Adsorption of an Ionic Complementary Peptide on the Hydrophobic Graphite Surface

Yuebiao Sheng,[†,‡] Wei Wang,[‡] and P. Chen*,[†]

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1, and National Laboratory of Solid State Microstructure, Institute of Biophysics, and Department of Physics, Nanjing University, 210093, China

Protein adsorption plays a crucial role in bioactive implant devices and drug delivery materials design. Ionic complementary peptides are novel nanobiomaterials with many biomedical applications. In this work, molecular dynamics simulations of the ionic complementary peptide EAK16-II on the hydrophobic HOPG surface were performed. The adsorption and initial assembly process of EAK16-II on the surface were revealed. Hydrophobic alanine residues are found to be energetically favorable when in contact with the HOPG surface. It is the hydrophobic interaction that drives the adsorption of the first peptide molecule. Both hydrophobic and electrostatic interactions contribute to the adsorption of the second peptide molecule. The effect of hydrogen bonding is found to be less significant in the adsorption. The adsorption of the peptide on the HOPG surface is stable. These results provide a basis for understanding the fundamentals of peptide adsorption on the surface, which is critical for peptide applications in biotechnology and nanotechnology.

Introduction

The interaction of proteins with solid surfaces is currently an active area of study, which is of great interest to physicists, biologists, and engineers. It plays an important role in many fields, such as interfacial engineering, tissue engineering, materials science, nanotechnology, and biomedicine.[1−9] For example, in drug delivery systems, the materials have to be compatible with the physiological systems and can interact/function on specific targets. A good understanding of the protein−surface interaction will help us design more effective delivery materials for cellular targeting and membrane penetration.

Over the past years, much effort has been made in protein adsorption, and a few models to understand the detailed protein−surface interactions have been developed.[7,10] The self-assembly and adsorption behavior of an amphiphilic ionic complementary peptide, EAK16-II, has been studied systemically in our group.[11−15] Although the experimental findings are very promising, it is still necessary for us to verify these findings theoretically and to investigate the mechanistic details of EAK16-II−surface interactions. This can assist us in modifying the peptide to make it more suitable for bioengineering applications. Molecular dynamics (MD) simulations provide one of the most efficient ways to investigate molecular behaviors and interactions in complex systems, such as in a system involving protein/peptide adsorption.

Many theoretical studies on protein adsorption have been reported over the past decades.[7,16−18] However, because of the complexity of protein−surface systems, most simulations were performed based on colloidal-scale models, which represent the protein molecule as a colloidal particle,[10,16−18] neglecting the details of the molecule. In recent years, atomic representations of proteins have been considered by researchers for protein adsorption simulations. However, as a compromise between model accuracy and computational cost, protein molecules are
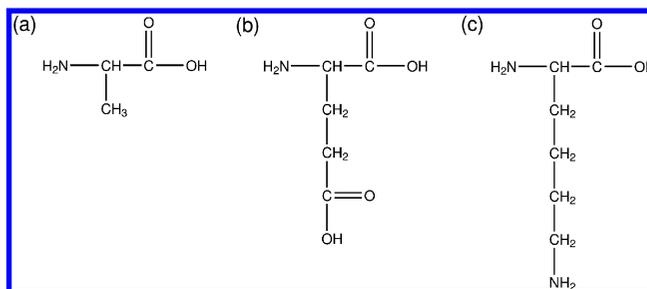

Figure 1. Chemical structures of alanine (a), glutamic acid (b), and lysine (c), respectively.

often treated as being rigid,[19−21] and the flexibility of proteins has been ignored.

In this study, we demonstrate the adsorption and initial assembly events of an ionic complementary peptide, EAK16-II, on the hydrophobic highly ordered pyrolytic graphite (HOPG) surface. An accurate all-atomic MD simulation of EAK16-II adsorption on HOPG was performed using the CHARMM22 empirical force field.[22] As EAK16-II can self-assemble, two molecules were involved in the simulation system. Obviously, this macromolecular system is complex with many intra- and intermolecular interactions. Roles of different interactions were investigated during the adsorption, and the interchain hydrogen bond forming and deforming were also analyzed. The aim of the studies is to obtain a more complete understanding of EAK16-II adsorption on the HOPG surface at the atomic level and to provide detailed interaction information for the peptide design.

