Enhancement of E6 Protein Binding on Binding-Orientation-Sensitive Mixed SAMs Molecules

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ABSTRACT

This study proposed the use of mixed SAMs of different chain lengths to bind the E6 protein molecules. Atomic Force Microscopy measurement and molecular dynamic simulation are performed to demonstrate the successful enhancement of the binding of E6 protein with mixed SAMs of proper mixing ratio of thiols (C9 and C5) and the determination of binding orientations.

Keywords: binding force, protein molecules, mixed SAMs, atomic force microcopy, Molecular Dynamic simulstions

1 INTRODUCTION

SAMs (Self Assembled Monolayers) are commonly employed binding molecules for protein micro array. However, SAMs-bound proteins (such as antibodies) have poor binding efficiency for the detecting proteins (such as antigens), owning to the large space blockage effect and the random orientations of the bound proteins. This study aims the study of orientation selectivity and binding enhancement for E6 protein to nano-structured mixed-SAMs surfaces of different chain lengths. The E6 protein is one of the major oncoproteins produced by the human papillomaviruses responsible for cervical cancers [1]. Mixtures of n-alkinethiol SAMs of different chain lengths: 1-Decanethiol (C9) and 1-Hexanethiol (C5) are the demonstrating examples in present work, which provides additional dimension for additional reaction area on limited area. The binding enhancement and orientation selectivity will implicate and help the viability of the detection of cervical cancer. Present study performs the molecular dynamics simulations on the binding energies of a single E6 protein molecule with mixed SAMs molecules and the binding force measurements by Atomic Force Microscope (AFM) for a cluster of E6 protein molecules on surfaces of same SAMs.

2 SIMULATION MODELS AND METHODOLOGIES

Molecular simulations were performed on a system consisting of E6 protein and SAMs molecules. The E6 protein structures were predicted by Robetta Automation Predication Server [2] using the ROSETTA fragment method [3]. The molecular dynamics simulation was performed using the MOLSIM package based on the force field of ENZYMIX parameters [4] in the united atom representation [5]. Each system was placed in a rectangular box of 9.98×8.64×15.00 nm^3 with periodic boundary condition and minimum image convention applied to x and y directions only. The SAM surfaces were constructed by a 20×20 array of the single chain with a sulfur-sulfur spacing of 0.499nm. The initial structure of the SAM chains has a zigzag configuration shown in Fig.1 . The potential function consists of bond, angle, dihedral and improper dihedral terms, as well as nonbonded van Der Waals and Coulombic interactions. The complete form of the potential function is given

$$U = \sum_{bonds} K_b (b - b_0)^2 + \sum_{angles} K_\theta (\theta - \theta_0)^2 + \sum_{dihedrals} K_\phi \left[1 + \cos(n\phi - \phi_0)\right] + \sum_{impropers} K_\chi (\chi - \chi_0)^2 + \sum_{vdW} \varepsilon_{ij} \left[\left(\frac{R_{ij}}{r_{ij}} \right)^{12} - 2 \left(\frac{R_{ij}}{r_{ij}} \right)^{6} \right] + \sum_{Coulombic} \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}$$

where K_b, K_θ, K_ϕ and K_χ are the bond, angle, dihedral, and improper dihedral force constants ; b_0, θ_0, ϕ_0 and χ_0 are the equilibrium values of bond length, bond angle, dihedral angle, and improper angle; ε_{ij} and R_{ij} are the Leonard-Jones energy and the distance parameters; and q_i is the partial atomic charge. In this work, binding energy is the summation of nonbonded, van der Waals and Coulombic interactions between E6 protein and SAMs surface.

The Beeman algorithm [6] was used for the integration of equation of motion with a time step of 1 fs. Initial velocities were assigned with the Maxwell-Bolzmann distribution at 300K and the simulation was performed at 300K.

3 SIMULATION RESULTS

A single E6 protein molecule structure is modeled by Robetta solver as shown in Figs. 2a and 2c. The binding orientations of E6 protein with respect to SAM surfaces are of interest and the binding performance is analyzed at opposite orientations of protein tips. Figures 2b and 2d show the amino acids of E6 protein adjacent to SAM molecules for E6 at orientations 1 and 2 (corresponding to

Figs.2a and 2c). Most of these amino acids are hydrophilic. The binding energy versus mixing ratio of C9 and C5 (Fig.3) indicates that maximum binding energy can be increased up to 2.5 folds or 1.5 folds for selective SAMs as mixing ratio of C5/(C9+C5)=0.5 or 0.25 for orientation 1 or 2, respectively. Thus the peak to average force can be used as indicator of the protein orientation in the experiments.

