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Native Mass Spectrometry, Ion mobility, and Collision-Induced Unfolding Categorize Malaria Antigen/Antibody Binding

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Abstract

Plasmodium vivax Duffy Binding Protein (PvDBP) is a promising vaccine candidate for *P. vivax* malaria. Recently, we reported the epitopes on PvDBP region II (PvDBP-II) for three inhibitory monoclonal antibodies (2D10, 2H2, and 2C6). In this communication, we describe the combination of native mass spectrometry and ion mobility (IM) with collision induced unfolding (CIU) to study the conformation and stabilities of three malarial antigen-antibody complexes. These complexes, when collisionally activated, undergo conformational changes that depend on the location of the epitope. CIU patterns for PvDBP-II in complex with antibody 2D10 and 2H2 are highly similar, indicating comparable binding topology and stability. A different CIU fingerprint is observed for PvDBP-II/2C6, indicating that 2C6 binds to PvDBP-II on an epitope different from 2D10 and 2H2. This work supports the use of CIU as a means of classifying antigen-antibody complexes by their epitope maps in a high throughput screening workflow.

Graphical Abstract

Introduction

Characterization of epitopes is important in the discovery and development of new therapeutics, vaccines, and diagnostics [1]. Owing to its sequencing capability, MS is one “read-out” for many functional epitope-mapping approaches [2, 3]. Moreover, MS-based footprinting (e.g., HDX-MS) have successfully bridged high and low-resolution structural epitope mapping methods by reliably localizing the binding site at the peptide level [4–8].

In parallel, native IM-MS is emerging for applications in the biophysical characterization of intact proteins [9–11]. Native MS introduces protein complexes up to the mega-Dalton range [12] into the gas phase in near-native states, while preserving non-covalent interactions. Ion mobility can be used to separate individual ions with similar m/z but different sizes and shapes. Drift times (DTs) provide information on conformational dynamics [13, 14], and folding/unfolding intermediates [15, 16]. Native MS and IM report on ligand-induced stability and conformational changes for protein-ligand and protein-protein interactions [17, 18].

CIU experiments can further characterize proteins by monitoring their unfolding. Following collisional activation, the drift times (DT) of activated ions are afford activation profiles as “fingerprint plots”. Such CIU experiments were first described for small monomeric protein ions [19], but they are now applied for stabilities and conformational changes even with ligand binding [20]. CIU can also report on the consequences of small-molecule attachments to large protein systems [21, 22], revealing cooperative binding mechanisms, overall stability of multi-protein systems [23], and antibody-isoform differentiation of disulfide bonding and glycosylation levels [24]. Software advances for CIU are allowing comparison between ions with subtle differences [25].

Although native MS, IM, and CIU have gained importance in biophysics, providing structural information for biomolecules, they have yet to see application in the field of antibody-antigen characterization. Herein, we differentiate antigen-antibody complexes with disparate binding topologies by using native IM-MS combined with CIU. We chose PvDBP, a leading vaccine candidate for preventing malaria caused by *Plasmodium vivax*. A conserved extracellular domain of this parasite cell-surface protein designated as region II (PvDBP-II) plays a key role in a critical step-wise interaction with Duffy antigen receptor for chemokines (DARC) on reticulocytes. This interaction is imperative for establishing successful invasion and subsequent host infection [26–28].

A panel of monoclonal antibodies against PvDBP was generated, and several inhibitory antibodies were described [29]. Epitopes for the inhibitory antibodies 2D10, 2H2, and 2C6 were structurally-defined by X-ray crystallography, small angle X-ray scattering (SAXS), site-directed mutagenesis, binding assays and MS-based HDX [29, 30]. All of these techniques show identical epitopes for two antibodies, 2D10 and 2H2, whereas antibody 2C6 binds to a different epitope (Figure 1) [30].

Experimental Section

PvDBP Region II and monoclonal antibodies were purified as described [29] and buffer exchanged into PBS. Stock antigen and antibodies solutions in PBS buffer were exchanged into 200 mM ammonium acetate solution (pH = 6.5–7). Before each experiment, the antigen and antibody were mixed and diluted to 5 μ M and 6 μ M, respectively. The sample solution was incubated at 25 °C for 30 min before MS analysis.

Samples were analyzed using a quadrupole ion-mobility time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Protein and complex ions were generated in a nESI source in the positive-ion mode. The source parameters were optimized to provide gentle ESI conditions. The traveling-wave IM separator was operated at a N₂ pressure of ~1.5 mbar; the IMS wave velocity was 650 m/s; and the IMS wave height 40 V. The ToF-MS was operated over a m/z range of 100–15,000. For a CIU experiment, the trap-collision voltage applied to the ions in the traveling-wave-based ion trap situated prior to the IM separator was increased from 10 to 200 V in 10 V increments, and ion-mobility mass spectra were acquired at each increment.

Mass spectra were processed with Masslynx V4.1 software (Waters). Arrival time distributions at each collision voltage were extracted in the Drift Scope (Waters) and plotted as a 2D contour plot using Origin 2015 (OriginLab, Northampton, MA).