Materials and Methods

Materials. The ionic complementary peptide EAK16-II ($C_{70}H_{121}N_{21}O_{25}$) has the sequence AEAEAKAKAEAEAKAK, where A corresponds to alanine, E glutamic acid, and K lysine. Figure 1 provides the chemical structures of alanine, glutamic acid, and lysine, respectively. At neutral pH, A is neutral, while E and K are negatively and positively charged, respectively.

* To whom correspondence should be addressed. E-mail: p4chen@uwaterloo.ca.
† University of Waterloo.
‡ Nanjing University.

Adsorption of EAK16-II on the HOPG Surface

J. Phys. Chem. C, Vol. 114, No. 1, 2010 **455**

Thus, the peptide chain has a charge distribution of $- - + +$ $- - + +$. To keep consistent with the experiment, the N-terminus and C-terminus of EAK16-II were protected by acetyl and amino groups, respectively.

The highly ordered pyrolytic graphite (HOPG) has a hexagonal crystal structure, which can serve as a model hydrophobic surface. A two-layered HOPG surface (0001) was constructed for this study using the program CHARMM.[22] Starting from a coordinate file of the primitive cell, the surface was initially generated by creating the appropriate images of the central unit cell using the IMAGE facility of CHARMM, with the surface on the *xy* plane. That is, from the primitive cell, a surface was created by replicating it 45×45 times according to the above image generating rule, and then the surface was trimmed to make the surface plane have the dimensions of 70.0×70.0 Å^2.

MD Simulations. Molecular dynamics simulations were performed using the program CHARMM with the all-hydrogen parameter set PARAM22.[22] Before the adsorption process was simulated, two short MD simulations were performed on each peptide molecule in vacuum (300 K, 100 ps, 1 fs time step), respectively, to generate two sets of initial random EAK16-II configurations. The two randomly structured peptide molecules were then placed 18.0 Å above the top layer of the HOPG surface in a head-to-tail mode, with the peptide chains parallel to the surface plane. The initial center-of-mass distance between the two peptide chains was 20.0 Å. TIP3P, transferable intermolecular potential 3 points, water molecules were then added surrounding the two peptide molecules to create a $70.0 \times 70.0 \times 40.0$ Å^3 solvent environment. Water molecules falling within a 2.8 Å radius from the center of non-hydrogen atoms were deleted to establish the simulation system. The periodic boundary condition and minimum image convention were applied in the *x* and *y* directions. After these preparations, the final system contains around 20 492 atoms, including 5688 water molecules; see Figure 2. Note that the number of atoms varies a little depending on the initial setup of the system. The snapshots were all constructed by the software VMD.[23]

Once the system was created, six independent runs of simulations were then carried out. For each run, the system was first energy-minimized using the steepest descent method for 50 steps without any restraint. Then the backbones of two peptide molecules were harmonically restrained with a force constant of 20.0 kcal/mol/Å^2, and the system was energy-minimized again using the adopted basis Newton−Raphson method for 500 steps, which was then repeated twice with a restraint force constant of 10.0 and 5.0 kcal/mol/Å^2, respectively. The entire system was further minimized for another 1000 steps without any restraint. After that, the system was heated to 300 K and stayed at this temperature for equilibrium for about 50 ps. Self-guided molecular dynamics (SGMD)[24,25] simulations were then performed at 300 K in the canonical (NVT) ensemble with a local sampling time of 0.2 ps and a guiding temperature factor of 1.0. During the simulations, the SHAKE algorithm was used to fix the lengths of bonds involving hydrogen atoms, and a 12 Å effective nonbonded cutoff distance was used. All the coordinates of the carbon atoms of the HOPG surface were fixed during the simulations. Configurations of the peptide molecule obtained from simulations were saved every 1.0 ps for analysis. The time step of integration for the potential energy of the system was taken as 1.0 fs. The length of each run of the simulations is 50.0 ns.