4 EXPERIMENTAL DEMONSTRATIONS

Interactions between macromolecules and macromolecular assemblies can be obtained using indirect (such as thermodynamic and kinetic approaches) and direct (such as the deflection of a spring supporting one for two separated particles) methods. Force measurement techniques of an AFM using a protein-modified probe tip has been a popular structural and functional tool for analyzing the actual interaction forces between protein and material surfaces with the force spectrum from several fN to nN at the molecular level. Kidoaki et al [7] has reviewed the applications in this issue, e.g. the measurement of single molecular forces between specific protein pairs such as avidin/biotin pairs or antigen/antibody pairs, of the unfolding force of single protein molecules, and of nonspecific adhesion forces between proteins and surfaces of material such as polystyrene or glass. Agnihotri et al [8] have extended the application to dual component protein films adsorbed on mica. The force-distance curve can reflect the interaction of the sharp tip of AFM and a surface at a distance of atomic dimension. Analysis showed that the force between them is mainly due to van der Waals force as the AFM tip in water. Present study aims to measure the binding force between E6-protein modified tip and mixed SAMs surface and identify the orientation of E6-protein molecule.

4.1 Experimental Procedures

Experimental process includes the preparation of AFM tip coated with E6 protein molecules, preparation of mixed SAM surfaces, and performing interaction force measurements for E6-protein molecules and mixed SAM molecules.

4.1.1 Preparation of AFM tip Coated with E6 Protein

The preparation of AFM tip includes three important layers as Au(111) with thickness of 20nm, COOH-terminal thiol, and E6 protein on silicon based AFM tip. Coating processes are briefly described as follows**:**

Au(111) Preparation. Before evaporating Au(111) on large surface by e-beam evaporation, the AFM tip were firstly cleaned by alcohol solution for removing any form of organic contaminants. Gold film of 20 nm was evaporated on a promoting adhesion layer of 2nm Titanium (Ti) in a high-vacuum evaporator under 2×10^{-6} Torr with deposition rate of 0.5Å/s and subsequently annealed at 200℃ for 120 min to enhance Au in (111) form.

Immobilization of proteins. Second layer of COOH-terminal SAMs was formed on gold-coated AFM tip by immersing into 1mM thiol solution diluted with absolute ethanol (99.5%) for 24 hours at room temperature (~20℃), and then rinsed with 95% ethanol and dried out under N_2 stream. The gold-coated slide of COOH-thiol coverage was activated by 20mM EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, 98%+(EDC)) mixed with 5mM Sulfo-NHS (N-hydroxysulfosuccinimide sodium salt) solution in sodium phosphate 0.1M. Finally, E6 protein was immobilized with different concentrations (0.0625, 0.125, 0.25, 0.50, 0.75 mg/ml) for the experiments for 2 hours and E6 protein is prepared from expression system in E. coli SG13009 cells containing the plasmid pQE30 (Qiagene) harboring the HPV-16 in our lab. E6 was coated on the functional tip of radius less than 17nm.

4.1.2 Preparation of mixed SAMs sufaces

Mixed SAMs of C9 and C5 are formed by immersing gold-coated substrate in diluted mixed thiol (1 mM) of different ratio combinations of C9 and C5 for 24 hours at at room temperature (~20 ℃), i.e. 1-Hexanethiol ($HS(CH_2)_5CH_3$, 95%) and 1-Decanethiol ($HS(CH_2)_9CH_3$, 96%) purchased from Aldrich. And then the surfaces are rinsed with absolute ethanol and dried out under N_2 stream. The gold-coated substrate is prepared by the same method in section 4.1.1.

4.1.3 Interaction force measurement

The force-distance curve measurements are made with atomic force microscopy system (Nanoscope multimode III, Digital Instruments, USA) with tip/cantilevers manufactured by Mikromasch (Russia). The cantilevers are made of silicon and are conic shaped with radius of 7 μm on the base and 15μm high. Radius of tip curvature is less than 10μm and the full tip cone angle is less than 30°. The schematic operation of the probe is illustrated in Fig.4 [9] and the trace pull-out force as indicated represents the binding force between the E6 protein clusters and SAM molecules.

In order to ensure the force measurement, two reference sets of measurements have been conducted before systematic measurements: for gold-coated AFM tip and COOH-terminated SAM on top of gold-coated AFM tip.

Figure 4 plots the AFM force-distance curve that illustrates schematic operation of the probe and the trace pull-in force as indicated represents the van der Waals force (binding force) between the protein clusters and SAM molecules. In the present study, each data point is obtained by force-distance measurements on 5 spots and 10 repeats at every spot. Two independent runs are performed on SAMs surfaces of five mixing ratios.

