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Atomistic simulation of compression of single human serum albumin molecule by AFM tip

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Abstract. The compression process of a single human serum albumin (HSA) molecule by an atomic force microscope (AFM) tip in ultra high vacuum (UHV) condition is studied by the molecular dynamics (MD) simulations with the all-atom empirical force field model. The temperature is assumed to be 0 and 300 K, and the force curves are calculated assuming that both the tip and surface are rigid. At T = 300 K the thermal motion is found to promote the relaxation inside the protein and reduce the normal force observed. Furthermore, the saw-tooth peaks observed in the force curves are found to originate from abrupt structural changes in the sidechains at the atomic level.

1. Introduction
In the research field of single molecule force spectroscopy of biomolecules, the atomic force microscope (AFM) tip has been used to investigate their mechanical properties such as force extension curves [1, 2, 3, 4, 5, 6, 7, 8, 9]. They have shown highly nonlinear features and saw-tooth patterns in many cases, which reveal the heterogeneous interactions inside the biomolecules. On the other hand, the measurements of force compression curves, have been performed only for a few biomolecules [10, 11, 12].

Correspondingly, theoretical simulations for the force extension curves have been performed for several biomolecules [13, 14, 15, 16] by adopting the scheme called the steered molecular dynamics (SMD) [17, 18]. They have contributed to understanding of the unfolding process of biomolecules at the atomic level. However, atomistic simulations of compression of biomolecules are very limited [19, 20], and the phenomena occurring under the AFM tip have not been well understood.

Our purpose in this work is to develop a simulation method of the compression process of biomolecules under an AFM tip. In this article, we choose a case study of a human serum albumin (HSA) molecule adsorbed on the mica surface in ultra high vacuum (UHV) condition, and examine the developed simulation method. The tip is modeled by a graphite sheet with a large radius of curvature. The whole calculations are performed using the program called NAMD 2.5 [21] with CHARMM 22 and CLAY force field parameters [22]. We found that the temperature effect shows up as a promoted relaxation inside the protein which can be measured as a reduction of normal force in the force compression curve. Furthermore, the saw-tooth peaks observed in this curve are found to originate from abrupt structural changes in the sidechains at the atomic level.
The rest of the paper is organized as follows. In §2 the computational model and simulation method will be described. In §3 the results of our calculations will be presented and the mechanism of the jump in the force curves will be analyzed at the atomic level. In §4 the paper will be summarized.

2. Model and Method

2.1. Adsorbed structure
The initial structure of the HSA molecule is prepared from the Protein Databank (PDB) code 1AO6. This molecule is composed of 578 amino acid residues whose dimensions are about 62 × 79 × 56 Å³. The substrate surface is modeled as a thin cleaved muscovite (mica) surface composed of Si, Al, O, and K atoms. Although the muscovite takes a multi-layered structure, i.e., tetrahedron, octahedron, and tetrahedron layers, only the top tetrahedron layer is considered in this work. Its lateral dimensions are 142.9 × 145.1 Å². The AFM tip is modeled by a graphite sheet bent so that it takes a radius of curvature of 150 Å, which results in the lateral dimensions of 141.4 × 141.9 Å² and the height of 18.0 Å. The number of atoms in each component is 9 155 (protein), 5 824 (surface), and 6 604 (tip), respectively, which amounts to 21 583 atoms in total. The total charge of the system is -15 e. The interatomic interaction is described by the all-atom force field called CHARMM 22 and CLAY with slight modification, i.e., the parameter for the K ion in the latter is substituted with that in the former. The van der Waals interactions are cut off beyond 12 Å. The whole calculations in the followings are performed using the program NAMD 2.5 [21] with no boundary conditions imposed. The relaxation of the surface and tip is not considered in this work.

Figure 1 illustrates the system in consideration drawn by the program called VMD [23]. The O* atoms with red color at the bottom of the surface are equivalent to O atoms except that their atomic charge is -1.2875 e. The HSA molecule is drawn by the NewCartoon representation with red, blue, and green colors each of which corresponds to the three domains of the HSA, i.e., domain I (Ser5 - Gln196), II (Arg197 - Ile388), and III (Lys389 - Ala582). The N- and C-terminal residues (Ser5 and Ala582) are drawn by the van der Waals representation with yellow and cyan colors, respectively. This adsorbed structure is obtained by the molecular
dynamics (MD) simulation at $T = 400$ K for 2.0 ns with the time step of 2 fs. Hereafter, the axes with red, green, and blue colors are defined to be x, y, and z axis, respectively.

Initially, the tip height $h$ is set to 70 Å measured from the substrate surface (see Fig. 1(a)). The force compression curves are calculated at $T = 0$ and 300 K with the method proposed by our group previously [19]. Namely, at $T = 0$ K, the molecule confined between the substrate and tip is optimized for 50,000 steps with the conjugate gradient method. Then, the normal force of the tip is calculated by summing the atomic forces over the whole tip atoms. At the next step all the tip atoms are moved downward by 0.25 Å, and a similar calculation is performed. Such procedures are repeated until the tip height reaches $h = 30$ Å. At $T = 300$ K, the molecular dynamics simulations are performed over 200,000 steps (0.4 ns) during which the atomic forces are monitored. Here, the bond length constraint is applied to hydrogen atoms and the temperature is controlled by a LANGEVIN damping parameter of $\gamma = 5$ ps$^{-1}$. The mean normal force is calculated by summing these force components over the whole tip atoms at each MD step, and then by averaging over these 200,000 MD steps. Next, the tip height is lowered by 0.5 Å and similar calculations are performed repeatedly until the tip height reaches $h = 30$ Å.