Results

We report the detailed study on the adsorption and initial folding/assembly events of EAK16-II on HOPG. The focus will
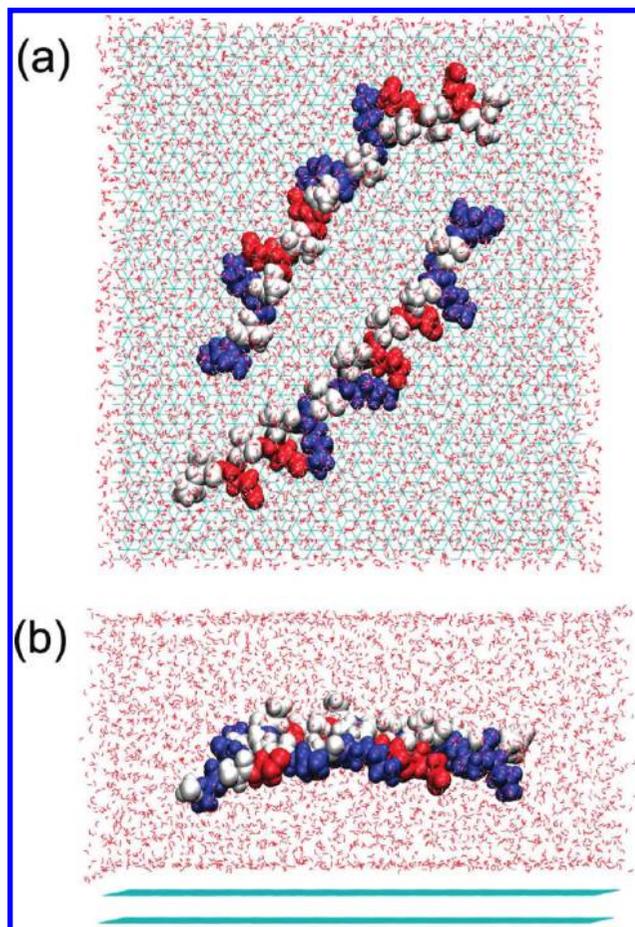

Figure 2. Snapshots of the peptide EAK16-II on the HOPG surface: (a) top view; (b) side view. The carbon atoms consisting of the HOPG surface are colored in cyan, and water molecules are colored in red. The two peptide molecules are displayed with vdW spheres, and the three residues alanine, glutamic acid, and lysine are colored in gray, red, and blue, respectively.

be on the interactions occurring during the adsorption of the peptide EAK16-II, and the analysis based on a typical run of the simulation will be presented. The reason for using two EAK16-II chains in our system is that EAK16-II itself can self-assemble in solution and on surface; thus, the interchain interactions during the adsorption and initial folding/assembly stage can also be important to study besides the peptide adsorption. Time evolution of different energy terms is used to analyze the interactions coming from hydrophobic and charged residues. Hydrogen bonding analysis is also provided. The radius of gyration R_g and solvent accessible surface area (SASA) are used to characterize the compactness of a conformation and the hydrophobic effect, respectively.

Adsorption of EAK16-II on the HOPG Surface. The distance between each peptide molecule and the HOPG surface as a function of time is presented in Figure 3. The calculation of the distance is based on the center of mass of each peptide chain and the surface plane. For the sake of convenience, the two peptide molecules are named chain I and chain II, respectively, in the analysis. The figure shows that the adsorption of chain I begins at about 1.25 ns, and after a short time period of about 0.45 ns, it is completely adsorbed on the surface. The adsorption of chain II is not as quick as chain I. Starting from almost the same time point as that of chain I, the adsorption of chain II proceeds slowly toward the surface and finally deposits on the surface at around 4.0 ns, with the total adsorption process of about 2.75 ns.
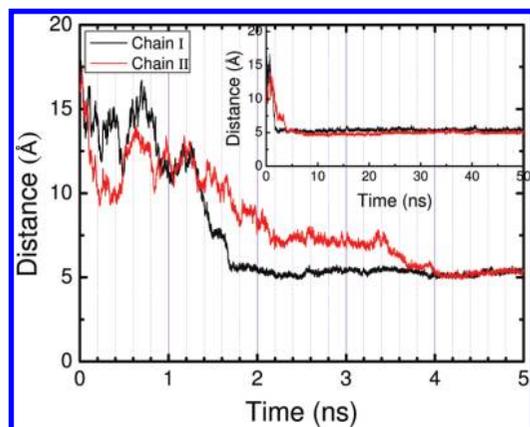
Figure 3. Time evolution of the distance between each peptide molecule and the HOPG surface. The inset shows the change of the distance within a prolonged time period until 50 ns.
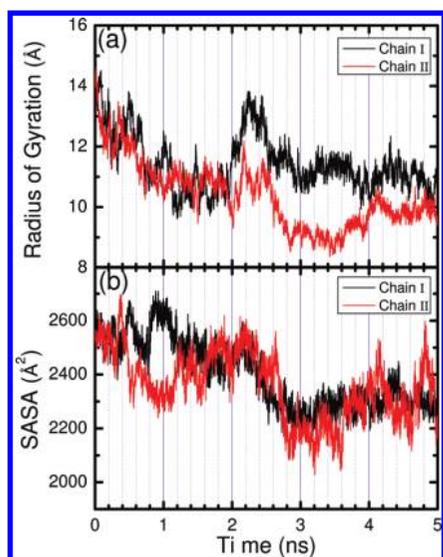

