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Sequential adsorption of proteins and the surface modification of biomaterials: A molecular dynamics study

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Abstract The sequential adsorption of two proteins of the same or of an unlike nature on a heterogeneous hydrophobic surface is investigated through atomistic molecular dynamics simulations. By modeling two real protein fragments having a different secondary structure (α-helices or β-sheets) on a graphite surface, the pre-adsorbed polypeptides are shown to modify the hydropathy of this substrate. Therefore, the graphite surface modified by the first adsorbed protein becomes more similar to a hydrophilic one in terms of both the interaction energy and the size of the second protein after the possible surface spreading.

1 Introduction

When a biomaterial is placed in a physiological environment, proteins do adsorb on its surface before cell adhesion takes place [1]. The first adsorbed proteins are the most abundant ones, due to their larger concentration, and/or the smaller ones, due to their larger diffusion coefficient, even though other factors may be at play on charged surfaces. However, the adsorbed proteins may slowly relax and spread on the surface, so that a few of them can be eventually released to the bulk solution. This process leads to a smaller surface coverage, or equivalently to a smaller number of adsorbed proteins, hence to a smaller mass per unit surface, giving rise to the so-called Vroman effect [2–5]. Such behavior is usually found on hydrophilic surfaces, and may be accompanied by the replacement of the proteins that were adsorbed earlier by other ones, more affine to the surface. In this case, there is an initial kinetic control of adsorption, eventually followed by a thermodynamic one. On the other hand, adsorption appears to be in general irreversible on hydrophobic surfaces [5, 6]. Accordingly, less abundant or larger proteins do interact with a modified surface because of the first layer of strongly bound proteins. This feature may be a natural event on the surface of an implanted biomaterial, but it may also be exploited to enhance or control its biomimetic performance through either physisorption or chemisorption of appropriate proteins or oligopeptides bound to the surface.

In the last few years, we have started a systematic study of protein adsorption on a surface through fully atomistic simulations using both Molecular Mechanics (MM) procedures, i.e. energy minimizations, and Molecular Dynamics (MD) methods. The main results can be found in a recent review paper [7]. The chosen methodology allows investigating the energetics of adsorption on different substrates, the protein surface rearrangements at the nanometer scale and the kinetics of spreading. Electrically neutral surfaces of different nature were taken into account, including a hydrophobic graphite surface [8–11] and a hydrophilic poly (vinyl alcohol) (PVA) surface [12]. Moreover, isolated fragments of globular proteins with an unlike secondary structure in the native state were considered: two human serum albumin subdomains, each formed by three α-helices, and a fibronectin type I module, containing three independent β-sheets. The present paper reports new simulation results about the sequential adsorption of these protein fragments on graphite, assuming that the pre-adsorbed fragment has already achieved the maximum surface spreading, and it is fully relaxed to the most stable arrangement as previously obtained in our group by similar methods [8–11]. This study includes the sequential adsorption of two fragments of the same or of an unlike

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nature on each other. In this way, the surface is shown to be greatly modified by the first adsorbed fragment, which drastically changes both the surface hydropathy and, to some extent, its topography, thus eventually controlling the adsorption strength of the second fragment. More specifically, in this simulation study the graphite surface was modified through a monolayer of aminoacids formed by the albumin subdomain, or through the pre-adsorbed fibronectin module, while the adsorption of a second fragment of the same or of an unlike nature was modeled starting from its native geometry at the physiological pH (=7.4). In both cases, very little rearrangements, if any, took place in the initial adsorption stage, so that the interaction energy was significantly depressed compared to what found on bare graphite. This result is fully consistent with what was obtained on the hydrophilic PVA surface [7, 12], leading to the conclusion that the pre-adsorbed fragment makes the graphite surface essentially hydrophilic. However, if the surface is only partially covered, slower readjustments of both fragments can take place, as indeed found through MD simulations at room temperature, possibly leading to strongly enhanced adsorption strengths. Some preliminary results of the present work were briefly mentioned in the above-mentioned review paper [7]. It should be added that only the thermodynamically most stable states at room temperature are investigated in the present paper. Kinetics aspects of the adsorption process due for instance to different protein concentrations, or to their size, which controls the diffusion in solution, are outside the scope of this study.