4.2 Experimental Results

Two separate runs of force-distance experiments have been conducted for E6 protein concentrations of 0.0625, 0.125, 0.25, 0.50, and 0.75 mg/ml. Higher concentration of E6 protein may imply the larger number of E6 molecules attached to COOH-terminated SAM covering the AFM tip and thus larger binding force. Independent experiment is conducted on a glass slide as a reference by

conjugating E6, primary rabbit-anti E6 and secondary rabbit-anti Cy-3 for the fluorescent UV intensity measurement. Figure 5 shows that the fluorescent intensity approaches saturated as the concentration higher than 0.25 mg/ml. Therefore, it may implicate that E6 protein may cover all surfaces of AFM tip. Figure 6 show the measured binding forces for mixed SAMs molecules with different C9 and C5 of combination ratios coated on the Au surface. These curves confirm that additional forces up to 3.0 folds as the mixture ratios are at 0.25, 0.50, and 0.75 comparing to pure C9 or C5, and the maximum increase is observed at mixture ratio of C5/C9=1.0. With the magnitude increase of the binding force and the mixing ratio of SAMs with different chain lengths, binding orientations can be obtained, e.g. the analysis of present data implies the ratio of binding E6 orientation at 2 to at 1 is 2.

Summarizing all data points, peak and averaged interaction forces are linearly increased with the increase of concentration of E6 protein until concentration of 0.25 mg/ml (Fig.7), which is consistent with the reference fluorescent experiment in Fig.5 and implicates that the excess E6 molecules do not increase the binding force of E6 protein and SAM molecules. The binding force is increased for SAMs surfaces with mixing ratio of one-to-one and is in between pure C9 and pure C5 for other mixing ratios. (Fig.8)

5 CONCLUDING REMARKS

Both experiments and simulations indicate that the proper design of mixed SAMs structure can enhance the binding efficiency with orientation selectivity for E6 protein and mixed SAMs surface and the mixing ratio of C9 and C5 as 1:1 is proposed for in this study.

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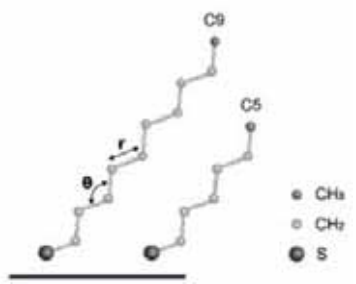


Fig.1 Schematic Diagrams of C9 and C5

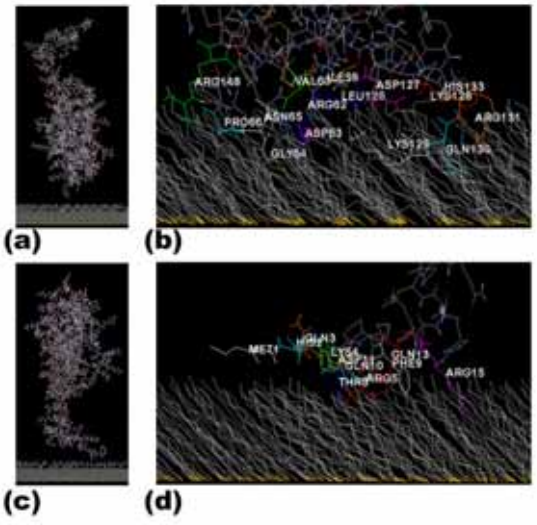


Fig.2 Snapshots of simulation Models for E6 Protein at Selected Orientations on SAM Surfaces (a) at t=0 and (b) at t=200ps for orientation 1 (c) at t=0 and(d) at t=200 ps for orientation 2