Figure 4. Time evolution of the radius of gyration (a) and solvent accessible surface area (b) for each peptide molecule.

To characterize the structure change during the adsorption, we further analyzed the radius of gyration R_g and SASA; see Figure 4. The compactness of a structure can be understood quantitatively by monitoring the radius of gyration R_g. The solvent accessible surface area of a protein or peptide molecule is a factor to measure the hydrophobicity of the molecule. The accessible surface represents the part of the complex peptide surface in direct contact with the solvent. The hydrophobic force is one of the most important forces that determine the adsorption of peptides. SASA is traced out by the center of the probe sphere with a radius of 1.6 Å as it rolls over the peptide molecules. From Figure 4a, we can see that there is an obvious structure rearrangement for chain I when it deposits on the surface; i.e., the chain first becomes less compact and then rearranges to a structure as compact as that prior to deposition, which takes place between 2.0 and 2.9 ns. At the same time, the structure rearrangement of chain II is also observed, although the change is not as large as that of chain I. At 2.5 ns, the R_g of chain II decreases dramatically, which indicates the structure of chain II becomes more compact. When chain II completely deposits on the surface at about 4.0 ns, another structure adjustment is observed. From the SASA plot in Figure 4b, we see that from 2.0 to 2.9 ns, both chain I and chain II become more hydrophobic corresponding to the structure adjustment. This is in accord with normal protein folding with a fast hydrophobic
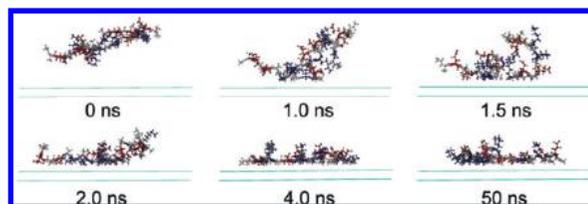

Figure 5. Side view of the snapshots of the peptide−surface system at different times, which are derived from a sample run. Water molecules are omitted for simplicity. Peptide molecules are displayed in CPK mode, and the three residues alanine, glutamic acid, and lysine are colored in gray, red, and blue, respectively.

Figure 6. Top view of the snapshots of the peptide−surface system at different times, which are derived from a sample run. Water molecules are omitted for simplicity. Peptide molecules are displayed in CPK mode, and the three residues alanine, glutamic acid, and lysine are colored in gray, red, and blue, respectively.
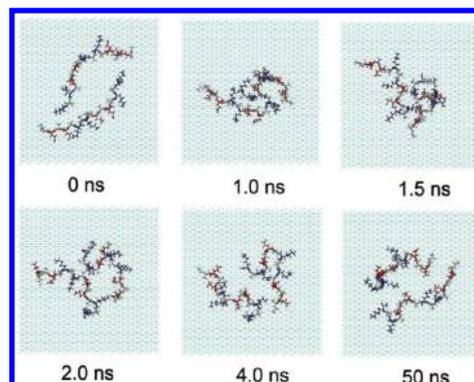
collapse at the initial stage.[26−30] However, due to the unique sequence of our ionic complementary peptide and the hydrophobic surface of HOPG, the peptide tends to proceed to form a hydrophobic contact with the surface of HOPG instead of forming a hydrophobic core.