In the following, after the section on the simulation methodology, earlier results about the adsorption of the individual protein fragments on graphite and on PVA are briefly reviewed for a comparison, and then the new results are discussed in two separate sections that correspond to the simulation protocol. More precisely, the first section describes the initial adsorption stage of the second protein fragment studied by full energy minimization. In this case, the pre-adsorbed fragments display little changes, as expected for a fully covered surface where the first fragment is constrained by its neighbors, and in practice only the second one may readjust its conformation. Afterwards, the second section presents the results obtained through the MD simulations at room temperature by allowing complete freedom of motion to both fragments. In this case, the first fragment may significantly modify its conformation to allow a stronger adsorption of the second one with the bare surface, especially if it belongs to a soft protein such as albumin [1]. Therefore, on a surface with little coverage both fragments may display large rearrangements to enhance the system stability by simultaneously optimizing their interactions both with the surface and between themselves. Finally, some general adsorption features are summarized, taking also into account for a comparison previous simulations of the adsorption of the same fragments on the hydrophilic PVA surface. The conclusion is that the modified surface is indeed made more hydrophilic by the pre-adsorbed polypeptide, and therefore more resistant to protein adhesion and spreading.

2 Simulation method

The simulations were performed with InsightII/Discover 2000 [13], using the consistent valence force field CVFF [14] with a Morse potential for the bonded atoms. This force field was chosen here, as in previous work, because it is well parametrized to deal with the conformations and energies of proteins and of the biomaterial surfaces taken into account. It should be noted incidentally that CVFF describes non-bonded interactions through van der Waals and Coulombic terms only, with no extra terms for the hydrogen bonds. The atomic coordinates used for the initial geometries of the proteins were taken from the experimental results deposited with the Protein Data Bank [15] (Human Serum Albumin, 1AO6, fibronectin type I module, 1FBR), and the hydrogen atoms were added in the calculated positions. Due to the large size of albumin, one subdomain (subdomain *A* of ref. [8]) formed by three α-helices was selected, while the fibronectin module contained only β-sheets [9]. In ref. [8], all the side groups of the albumin subdomains were assumed to be in a neutral, uncharged form, with the terminal groups in a zwitterionic form, but later work showed that the final adsorption state was independent from this choice, being the same when the correctly charged side groups at pH = 7 were accounted for [11]. Conversely, the fibronectin module was always modeled with the appropriate charges at the same pH. In the present paper, all the protein fragments were modeled throughout with the appropriate charges at the physiological pH = 7.4.

In analogy to what done for the isolated fragments on graphite [7, 8–11], the initial adsorption of the second fragment was studied by placing it in different trial orientations (see also later) close to the first one on graphite. In turn, the latter fragment was taken in the most stable surface arrangement found in previous work [8, 9] after the MD runs at room temperature and final energy minimizations. The energy of both fragments was then minimized with respect to the coordinates of all the atoms up to a gradient lower than 4×10^{-3} kJ mol^{-1} Å^{-1} with implicit solvent in an effective dielectric medium mimicking water. The MD simulations were performed in the same medium at a constant temperature (T = 300 K) controlled through the Berendsen thermostat. The size of the graphite surface was initially taken as equal to 84 × 59 Å, but in view of the large spreading of the fragments it was eventually increased to 110 × 90 Å to avoid any edge effect. The length of the MD runs was dependent on the system, and lasted for 1 ns on the smaller surface, and then for a further 1.5 ns or 2.5 ns on the larger one. Integration of the dynamical equations was carried out with the Verlet algorithm with a

time step of 1 fs. Within the MD runs, the total and potential energy and the distance between the center of mass of the second fragment and the surface showed an initial decrease, possibly with a few separate kinetic stages, and then fluctuated around a constant value, indicating achievement of an equilibrium state. Final geometry optimizations were then carried out as before in search of the most stable adsorbed state.

3 Results and discussion

Some previous results about the absorption of isolated protein fragments in a dielectric medium and in water are first briefly reviewed, considering the bare surfaces of hydrophobic graphite and of hydrophilic PVA. Through the MD simulations at room temperature, the adsorption of the albumin subdomain on graphite eventually yielded a monolayer of aminoacids with a parallel ordering of the distant strands, a result that was independent from the hydropathy of the fragments and, largely, from pH [8, 10, 11]. In this way, the system optimized both the surface interaction of all the aminoacids through dispersion forces, and the interaction among the residues of different strands (mainly dipolar and ion-pair interactions). At the same time, a complete hydration of the backbone and of the polar residues was achieved thanks to the large surface spreading that exposed all the residues to the solvent. The fibronectin module displayed again a strong adsorption with some parallel ordering of distant strands [9, 10], but its full surface spreading was prevented by the presence of some disulfide bridges acting as chemical cross-links. In fact, if the adsorption of the same fragment was modeled after reduction of the disulfide groups, a monolayer of aminoacids on graphite was again obtained [7, 10].