Results and Discussion

We introduced the antigen-antibody complexes into the gas phase by native MS, presumably preserving the non-covalent interactions (see supporting information for PvDBP-II and 2D10.). The relatively few and low charge states (from +23 to +28) strongly suggest that compact, near-native structures are maintained in the gas phase. Mass spectra clearly indicate that the binding stoichiometry between the antigen and antibodies is 1:1. We then

selected the +26 ions for IM analysis because they are of high abundance and show low interference.

CIU fingerprints for complexes of PvDBP-II with antibodies 2D10, 2H2 and 2C6 were generated with collision voltage ranging from 20 to 200 V (Figure 1). Although charge-state selection can influence the CIU fingerprints, and high charges lead to some Coulombic unfolding prior to collisional activation [21, 31, 32], such effects do not compromise the comparison shown here because the same charge state was selected for all three complexes.

The unfolding pathways, illustrated by increasing drift times for the three complexes demonstrate a similar general trend over the entire voltage range. They all start from an initial compact state with short drift times. At higher accelerating voltages, the complexes undergo CIU and exhibit a gradual transition to a more elongated, unfolded state (increases in drift times). From that point on, the unfolded species dominate the CIU fingerprint, and only slight increases in drift times occur until the voltage reaches the maximum achievable.

A close examination reveals variations between complexes in the CIU fingerprints. At 100% relative intensity on each drift time spectra, the complexes with 2D10 and 2H2 exhibit identical compact state drift times (~7.1 ms) and unfolded state drift times (~8.5 ms). In contrast, the complex with 2C6 exhibits shorter drift times for both compact and unfolded states (~6.8 ms and 8.2 ms, respectively). This observation indicates that the orientationally averaged collision cross section (CCS) for PvDBP-II/2D10 and PvDBP-II/2H2 are comparable but significantly larger than that of DBP-II/2D6, indicating a different overall shape of the latter complex.

This observation is consistent with other evidence that PvDBP-II adopts an elongated structure with the epitopes for 2D10 and 2H2 located on one end of the molecule whereas the epitope for 2C6 is in the middle of the molecule [30] (Figure 1). By interacting with antibodies 2D10 and 2H2 at one end of the lengthy molecule, the complexes become more extended, whereas the interaction with 2C6 results in a more compact shape. Additional evidence is provided by small angle X-ray scattering (SAXS) that shows an elongated “boomerang-like” complex envelope for 2D10 and 2H2, but a more compact “T-shaped” envelope for 2C6 [30]. Although SAXS experiments were done with Fab fragments whereas CIU-IM was performed on full-length antibodies, the variation in complex shapes caused by different binding topology is consistent.

A clear stability shift was also observed. The complex of PvDBP-II/2C6 starts to unfold at collision energies corresponding to just under 60 V applied to the ions in the traveling-wave-based ion trap prior to IM, whereas the compact states of PvDBP-II/2D10 and PvDBP-II/2H2 persist over larger voltage ranges and do not unfold until the collision energies are 70 V, clearly requiring more activation voltage to undergo CIU. Although the complex with 2D10 shows a more gradual transition compared to that with 2H2, both can be considered highly comparable in contrast to the larger difference shown by the complex with 2C6. Thus, the CIU stability also supports the conclusion that 2C6 binds to a different epitope and brings less stabilization to the complex.

Encouragingly, HDX-MS reports that deuterium uptakes are increased significantly in many segments of DBP-II remote from the epitope upon binding to 2C6, indicating conformational changes that destabilize part of the molecule, whereas no such effect is detected for the antigen bound to either 2D10 or 2H2 [30]. Therefore, CIU and HDX results complement each other on the overall stabilities and different bindings of the complexes, and these insights are difficult to obtain via other epitope mapping methods.

In summary, we demonstrate for the first time that native IM-MS in combination with CIU can be used to distinguish complexes formed by one antigen (PvDBP-II) and various antibodies (2D10, 2H2 and 2C6) binding at different epitopes. Native MS allows complexes to be detected with sufficiently accurate mass to afford stoichiometric information. IM and CIU provide insights into the size, shape and stability of the complexes. CIU patterns for PvDBP-II bound to 2D10 and 2H2, and a different complex with 2C6 appear to classify the epitope for 2D10 and 2H2 vs. that of 2C6. This result is consistent with an extensive array of X-ray crystallography, SAXS, mutational mapping and HDX-MS. This outcome reinforces the concept that relevant solution protein-complex structures can be maintained in the gas phase.

CIU may become a high throughput screening for multi-pronged epitope-mapping. Facing a collection of antibody candidates for one antigen, we may be able to categorize antibody binding at different epitopes with high throughput and low sample consumption. Although this approach does not locate the epitope, it may in the future by top-down sequencing [33, 34].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Epitopes of 2D10 and 2H2 in red, 2C6 in green, mapped on PvDBP-II (PDB ID: 3RRC).

Figure 2. CIU fingerprints projected as contour plots for the +26 PvDBP-II in complex with antibodies 2D10, 2H2, & 2C6. Normalized intensities for the features are denoted by color-coding as per legend.