The results above indicate that the adsorption of the two peptide chains is in a cooperative mode, and the deposition of the first chain on the surface will affect the adsorption of the second one, resulting in a structure adjustment of both chains due to the interchain interactions. From the 50 ns plots of peptide−surface distance, it can be concluded that once the peptide molecules deposit on the surface, they will stay there until the simulation stops, indicating that the adsorption of peptide EAK16-II molecules on the HOPG surface can be stable. Figures 5 and 6 give intuitive demonstration snapshots of the conformations of the peptide−surface system at different times.

Interactions during the Adsorption and Initial Assembly Process. The different interactions are represented with the corresponding energy terms, which are provided in Figure 7. Figure 7a shows the time evolution of the potential energy of each peptide chain with the surface; the dramatic energy decrease of chain I is consistent with the above result for the distance change analysis (Figure 3). Two different decreasing rates in the plot are observed; the first one is slower, which lasts from about 1.25 to 1.5 ns, and the second one is much faster, which lasts from about 1.5 to 1.7 ns. At the transition point around 1.5 ns, a corresponding short plateau in the chain I−surface distance plot (Figure 3) is also observed, which lasts from 1.46 to 1.65 ns. Meanwhile, a significant structure change is not observed in Figure 4; this implies that there is a conformational rotation taking place from this time point, to make the hydrophobic residues closer to the surface, facilitating the adsorption. This inference can be verified through the change of the energy between hydrophobic alanine residues and the
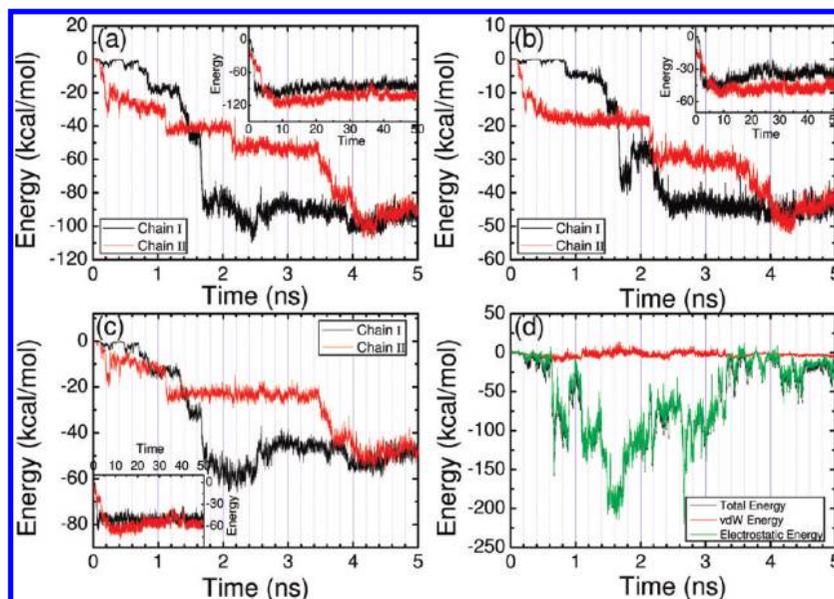
Adsorption of EAK16-II on the HOPG Surface

J. Phys. Chem. C, Vol. 114, No. 1, 2010 **457**

Figure 7. (a) Time evolution of the energy between each peptide molecule and the HOPG surface. (b) Time evolution of the energy between the alanine residues of each peptide molecule and the HOPG surface. (c) Time evolution of the energy between the charged residues of each peptide molecule and the HOPG surface. (d) Time evolution of the total energy, the vdW energy, and the electrostatic energy between the two peptide molecules; as the interchain interactions are contributed mainly by the electrostatic interaction, the curves for electrostatic and total energies are almost overlapping. The insets in (a)−(c) show the change within a prolonged time period until 50 ns.

surface in Figure 7b and that between charged residues and the surface in Figure 7c. Starting from 1.65 ns, the energy between alanine residues of chain I and the surface decreases dramatically, which indicates that the interactions between alanine residues and the HOPG surface intensify quickly. During the same time period, the interactions between charged residues of chain I and the surface also intensify and induce a decrease in energy; see Figure 7c. When chain I deposits on the surface at about 1.7 ns, the energy between hydrophobic residues and the surface increases and that between charged residues and the surface decreases, which indicates that there is an overrotation of the alanine side chains during the adsorption process, and adjustments for the side chains of both hydrophobic and charged residues are made in succession.