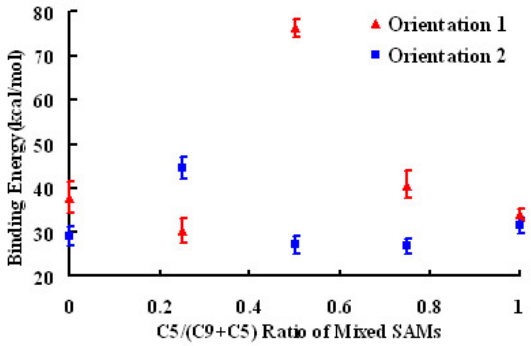


Fig. 3 Binding Energy vs Mixed Ratio of SAMs for orientation 1 and orientation 2

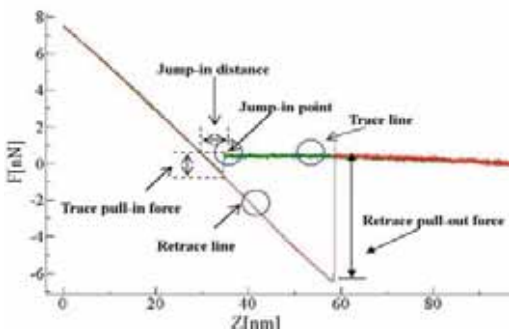


Figure 4 Illustration of AFM probe operation and the force distance curve

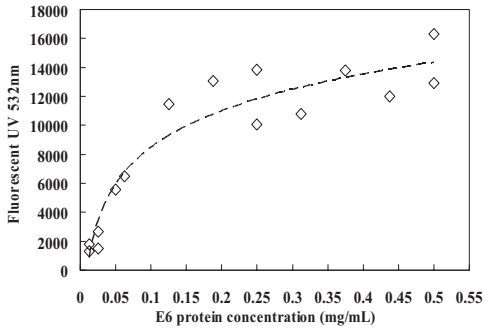
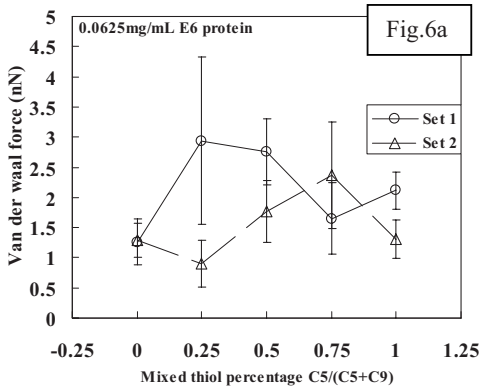
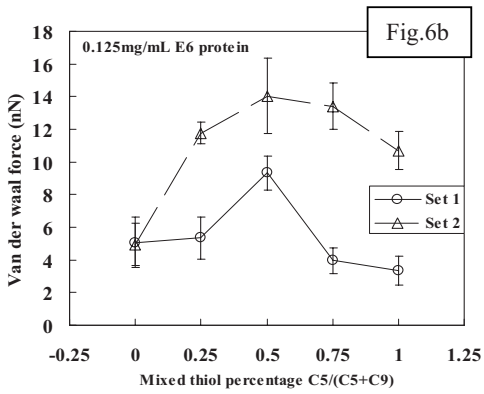
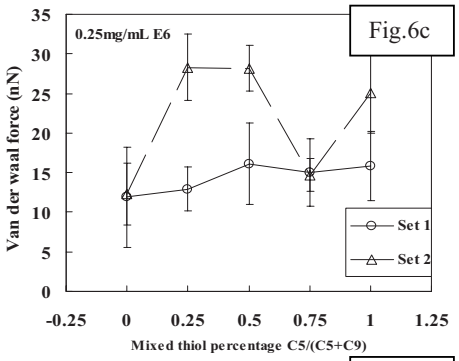


Figure 5 Fluorescent UV measurement for E6-binding experiments







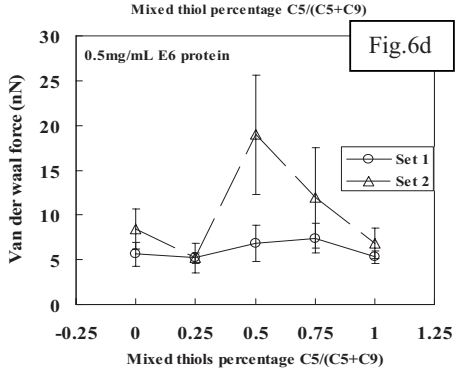


Fig. 6 AFM force-distance curve for various E6-protein concentrations (a) 0.0625 (b) 0.125 (c) 0.25 (d) 0.5 mg/ml

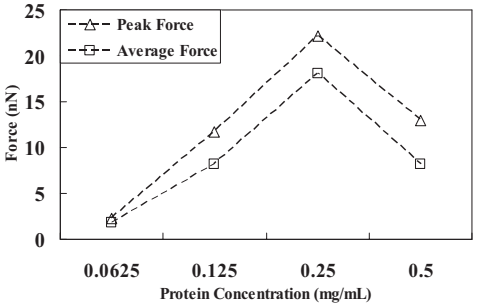


Fig.7 Averaged and peak binding forces for E6-protein molecules on all mixed SAMs surfaces

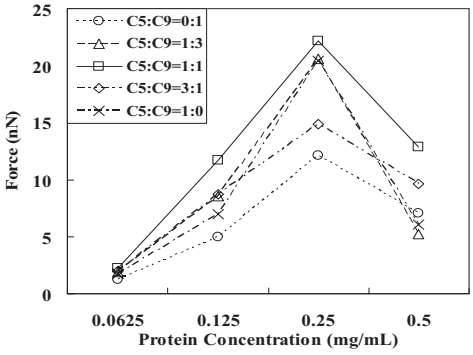


Fig.8 Averaged binding forces for E6-protein molecules of different concentrations on mixed SAMs surfaces of different mixture ratios of C9/C5