Functional Contacts between MPER and the Anti-HIV-1 Broadly Neutralizing Antibody 4E10 Extend into the Core of the Membrane

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Abstract

The exceptional breadth of broadly neutralizing antibodies (bNAbs) against the membrane-proximal external region (MPER) of the transmembrane protein gp41 makes this class of antibodies an ideal model to design HIV vaccines. From a practical point of view, however, the preparation of vaccines eliciting bNAbs is still a major roadblock that limits their clinical application. Fresh mechanistic insights are necessary to develop more effective strategies. In particular, the function of the unusually long complementarity-determining region three of the heavy chain (CDRH3) of 4E10, an anti-MPER bNAb, is an open question that fascinates researchers in the field. Residues comprising the apex region are dispensable for engagement of the epitope in solution; still, their single mutation profoundly impairs the neutralization capabilities of the antibody. Since this region is very hydrophobic, it has been proposed that the apex is essential for anchoring the antibody to the viral membrane where MPER resides. Herein, we have critically examined this idea using structural, biophysical, biochemical, and cell-based approaches. Our results demonstrate that the apex region is not just a "greasy" spot merely increasing the affinity of the antibody for the membrane. We demonstrate the three-dimensional engagement of the apex region of the CDRH3 with the conglomerate of gp41 epitope and membrane lipids as a means of effective binding and neutralization of the virus. This mechanism of recognition suggests a standard route of antibody ontogeny. Therefore, we need to focus our efforts on recreating a more realistic MPER/lipid immunogen in order to generate more effective anti-HIV-1 vaccines.

Introduction

The broadly neutralizing anti-HIV-1 antibody 4E10 targets the highly conserved membrane-proximal external region (MPER) of the membrane protein gp41, exhibiting one of the greatest neutralization breadths described so far [1–3]. There is genuine interest to understand the molecular basis by which the antibody 4E10 and other broadly neutralizing antibodies (bNAbs) recognize such a broad repertoire of HIV-1 subtypes because this knowledge could translate into novel and more efficient vaccination strategies. In particular, previous studies made clear that hydrophobic and aromatic residues belonging to the apex of the heavy-chain complementarity-determining region three of the heavy chain (CDRH3) of 4E10 are critical for viral neutralization [4–6], although their mechanism of action at the molecular level is still awaiting clarification. Specially, it is unclear how these residues contribute to the recognition of the epitope in the context of the viral membrane where MPER resides. These questions are more relevant in