During the side-chain adjustment of chain I from 1.7 to 2.15 ns, the electrostatic interaction between the two peptide molecules is very intensive (Figure 7d), and some positively charged lysine residues get closer to negatively charged glutamic acid residues. As the energy between charged residues of chain II and the surface does not change during this time period from 1.7 to 2.15 ns (Figure 7c), we can infer that at this stage it is mainly the charged side-chain terminals of chain I going deeper into the solvent causing the ionic carboxyl and amino groups to interact with the charged side chains of chain II. It should be noted that the side chain of lysine contains four methylene groups; once the positive charge of the side-chain amino group is screened when it interacts with the negatively charged glutamic acid, the methylene groups in the lysine side chain will result in strong hydrophobic effect and energy change. Therefore, a corresponding energy decrease between the charged residues of chain I and the surface is observed, indicating the interaction between side-chain methylene groups and the surface intensifies; see Figure 7c. After the above structure adjustment at around 2.15 ns, another structure change for alanine residues of both chain I and chain II takes place, the hydrophobic side chains get closer to the HOPG surface, and the interactions between alanine residues and the surface intensify quickly and hence a decrease in energy is observed (Figure 7b). Chain II deposits on the surface finally at around 4.0 ns.
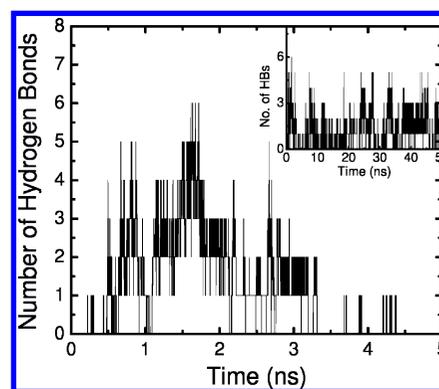

Figure 8. Time evolution of the number of hydrogen bonds between the two peptide molecules. The inset shows the change of the number of hydrogen bonds within a prolonged time period until 50 ns.

The change of different energy terms (potential energy, vdW energy, and electrostatic energy) between two peptide molecules with respect to time shown in Figure 7d indicates that the main interchain interactions affecting the adsorption come from the electrostatic interactions. Interchain hydrogen bonding may also form during the adsorption process, but it is much weaker compared to the Coulomb interaction; see below.

Hydrogen Bonding Analysis. Hydrogen bonding between the two peptide molecules is also investigated in this work. A hydrogen bond is considered to form if the donor−acceptor distance is less than 2.4 Å and the hydrogen donor−acceptor angle greater than 110°. Figure 8 shows the time evolution of the number of interchain hydrogen bonds during the adsorption and initial folding/assembly process. During the adsorption of chain I, several hydrogen bonds form; however, it should be noted that these formed hydrogen bonds are not stable, and the forming and deforming take place frequently. We consider that this frequent breaking up of hydrogen bonds is due to the structure change accompanying the adjustment of residue−residue electrostatic interactions. At the latter adsorption stage of chain II, say after 3.3 ns, most of interchain hydrogen bonds break up to ensure the configuration of chain II to be more favorable on the HOPG surface.

458 *J. Phys. Chem. C, Vol. 114, No. 1, 2010*

Sheng et al.

TABLE 1: Interchain Residue Pairs for Which Long Lasting Hydrogen Bonds Are Frequently Observed between 0 and 5 ns and between 10 and 50 ns, Respectively[a]

time (ns)	residue pairs	lasting time (ps)
0−5	Glu4-Lys14	137
	Glu10-Lys14	77
	Glu2-Lys14	75
	Glu2-Lys16	72
10−50	Glu10-Lys14	755
	Glu2-Lys8	630
	Lys8-Glu10	176
	Lys6-Glu10	112

[a] The residues are represented by combining the three-letter amino acid abbreviations and the residue's sequence number in the peptide chain. For example, the pair Glu4-Lys14 denotes the hydrogen bond forming between the 4th glutamic acid residue in one peptide chain (donor) and the 14th lysine residue in another peptide chain (acceptor).

