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AFM force measurements of the gp120–sCD4 and gp120 or CD4 antigen–antibody interactions

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ABSTRACT

Soluble CD4 (sCD4), anti-CD4 antibody, and anti-gp120 antibody have long been regarded as entry inhibitors in human immunodeficiency virus (HIV) therapy. However, the interactions between these HIV entry inhibitors and corresponding target molecules are still poorly understood. In this study, atomic force microscopy (AFM) was utilized to investigate the interaction forces among them. We found that the unbinding forces of sCD4–gp120 interaction, CD4 antigen–antibody interaction, and gp120 antigen–antibody interaction were 25.45 ± 20.46, 51.22 ± 34.64, and 89.87 ± 44.63 pN, respectively, which may provide important mechanical information for understanding the effects of viral entry inhibitors on HIV infection. Moreover, we found that the functionalization of an interaction pair on AFM tip or substrate significantly influenced the results, implying that we must perform AFM force measurement and analyze the data with more caution.

1. Introduction

HIV infection of target cells is a multi-stage process involving the entry, replication, and budding of virus. Presently, many strategies have been developed for HIV therapy depending on distinct stages of the process, among which blocking HIV entry is a well-known, important one. Many entry inhibitors or drugs have been developed in the treatment of HIV infection by blocking the interactions of HIV envelop glyprotein gp120 with cell-surface CD4 or coreceptors, or HIV envelop protein gp41-mediated membrane fusion [1–4]. However, the interactions of the inhibitors or drugs with CD4 or gp120 or gp41 remain poorly understood.

Soluble CD4 (sCD4) and CD4-mimetic compounds are well known to inhibit HIV entry in vitro or in vivo [5–8]. It has been reported that sCD4 selectively inhibited HIV replication and syncytium formation [9] or inactivated HIV by inducing the release of gp120 [10,11]. Recently, sCD4 and CD4 mimics were found to inhibit HIV infection by inducing a short-lived activated state of gp120 and spontaneously and irreversibly transforming gp120 into a non-functional conformation from the relatively long-lived activated intermediate induced by cell-associated CD4 [12]. Neutralizing antibodies against gp120 or cell-associated CD4 are also well-known HIV entry inhibitors and antibody-based vaccines [13–15]. These antibodies inactivate or neutralize or block the invading HIV virus by interacting with gp120 on viral surface or cell-surface CD4 on CD4+ lymphocytes.

Until now, unfortunately, no safe, effective vaccine against HIV-1/AIDS has been found [16,17]. Therefore, the development of safe, effective vaccines is a top priority in HIV/AIDS research field. Accordingly, to investigate the interactions between HIV vaccines or inhibitors and their corresponding target molecules is very important for understanding the antiviral mechanisms of vaccines or inhibitors.

Recently, atomic force microscopy (AFM) has been widely applied in biological and viral studies [18–20]. AFM also has been used to image HIV viral particles and HIV-infected lymphocytes [21,22]. Chang et al. investigated the HIV-1 gp120–receptor interactions in living cells [23]. More recently, the kinetics of gp41 (HIV fusion protein) interaction with lipid membranes was detected by AFM [24]. To date, however, there are no reported AFM studies on interaction forces between HIV inhibitors (e.g. sCD4, anti-CD4 or anti-gp120 antibody, etc.) and their target molecules. In this study, we recruited AFM force measurement to detect the sCD4–gp120 interaction and gp120 or CD4 antigen–antibody interaction.

2. Materials and methods

2.1. Reagents

Human soluble CD4 (Affinity BioReagents, Golden, CO), mouse monoclonal IgG1 against CD4 (Ab-2, clone 1F6; NeoMarkers, Inc., Fremont, CA), recombinant HIV-1_{MN} envelope glycoprotein gp120

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(Advanced Biotechnologies Inc., Columbia, MD), and mouse monoclonal IgG1 against HIV-1 gp120 (Clone ED8.D4; Abcam, Cambridge, MA) were purchased from different companies. 3-Aminopropyltriethoxysilane (APTES), Bovine Serum Albumin (BSA), glutaraldehyde, and others were from Sigma.

2.2. Functionalization of AFM tips and substrates

The method for tip and sample functionalization was modified from previous studies [25–27]. Briefly, all Silicon Nitride tips and freshly cleaved micas were incubated in 1% (v/v) 3-aminopropyltriethoxysilane (APTES; Sigma) in toluene for 2 h, and rinsed in toluene for 5 min. Subsequently, they were incubated with 0.2% (v/v) glutaraldehyde in distilled water for 30 min, and then rinsed with distilled water for 5 min. In above steps, all tips and micas were always functionalized simultaneously in the same solutions. When functionalized with different proteins, these tips and micas were modified separately in 50 and 10 μl protein solution for 1 h, respectively. sCD4 (1 mg/ml), 1 or 0.01 mg/ml HIV-1 gp120, 1 mg/ml anti-CD4 or anti-gp120 antibody, 1 mg/ml monkey serum or 1 mg/ml BSA, or various mixtures of these proteins in distilled water were used for modification. After protein modification, all tips or micas were treated with glycine to block free aldehyde groups. All of them were rinsed with distilled water and then incubated in pH 7.4 PBS buffer for use (generally within 12 h).