3. Results

3.1. General trend in compression

The solid and dotted curves in Fig. 2 show the normal force at $T = 0$ and 300 K, respectively. At $T = 0$ K, first, an attractive normal force sets in at $h \sim 66$ Å and its maximum appears at $h = 61$ Å indicating the attractive van der Waals interaction between the tip apex and the highest turn structure in the domain III, Ala553 - Glu565. Although the normal force turns to be repulsive at $h \sim 58$ Å, its value does not increase until $h \sim 54$ Å. This can be explained by the cancellation between two attractive and repulsive components, i.e., the domain III starts to be pushed down resulting in the repulsive force, while the highest turn structure in the domain I, Thr166 - Asp183, starts to approach the tip resulting in the attractive force. Then the normal force increases almost linearly until $h = 47.5$ Å followed by an oscillatory behavior with a succession of saw-tooth peaks in the region of $40 < h < 47.5$ Å. After this, the slope of the force curves gets steeper, once falls down to 6.46 nN at $h = 35.75$ Å, and finally reaches 15.5 nN at $h = 30$ Å.

A similar trend is found in the force curve at $T = 300$ K. It shows a maximum in the attractive force region at $h = 62.5$ Å, and then turns to be repulsive, but it takes an attractive value again at $h = 51$ Å. Then it starts to increase up to 2 nN, but drops to 0.69 nN at $h = 44$ Å. After this, it suddenly increases with a steeper slope, but becomes almost flat at $30 < h < 35$ Å, and finally takes 8.35 nN at $h = 30$ Å.
Figure 5. (a) Closeup of Tyr401, Asp549, and Ala552 relevant for saw-tooth peak a in Fig. 2. (b) (solid) Distance between H1 and the carboxyl oxygen of Asp549. (dotted) Distance between H1 and the backbone carbonyl oxygen of Ala552. The atom H1 is the hydroxyl hydrogen of Tyr401.

The reason of the reduced normal force at T = 300 K is the thermal motion of the residues which promotes relaxation of the HSA confined between the tip and surface. This can be understood by comparing the snapshots at both temperatures. Figures 3 and 4 show the snapshots at T = 0 and 300 K, respectively, at the tip height $h = (a)$ 45 and (b) 30 Å. At both temperatures, as the tip height is decreased, the highest domain III (green) moves its head with large amplitude so that it falls down toward the mica surface, and the upper half of the domain I (red) changes their height significantly. However, a few striking difference is observed between the two temperatures. At T = 300 K, the $\alpha$-helix in the left edge of the domain III decreases their height at $h = 30$ Å. In addition, the $\alpha$-helix enclosed in the dotted circles (domain II) decreases their height so that its axis is inclined to be almost parallel to the surface.

3.2. Origin of Discontinuity

As denoted in the previous subsection, the force curve at T = 0 K shows saw-tooth peaks at several tip heights. Although even at T = 300K a similar feature is observed, it is difficult to distinguish the purely mechanical effects from the thermal fluctuation at this stage. Thus, in this subsection, we will discuss the origin of the saw-tooth peaks at T = 0 K. As an illustrated example, we choose the peak position a in Fig. 2. From the top ($h = 44.5$ Å) to the bottom ($h = 44.25$ Å) of this saw-tooth peak, it turns out that sudden structural changes occur independently at the two positions, i.e., the turn structure in the domain I, Thr166 - Leu179, and the turn structure in the domain III, Cys392 - Lys402.

In the former position, roughly speaking, the backbone chain seems to displace toward the $[010] \sim [110]$ direction. At the atomic level, the maximum displacement 3.54 Å is observed at a hydrogen atom bonded to the $C_\gamma$ atom of Thr166. This residue and its adjacent residue Leu178 lower their heights while Phe165 sandwiched between the above two residues slightly raises its height. The steric repulsion formed on Phe165 is considered to be released by rotating its phenyl ring by 23.2 and 18.4 degrees around its $C_\alpha-C_\beta$ and $C_\beta-C_\gamma$ bonds, respectively.

In the latter position, the turn structure seems to displace toward $[100]$ direction. The maximum displacement 5.02 Å is observed at the hydroxyl hydrogen atom (H1) of Tyr401. This residue rotates its phenyl ring by 72.3 and 9.4 degrees around its $C_\alpha-C_\beta$ and $C_\beta-C_\gamma$ bonds, respectively. Accompanied with this, the surrounding bonding environment changes dramatically. Figure 5(a) shows a closeup of the area enclosed by the lines with orange color.
in Fig. 1(b). The solid curve in Fig. 5(b) represents the distance between the atom H1 and the carboxyl oxygen atom of Asp549, and the dotted curve represents the distance between H1 and the backbone carbonyl oxygen of Ala552. This indicates that the hydrogen bond between Tyr401 and Asp549 is abruptly broken at this tip height, and H1 comes close to the vicinity of the C-terminal of Ala552.

A similar analysis can be performed at the other saw-tooth peaks. We find that abrupt structural changes in the sidechains at the atomic level cause the collective sliding motion of the backbone, which results in the saw-tooth peaks.

4. Summary
The compression process of a single HSA molecule by an AFM tip is studied in the UHV condition by the molecular dynamics (MD) simulations based on the all-atom empirical force field model. The temperature is assumed to be 0 and 300 K, and the force curves are calculated assuming that both the tip and surface are rigid. At T = 300 K the thermal motion is found to promote the relaxation inside the protein and reduce the normal force observed. Furthermore, the saw-tooth peaks observed in the force curves are found to originate from abrupt structural changes in the sidechains at the atomic level. We expect that the saw-tooth peaks will be detected through an experimental measurement of force compression curves of a single protein molecule.

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