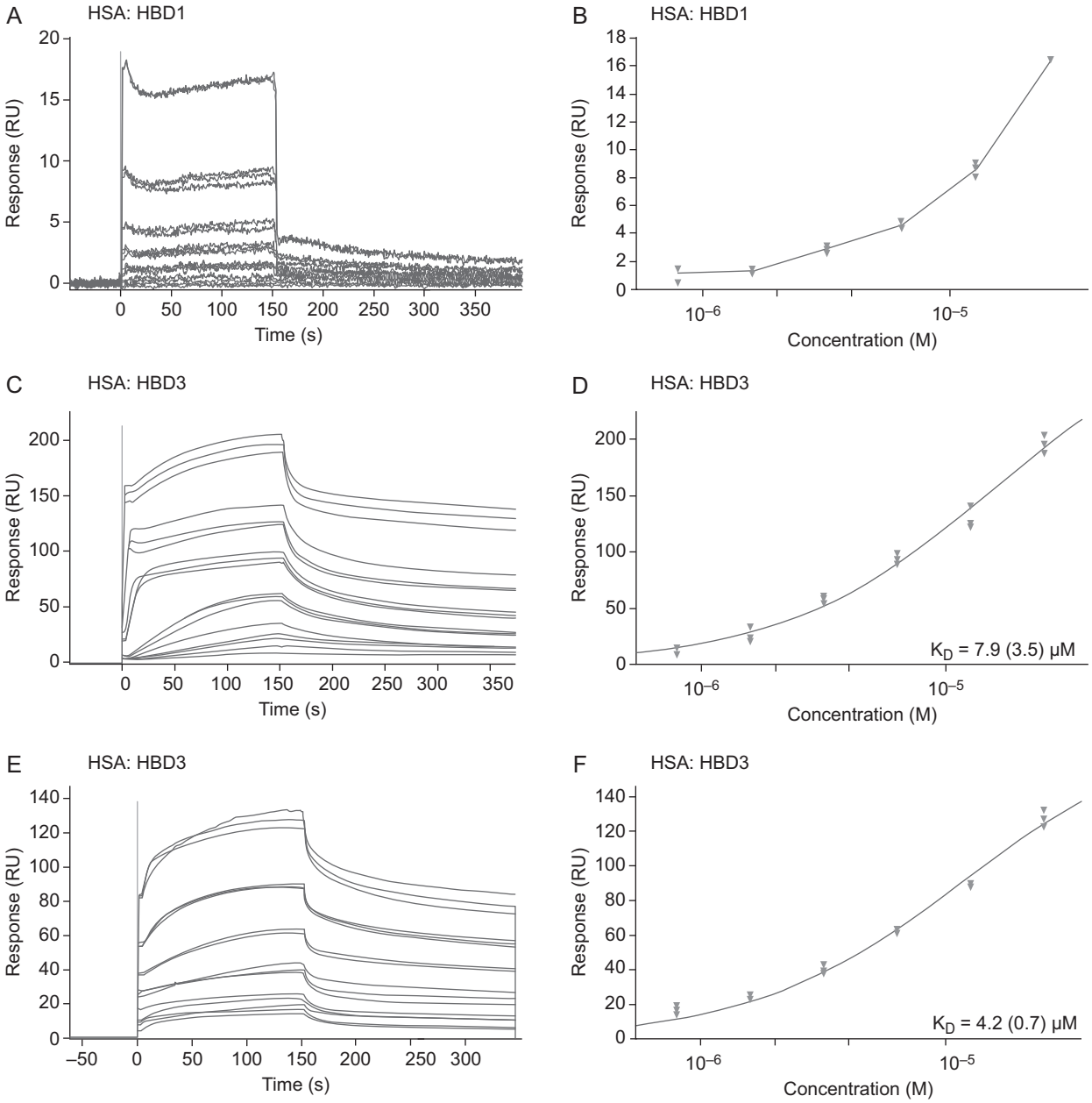

Fig. 2. SensíQ Pioneer Sensograms of HBD1 and HBD3 binding to immobilized human serum albumin (HSA) showing (A) HBD1 binding to HSA in 0.15 M NaCl and (B) the corresponding dose response; (C) HBD3 binding to HSA in 0.15 M NaCl and (D) the corresponding dose response; and (E) HBD3 binding to HSA in 0.3 M NaCl and (F) the corresponding dose response.

pH 5.0 for HSA). Activated surfaces were capped by injecting 1.0 M ethanolamine pH 7.5 for 3 min. FC2 was left unmodified to serve as a reference for non-specific binding to the surface chemistry.

Dilution series of HBD1 and HBD3 peptides was prepared in running buffer including concentrations: 25.4, 12.7, 6.4, 3.2, 1.6, 0.8 μM and a buffer blank. Each solution was injected over Pioneer's three FC for 2.5 min contact time and the dissociation of bound peptide was observed under buffer flow for 3 min.

Regeneration of the immobilized Bla g2 was performed by injecting 2.0 M NaCl for 1 min after each HBD injection. The HBD samples were tested in triplicate and the order of testing was randomized.

A third assay was performed as described above where the HBD3 peptide was prepared in a similar dilution series in running buffer with 0.3 M NaCl. The running buffer for the instrument was also modified to contain 0.3 M NaCl. The peptide samples were tested as described above.

2.3. Analysis

SPR response curves from the true reference channel (FC2) were subtracted from the Bla g2 (FC1) and HSA (FC3) channel curves. Buffer blank curves were then averaged and subtracted from the HBD curves. Dose–response plots were constructed by plotting the equilibrium response reached during sample injection vs. the peptide concentration. The "one site-total" model was fit to the data to determine maximum response (R_{max}) and K_D for each assay with a term for linear non-specific binding ($Y = R_{max} \times X/(K_D + X) + NS \times X$. NS is the slope of the linear non-specific binding component). The

HBD1 interactions demonstrated a very weak affinity and due to the low concentrations tested, the K_D could not be estimated.