On the other hand, by adopting the same simulation methodology the globular shape of the fragments was shown to be preserved on the hydrophilic PVA surface with only minor changes in size and anisotropy, albeit with an extensive, though incomplete, denaturation [7, 12], consistent with experimental results [16, 17]. The interaction energy per residue in contact with the PVA surface was significantly smaller than on graphite, and less residues did adhere to the surface [7, 12], so that the adsorbed fragments could be displaced much more easily by an external force (a flowing fluid, for instance), again in keeping with experiment [18].

3.1 The initial adsorption stage of the second protein fragment

This first section describes the initial adsorption of a second protein fragment on a pre-adsorbed one after their full energy minimization. The trial starting arrangements chosen for the second fragment (the albumin subdomain or the fibronectin module) being adsorbed on a previous fragment of the same or of an unlike nature were the same as those adopted for the isolated fragments on bare graphite [8, 9]. More precisely, considering the different sides of these fragments eight possible orientations for the albumin subdomain were chosen, and six for the fibronectin module (Fig. 3 of ref. [8] and Fig. 2 of ref. [9], respectively). As already anticipated, the second fragment did adsorb on a modified surface that was made more hydrophilic by the residues of the first one. In fact, the energy minimizations showed that the new fragments retained in all cases the globular shape and in particular the native secondary structure consisting of α-helices or β-sheets. As typical cases, Fig. 1 reports the initial trial arrangements (at left) that yielded the best adsorption geometry in the initial stage after full energy minimization (at right). The other trial arrangements are not shown for brevity, but they did not display major conformational changes, either for the second fragment compared to its native state, or for the first fragment with respect to its most stable surface arrangement. In a few cases, however, some local order was induced, as shown for instance by the formation of a short intermolecular β-sheet involving a few residues of unlike fragments (central panel of Fig. 1). Adsorption was favorable, but quite weak in all cases, because only a few residues of the second fragment did interact with the pre-adsorbed ones or with the graphite surface, and the interaction energy per residue was also quite small. In other words, the intramolecular interactions dictated the overall geometry of the new fragments, which interacted only weakly with the pre-adsorbed ones through the outer envelope, forming only few intermolecular hydrogen bonds, if any. In order to have a quantitative estimate of the adsorption strengths, it is useful to calculate the interaction energy E_{int}, defined as $E_{\text{int}} = (E_{\text{free}} + E_{\text{pre-ads}}) - E_{\text{tot}}$, as done before [7–9]. Here, E_{free} is the energy of the free native fragment, $E_{\text{pre-ads}}$ is the energy of the pre-adsorbed fragment on graphite, and E_{tot} is the energy of the whole system. According to this definition, $E_{\text{int}} > 0$ is the energy required to desorb the second fragment from the modified surface and bring it back to the free, native state. Figure 2 reports the interaction energies of the optimized new fragments in the local energy minima found starting from the chosen different orientations as a function of the number of aminoacids in contact with the modified surface, $n_{5\,\text{Å}}$, where 5 Å was conventionally taken as the upper limit for a contact distance [7–9]. The figure shows a linear correlation between E_{int} and $n_{5\,\text{Å}}$, a result consistent with the fact that adsorption is driven by dipolar interactions and dispersion forces, with very few hydrogen bonds between the two fragments. The best-fit line through the origin shown in Fig. 2 is given by

$$E_{\text{int}} = 22.8(9) \cdot n_{5\,\text{Å}}\ \text{kJ mol}^{-1} \quad (1)$$

the figure in parentheses being the estimated standard error on the last significant digit. Therefore, a single regression line