The interchain residue pairs for which long lasting hydrogen bonds are frequently observed are also presented in Table 1. The longest lasting hydrogen bond during the adsorption process forms between Lys14 (chain I) and Glu4 (chain II) at around 2.18 ns and lasts for 137 ps, which further verifies the inference in the above subsection that the charged side-chain terminals of chain I get closer to the charged side chains of chain II at that time point, inducing the intensification of the peptide−peptide electrostatic interaction.

As the HOPG surface cannot form hydrogen bonds with the peptide and water molecules, the role of hydrogen bonding in EAK16-II adsorption is nonsignificant. After the adsorption, hydrogen bonding will play an important role in the succeeding assembly process. The longest lasting hydrogen bond observed between 10 and 50 ns lasts for 755 ps; see Table 1. Although there are still many wrongly paired interchain hydrogen bonds formed during this process, the peptide molecules are conducting a search guided by the free energy to find the optimal hydrogen bonding patterns to assemble them into a dimer on the surface.

Discussion

The results of the simulations above show the roles of different interactions played in EAK16-II adsorption and initial assembly, which gives an important support to our previous experimental findings.[14,15] As presented above, the adsorption of the chain II is significantly affected by the previously deposited chain I. Among different interactions, this effect comes mainly from the peptide−peptide electrostatic interactions. The deposition of the first chain is driven mainly by the hydrophobic effect, and both noncharged alanine residues and charged lysine and glutamic acid residues contribute to this hydrophobic effect, while the adsorption of the second one is the result of a competition between the interactions from the peptide chains and the surface and the interactions from the two chains. During the adsorption, the contribution of hydrogen bonding is weak compared to other interactions. Electrostatic interaction is a very strong interaction which drives the peptide molecules closer to each other, and it is mainly the hydrogen bonding interaction which contributes to the subsequent interactions between the two chains to assist the peptide in self-assembling to dimers and eventually fibers after the peptide chains deposit on the surface, consistent with the earlier experimental work.[14]

After the deposition of the peptide on the HOPG surface, the hydrophobic alanine residues tend to stay close to the HOPG surface, while side chains of charged residues prefer to stay deeper into the solvent (Figures 5 and 6), which is consistent with our previous experimental findings.[14,15] In addition, the alanine side chains have a potential to be closer to the carbon atoms of the surface in order to attain the largest hydrophobic contact and achieve more stable configurations, resulting in the peptide molecules covering as many surface carbon atoms as possible. Because of the hexagonal structure of the HOPG lattice, particular orientations of the peptide principal axes and the assembled peptide fiber axes are thus observed accordingly in experiment.[14,15] We examined the peptide structures from the trajectory files and found that after depositing on the surface, most of the adjacent residues have a tendency to cover the surface in a manner parallel to the three horizontal crystallographic axes of the HOPG surface; see Figures 5 and 6.

Although an all-atomic representation of the peptide−surface system is used, and much detailed information is obtained in the simulations, the limitation of our model still exists. This lies in the fact that under the consideration of the computational cost, the model system is not large enough to adequately consider the diffusive behavior of peptide chains in solution. Nevertheless, the consistency with experiment in peptide side-chain arrangement and surface covering orientation implies that our simulations have captured the essence of the adsorption of EAK16-II on the HOPG surface. The behavior of chain I may be representative of the adsorption for a range of similar peptide molecules at low concentrations. At high concentrations, however, due to the more crowded environment, more peptide molecules will behave with respect to the adsorption in a manner like chain II as the interchain interactions become significant.

Conclusions

In this work, all-atom MD simulations were performed and different interactions during the adsorption and initial assembly process were analyzed. The adsorption behavior of the peptide EAK16-II on the HOPG surface has been characterized in detail, and the results give an understanding of the roles of each component of the interactions played during the adsorption process. The deposition of chain I is governed mainly by the hydrophobic interaction with the surface, while both electrostatic and hydrophobic interactions contribute to the adsorption of chain II. The effect of hydrogen bonding is nonsignificant during peptide adsorption, but it plays an important role in the succeeding peptide assembly process on the surface. The knowledge of these interactions during the adsorption will assist us in obtaining a more complete peptide−peptide and peptide−surface interaction picture, which will be beneficial for designing useful peptide molecules more effectively, e.g., to be applied in drug delivery materials and bioactive implant devices.