3. Results

HBD1 demonstrated a very weak affinity for Bla g2, which was not quantified at the concentrations of peptide tested in this assay (Fig. 1A and B). HBD3 was observed to bind with a stronger affinity for Bla g2 (Fig. 1C–F). HBD3 was also observed to bind non-specifically to the reference channel and as a result an additional assay was performed where the NaCl concentration was increased to reduce this artifact. This non-specific binding was hypothesized to be due to the peptide's electropositive nature in neutral buffer conditions. The difference in binding response is primarily due to the reduction in nonspecific binding to the reference channel in the 0.3 M NaCl assay (Fig. 1C vs. E). We observed that when 2.0 M NaCl was injected over a channel with bound HBD3 (Bla g2 or reference channel) the majority of bound peptide was eluted which indicates that HBD3 binding to Bla g2 is mostly ionic in nature.

The binding curves were analyzed assuming the injections had reached steady state. Since most of the curves were at equilibrium this is considered an acceptable approximation although longer injection times will likely return a more accurate K_D value. The apparent K_D value for HBD3 binding Bla g2 was 5.9 ± 2.1 μM (0.3 M NaCl, Fig. 1F). The appearance of the HBD3 binding responses suggests that the peptide is binding Bla g2 in a heterogeneous manner. The inclusion of a non-specific binding term should better analyse the specific binding between HBD3 and Bla g2. For the ~780 RU of immobilized Bla g2 we would expect ~107 RU of peptide binding at maximum (for a 1:1 interaction) and the estimated R_{max} from the model fit was 87 RU. This supports the hypothesis that the non-specific term in the model accounted for linear non-specific binding and accurately characterized the specific interaction.

An additional control was included to test the binding of the HBD peptides to HSA (Fig. 2). HSA is a common control protein so it was used to demonstrate potential non-specific protein binding. Similar equilibrium K_D values of HBD binding to HSA, as with Bla g2, were observed. The apparent K_D for HBD3 binding HSA was 4.2 ± 0.7 μM (0.3 M NaCl, Fig. 2F).

4. Discussion

HBD1, HBD2, and HBD3 are all known to bind with different affinities to a variety of microbial and viral antigens [5,7,13,16–18,21]. Recently, we observed that HBD1, HBD3, and histatin 5 bind specifically to immobilized *P. gingivalis* hemagglutinin B (HagB) [2]. Binding occurred in very specific domains on a modeled HagB molecule. This led us to hypothesize that HBD1 and HBD3 would also bind similarly to Bla g2. In this short communication, we report that HBD1 and HBD3 bind to both immobilized Bla g2 and HSA. The exact mechanism for the binding of HBDs to Bla g2 is not readily known but is likely related to the unique properties of these defensins and the composition of Bla g2 allergen. It could be a non-specific binding based on electrostatic interactions between the defensin analyte and the immobilized adhesin ligand. Binding was influenced by the NaCl concentrations and occurred more readily in 0.15 M NaCl than 0.3 M NaCl conditions. HBD1 has a net ±3 charge and HBD3 has a net ±11 charge. Electrostatic interactions were thought to play a role on our results. It is also possible that there may be defensin-specific binding domains on this allergen that favors higher affinity binding of one defensin over another defensin. This remains to be determined.

HBDs are expressed throughout the oronasal cavity and respiratory system [4,10,11] and may serve to protect these tissues from allergen exposure. Although early in this concept, it is tempting to speculate that polymorphisms in defensins or differences in copy number of defensins in some individuals may decrease the ability of defensins to protect mucosal sites from allergen exposure and may increase the susceptibility of those individuals to inflammation. Copy number polymorphisms and expression level variations of β-defensins [22] may lead to different defensin profiles. Specifically for HBD3, the three-copy number variant was the most frequent genotype occurring in 65.9% of 44 subjects followed by the two-copy number variant in 30.5% of 44 subjects and the four-copy number variant in 13.6% of 44 subjects [3]. Furthermore, there are numerous reports that HBD expression and production are down regulated in individuals with allergies.

Whether the binding of defensins to cockroach allergen Bla g2 attenuates its ability to induce localized allergic and/or inflammatory responses is not yet known. There is a good chance this may occur. Recently, HBD3 was found to attenuate chemokine and proinflammatory responses in dendritic cells treated with recombinant HagB from *P. gingivalis* strain 381 [12,18].

5. Conclusions

The binding of HBD1 and HBD3 to immobilized Bla g2 was characterized with the SensíQ Pioneer SPR biosensor system. HBD1 was observed to bind weakly to Bla g2 and the affinity constant was undetermined due to low analyte concentrations being used. The binding of HBD3 to immobilized Bla g2 was tested at 0.15 M and 0.3 M NaCl concentrations. Increased salt content sufficiently reduced non-specific binding to the reference channel to improve the signal of HBD3 binding the protein channels. The apparent K_D of HBD3 binding Bla g2 was 5.9 ± 2.1 μM and for binding HSA was 4.2 ± 0.7 μM, respectively. Both HBD1 and HBD3 bound to immobilized HSA similar to Bla g2 in terms of absolute response and kinetic shape of binding.

Conflict of interest

Deborah E. Dietrich and Kim A. Brogden declare no competing financial interests in the findings of this study or with SensíQ Technologies Inc., 800 Research Parkway, Suite 100, Oklahoma City, OK 73104, USA. Aaron D. Martin works for SensíQ Technologies Inc.

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