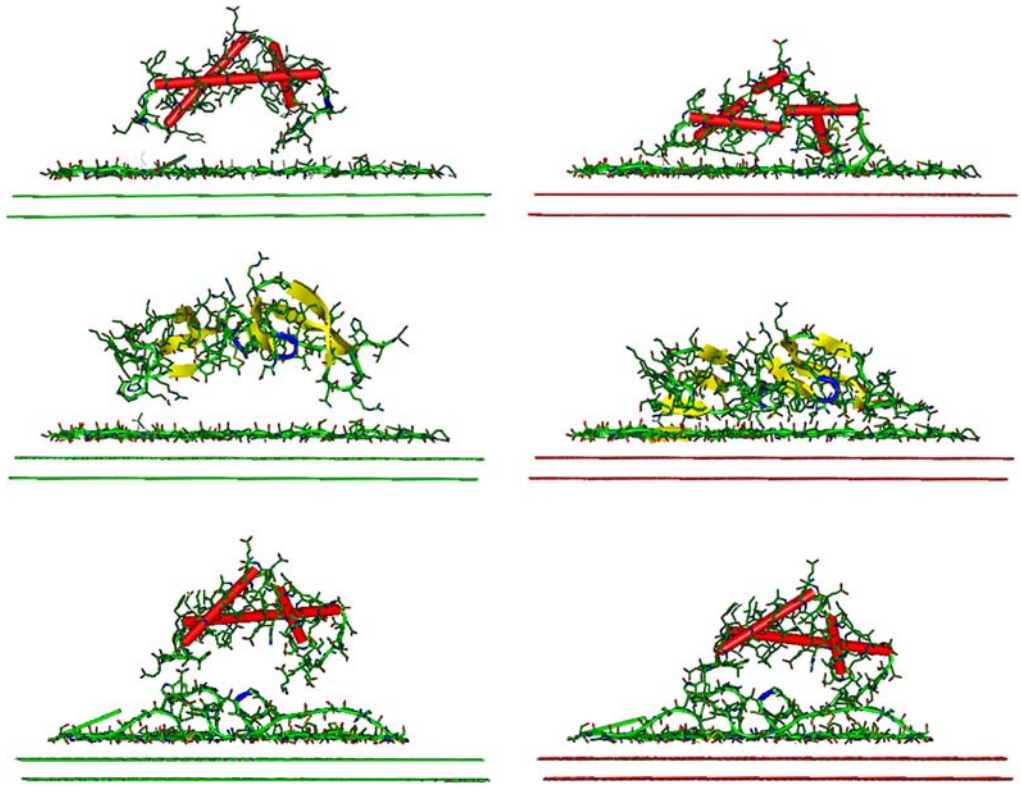

Fig. 1 The trial geometries of the protein fragments on a pre-adsorbed one over graphite (at left) leading to the most stable initial geometries (at right) obtained by full energy minimization. The other geometries are not shown for brevity. Top: the albumin *A* subdomain on the monolayer formed by an equal subdomain. Center: the fibronectin module on the monolayer formed by the albumin *A* subdomain. Bottom: the albumin *A* subdomain on the pre-adsorbed fibronectin module. The two upper planes of carbon atoms of graphite are also shown in a side view in different colors for clarity. The red cylinders indicate the α-helices and the yellow arrows the β-sheets

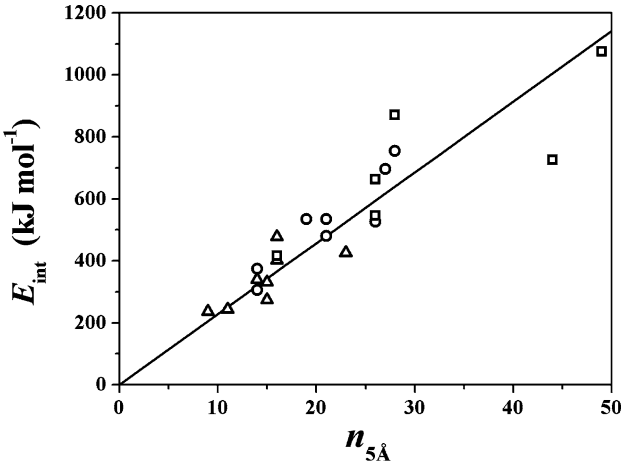

Fig. 2 The interaction energy E_{int} plotted as a function of the number of residues in contact with the modified surface, $n_{5\,\text{Å}}$ (see the text for their definition). The circles indicate the results obtained by energy minimization in different trial orientations of the albumin *A* subdomain on the monolayer formed by an equal subdomain, the squares those for the fibronectin module on the monolayer formed by the albumin *A* subdomain, and the triangles those for the albumin *A* subdomain on the pre-adsorbed fibronectin module. The straight line is the best-fit line through the origin obtained from all the data points and its equation is reported in Eq. (1) ($R = 0.898$)

describes the interaction energy calculated after full geometry optimization of the two protein fragments considering all the starting initial orientations. This result is not surprising if one considers that the same natural aminoacids are involved. Accordingly, the slope of the best-fit line through the origin is the same to within 1.5 kJ mol^{-1} if the three different sets of data were independently fitted. It must be pointed out that the overall interaction energy of the new fragments with the pre-adsorbed ones on graphite was rather close to what was found for the adsorption of the same isolated fragments on the hydrophilic PVA, namely 14.5(6) kJ per mole of residue in contact with the surface [12]. In the present case, however, a small number of residues of the second fragment interacted also with graphite, where adsorption is stronger, and therefore the interaction energy per residue, given by the numerical coefficient in Eq. (1), was somewhat larger than on PVA. On the other hand, the present value is definitely much smaller than the value of 58(1) kJ per mole of residue in contact with the surface found on hydrophobic graphite at pH = 7 [11].