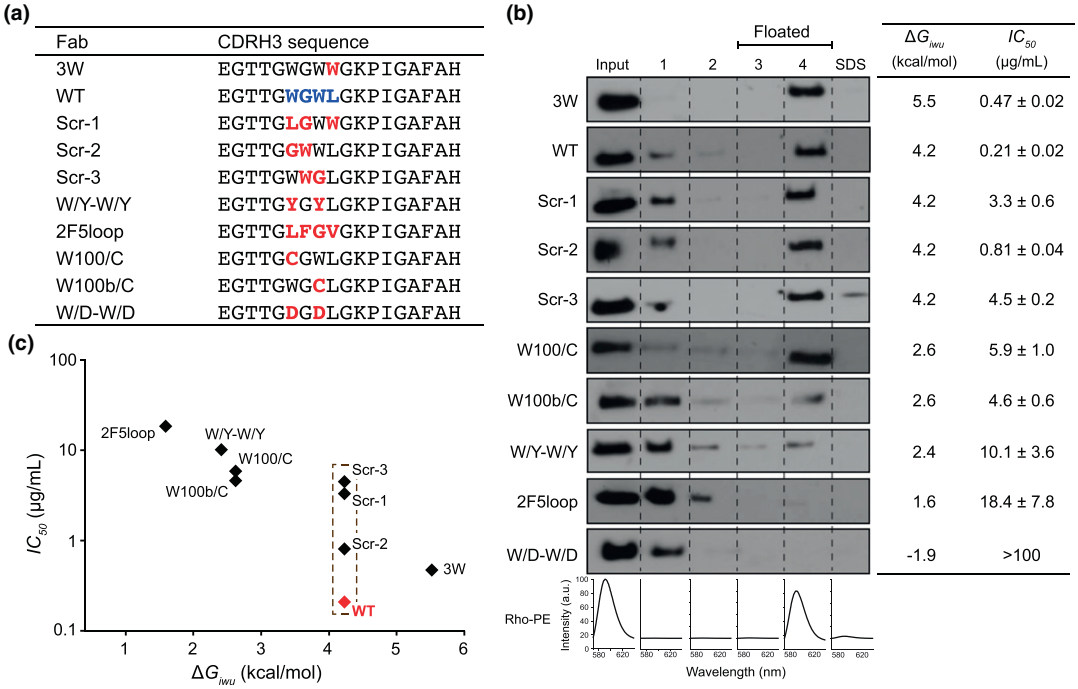

Fig. 1. Multivariate mutagenesis analysis of the apex loop of CDRH3. (a) List of sequences of CDRH3 examined. The mutated residues of the apex of the CDRH3 loop (defined as residues ^{100}WGWL^{100c}) are indicated in red. The two key tryptophan residues present in the apex are located at positions 100$_{HC}$ and 100b$_{HC}$. (b) For each variant of Fab, three properties were analyzed: binding of Fab to VL vesicles by the flotation method (left), hydrophobicity-at-interface [18] (center), and neutralization potency against HXB2 PsVs (right). The co-localization of vesicles and Fab was demonstrated by comparing the position of the protein as shown by western blot, with the fluorescence signal of the lipid 1,2-dioleoyl-*sn*-glycerol-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rho-PE) (bottom). Lanes 1–4 of the western blot correspond to samples of each of the four 250-μl fractions collected from the centrifugation tube, from bottom to top. The material adhered to the tubes was collected into a fifth fraction (labeled SDS). (c) The hydrophobicity at interface for each variant of Fab is plotted *versus* its viral neutralization potency. The mutant W/D-W/D does not display a measurable neutralization and therefore was not included in this plot. The hydrophobicity of the constructs enclosed in the dashed line box is constant, but their inhibitory potency varies significantly.

view that the hydrophobic residues of the apex region of the CDRH3 remain in a conformation exposed toward the solvent and not oriented toward the bound peptide as it could be expected from their critical role in the virus neutralization by the antibody [7–12]. In contrast, the functionally similar bNAb 10E8 positions the apex of the CDRH3 loop in close proximity to the MPER peptide [13,14], thus rationalizing its neutralization potency in terms of binding affinity to the epitope in membranes.

The unusual length and hydrophobicity of the CDRH3 are shared by both 4E10 and 10E8 antibodies, suggesting that the recognition mechanism of the MPER epitope in the environment of the viral membrane could be similar to each other. In the specific case of 4E10, the recognition of the epitope may be even preceded by the integration of the CDRH3 in the viral membrane, facilitating the reconfiguration of the CDRH3 apex with respect to the conformation observed in the crystal structure of complexes Fab–peptide [8,9,11]. In support of this hypothesis, a recent crystallographic study evidenced that the presence of lipids triggers a conformational change of the CDRH3 of 4E10, facilitating the engagement of the full-length epitope by the antibody [10].

Herein, we sought to determine two critical aspects, which have not been addressed before and are necessary for the functional engagement of 4E10 to the epitope in a membrane-mimicking environment. First, we investigated the molecular principles of the interaction of the CDRH3 of 4E10 with membranes, such as the partition of the antibody into proteoliposomes, the conformational change of CDRH3, and the formation of intermolecular contacts mediated by the apex of the CDRH3. Second, we evaluated the importance of these molecular contacts for the neutralization potency of the antibody. In particular, we monitored the physicochemical environment at two key positions of the apex of the CDRH3 (Trp100$_{HC}$ and Trp100b$_{HC}$) upon binding to membranes using a reporter probe. The