References and Notes

(1) Schamberger, P. C.; Gardella, J. A., Jr. *Colloids Surf., B* **1994**, *2*, 209.

(2) Larsen, C. C.; Kligman, F.; Kottke-Marchant, K.; Marchant, R. E. *Biomaterials* **2006**, *27*, 4846.

(3) Lahann, J.; Klee, D.; Pluester, W.; Hoecker, H. *Biomaterials* **2001**, *22*, 817.

(4) Chinn, J. A.; Sauter, J. A.; Phillips, R. E.; Kao, W. J.; Anderson, J. M.; Hanson, S. R.; Ashton, T. R. *J. Biomed. Matter Res.* **1998**, *39*, 130.

Adsorption of EAK16-II on the HOPG Surface

J. Phys. Chem. C, Vol. 114, No. 1, 2010 **459**

(5) Toublan, F. J.; Boppart, S.; Suslick, K. S. *J. Am. Chem. Soc.* **2006**, *128*, 3472.

(6) Willett, R. L.; Baldwin, K. W.; West, K. W.; Pfeiffer, L. N. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 7817.

(7) Hlady, H.; Buijs, J. *Curr. Opin. Biotechnol.* **1996**, *7*, 72.

(8) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.

(9) Kane, R. S.; Deschatelets, P.; Whitesides, G. M. *Langmuir* **2003**, *19*, 2388.

(10) Gray, J. J. *Curr. Opin. Struct. Biol.* **2004**, *14*, 110.

(11) Fung, S. Y.; Keyes, C.; Duhamel, J.; Chen, P. *Biophys. J.* **2003**, *85*, 537.

(12) Hong, Y.; Legge, R. L.; Zhang, S.; Chen, P. *Biomacromolecules* **2003**, *4*, 1433.

(13) Jun, S.; Hong, Y.; Immamura, H.; Ha, B. Y.; Bechhoefer, J.; Chen, P. *Biophys. J.* **2004**, *87*, 1249.

(14) Yang, H.; Fung, S. Y.; Pritzker, M.; Chen, P. *J. Am. Chem. Soc.* **2007**, *129*, 12200.

(15) Yang, H.; Fung, S. Y.; Pritzker, M.; Chen, P. *PLoS ONE* **2007**, *2*, e1325.

(16) Malmsten, M. *J. Colloid Interface Sci.* **1998**, *207*, 186.

(17) Morra, M. *J. Biomater. Sci. Polym. Ed.* **2000**, *11*, 547.

(18) Roth, C. M.; Lenhoff, A. M. *Surfact. Sci. Ser.* **1998**, *75*, 89.

(19) Lu, D. R.; Park, K. *J. Biomater. Sci. Polym. Ed.* **1990**, *1*, 243.

(20) Noinville, V.; Vidalmadjar, C.; Sebille, B. *J. Phys. Chem.* **1995**, *99*, 1516.

(21) Ravichandran, S.; Madura, J. D.; Talbot, J. *J. Phys. Chem. B* **2001**, *105*, 3610.

(22) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.

(23) Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graphics* **1996**, *14*, 33.

(24) Wu, X.; Wang, S.; Brooks, B. R. *J. Am. Chem. Soc.* **2002**, *124*, 5282.

(25) Wu, X.; Brooks, B. R. *Chem. Phys. Lett.* **2003**, *381*, 512.

(26) Mirny, L.; Shakhnovich, E. *Annu. Rev. Biophys. Biomol.* **2001**, *30*, 361.

(27) Wolynes, P. G.; Onuchic, J. N.; Thirumalai, D. *Science* **1995**, *267*, 1619.

(28) Daggett, V.; Fersht, A. R. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 497.

(29) Jackson, S. E.; elMasry, N.; Fersht, A. R. *Biochemistry* **1993**, *32*, 11270.

(30) Sheng, Y.; Wang, W. *Phys. Rev. E* **2006**, *73*, 021915.