2.3. Force measurements by AFM

AFM data were collected using an Explorer AFM (Veeco, Santa Barbara, CA). The spring constants of the Si_3N_4 cantilevers were 0.01–0.03 N/m. All force measurements of antigen–antibody or ligand–receptor unbinding interaction were performed in 100 μl PBS buffer (pH 7.4) at room temperature.

During AFM measurements, we found that the experimental results may change dramatically with alternations of tips, substrates, sites, points, and even different times of measurement on the same point. To make the results more objective and accurate, we established the following guide lines in our AFM experiments: (a) only one force–distance cycle was done at each point; (b) around 20 force measurements were performed randomly in each 5 μm × 5 μm site; (c) more than 10 sites at intervals of <100 μm were measured following a detecting path (for each independent experiment, more than 10 sites were observed on a protein-immobilized substrate in a from-center-to-edge-and-then-back-to-center manner); (d) more than two functionalized AFM tips were used to detect proteins on a same substrate, and more than two substrates were measured for each pair of force interaction; (e) data shown in each histogram were obtained using more than three different AFM probes and independent samples.

2.4. Data processing and statistics

The data obtained for the unbinding force was used to generate a frequency distribution. This was then analyzed by fitting multi-Gaussian distributions. The validity of distinct unbinding distributions was tested and confirmed using one-way ANOVA analysis of statistical variance, and by Student *t*-test.

3. Results

3.1. AFM force measurements detected the specific interactions between sCD4 and gp120

Prior to the force measurements of ligand–receptor and antigen–antibody specific interactions, we detected the non-specific interactions between glutaraldehyde- or glycine- or sCD4-functionalized AFM tips and glutaraldehyde- or glycine- or BSA (Bovine Serum Albumin)-modified micas. We found that most of the control non-specific interactions have a force of 0 pN (Panel 1 of Fig. 1 and data not shown) except for the non-specific interactions of glutaraldehyde-functionalized tip or mica with other molecules. For instance, the mean value of the non-specific interaction between the glutaraldehyde-functionalized tip and the glutaraldehyde-modified mica was 432.54 ± 233.98 pN (n = 735).

Then, the sCD4-functionalized AFM tips were utilized to detect gp120 molecules (1 mg/ml in PBS) on micas. The histogram in Panel 2 of Fig. 1 showed a narrow distribution of sCD4–gp120 interaction forces with a generalized extreme value at 25.45 ± 20.46 pN and with a mean value at 42.25 ± 34.14 pN (n = 705). We found that most force–distance curves for sCD4–gp120 interaction have only one peak of ~30 pN which represent the unbinding force between sCD4 and gp120 (Panel 3a and 3b of Fig. 1). Occasionally, simultaneous unbinding of two or more sCD4–gp120 specific interactions (Panel 3c and 3e of Fig. 1, respectively) and sequent unbinding of two or more sCD4–gp120 specific interactions (Panel 3d and 3f of Fig. 1, respectively) were evident in an individual force–distance curves.

We also used the gp120-functionalized AFM tips to detect sCD4 molecules on micas. The data showed that the generalized extreme value and the mean value of sCD4–gp120 interaction force were 49.13 ± 32.41 and 69.23 ± 66.17 pN (n = 331), respectively, which were larger than those obtained by sCD4-functionalized tip and gp120-modified mica.

To further investigate the specificity of sCD4–gp120 interactions and the sensitivity of AFM force measurements, low-abundant (0.01 mg/ml) gp120 molecules mixed with 1 mg/ml monkey serum were measured by sCD4-functionalized tips (Fig. 2A). The data indicated that only 7.5% measurements displayed no interactions and that the generalized extreme and mean values of specific sCD4–gp120 interaction were 21.59 ± 20.19 and 39.11 ± 35.78 pN (n = 1243), respectively. 0.01 mg/ml denatured gp120 (heated at 100 °C for 3 min) mixed with 1 mg/ml monkey serum were also measured by sCD4-functionalized tips (Fig. 2B). As expected, the force measurements without specific interactions increased to 26.2%. However, the generalized extreme and mean values (25.56 ± 34.66 and 49.88 ± 49.78 pN, respectively; n = 1000) of detectable sCD4–gp120 interaction forces were similar to those in Fig. 2A and Panel 2 of Fig. 1. Additionally, ~83.0% measurements displayed no interactions for gp120-free BSA samples detected by sCD4-functionalized tips (Fig. 2C).

3.2. AFM force measurements detected the specific interactions between CD4 or gp120 and anti-CD4 or anti-gp120 antibody

Subsequently, we functionalized the AFM tips with CD4 molecules and the micas with anti-CD4 antibodies, and then performed AFM force measurements in liquid. The data showed that the generalized extreme and mean values of specific interaction between CD4-tip and anti-CD4 antibody-mica were 144.91 ± 84.18 and 220.85 ± 156.29 pN (n = 1135), respectively (Fig. 3A). At the same time, anti-CD4 antibody-modified tips were used to obtain the interaction forces again by detecting CD4 molecules modified on micas. The statistical analysis indicated that the generalized extreme and mean values of specific interaction between anti-CD4 antibody-tip and CD4-mica were 51.22 ± 34.64 and 79.72 ± 59.36 pN (n = 1114), respectively (Fig. 3B).

Similarly, we found that the generalized extreme and mean values of specific interaction between anti-gp120 antibody-tip and gp120-mica were 108.84 ± 83.26 and 185.44 ± 155.21 pN (n = 1724), respectively (Fig. 3C), whereas the generalized extreme and mean values of specific interaction between gp120-tip and