These results were obtained for the initial adsorption stage, but they also correspond to the final stage at a large surface coverage, when large rearrangements of the pre-adsorbed

fragments are hindered by neighboring ones, even though some minor changes at long times cannot be ruled out. However, the rather close similarity of the present results with those obtained on the PVA surface strongly indicate that a pre-adsorbed protein or oligopeptide, more generally, effectively modify the behavior of the graphite surface, making it more hydrophilic. Therefore, the conclusion is that in view of the weak adsorption strength and of the overall geometry of the second fragment, mainly dictated by its intramolecular interactions (hydrogen bonds, etc.), the initial adsorption on the modified surface is quite negligible, and in any case it is fully reversible. Such results are in satisfactory agreement with experimental results, including for instance the use of albumin as a passivating agent [19] and the coating of hydrophobic polystyrene with hydrophilic PVA for protein chromatography [18]. On the other hand, a different picture may emerge at a small surface coverage, when the adsorbed fragments can spread and leave room for the new fragment to optimize its interaction with the surface. Such situation is dealt with in the next section.

3.2 The final adsorption stage of the second protein fragment

The results of the previous section describe the final adsorption stage at a large surface coverage. However, if the surface coverage is far from complete and the first fragments do only form isolated islands on the surface, a second fragment may adsorb either on the bare surface, or on a pre-adsorbed fragment. In the latter case, the previous results do only provide the initial adsorption stage. However, at longer times both the pre-adsorbed fragment and the subsequent one may display extensive rearrangements to optimize the interaction energy both with the surface and with each other by maximizing the number of residues involved in these interactions. The most stable geometries eventually achieved by the two fragments on graphite after the MD runs and the final geometry optimizations are shown in Fig. 3. The freedom of rearrangement of both fragments was again very important, since the intramolecular cross-links due to the disulfide bridges involving topologically distant residues may provide a significant constraint to surface spreading. Such constraint was found to be effective in the fibronectin module, as already pointed out, whereas it was absent in practice in the albumin subdomain, where a single disulfide bridge involves two neighboring residues only. Therefore, the second albumin fragment initially adsorbed on the monolayer of a similar pre-adsorbed subdomain showed a large denaturation and spreading in the MD runs, together with large-scale rearrangements of the monolayer, in keeping also with the soft nature of albumin [1]. As a result, both albumin subdomains eventually maximized the interaction with graphite achieving the largest possible surface coverage (see Fig. 3 at top) with a sharp energy decrease. The kinetics of this process showed two rather fast changes and at least three well-defined states, but eventually the center of mass of the backbone of the second subdomain achieved the same distance from the graphite surface (5.3 Å) as the first one (4.4 Å). On the other hand, the top geometry in Fig. 3 shows that the pre-adsorbed fragment (displayed in red) strongly increased its size parallel to the surface, thus achieving a much larger radius of gyration of 32.3 Å (Table 1). This large increase was mainly due to the components parallel to the surface, as shown by

Table 1 The interaction energy E_{int} in MJ mol^{-1}, and the molecular size, in Å, expressed through the radius of gyration R_g and the principal axes λ_1, λ_2, λ_3, arranged in decreasing order for the final most stable states

	E_{int}	R_g	λ_1	λ_2	λ_3
Native albumin subdomain[a]	–	12.1	9.6	5.6	4.9
Native fibronectin module[b]	–	16.1	14.3	5.8	4.5
Albumin subdomain on graphite[a]	3.44	21.5	19.3	9.3	1.0
Fibronectin module on graphite[b]	3.90	18.9	16.2	9.4	2.7
Albumin subdomain on PVA[c]	1.57	12.4	10.1	6.1	3.8
Fibronectin module on PVA[c]	1.79	14.7	12.5	6.4	4.4
Albumin subdomain on albumin subdomain	3.03	**22.4**	**18.3**	**12.9**	**1.7**
pre-adsorbed on graphite[d,e]		*32.3*	*28.1*	*15.9*	*0.9*
Fibronectin module on albumin subdomain	2.35	**17.7**	**15.7**	**7.3**	**3.6**
pre-adsorbed on graphite[d,e]		*23.4*	*18.8*	*13.9*	*0.9*
Albumin subdomain on fibronectin module	1.28	**12.5**	**10.2**	**6.2**	**3.6**
pre-adsorbed on graphite[d,e]		*19.7*	*16.9*	*9.7*	*2.7*

[a]Refs. [8, 11]
[b]Ref. [9]
[c]Ref. [12]
[d]Present paper
[e]The boldface values apply to the size of the second fragment, and the italic ones to the pre-adsorbed fragment.

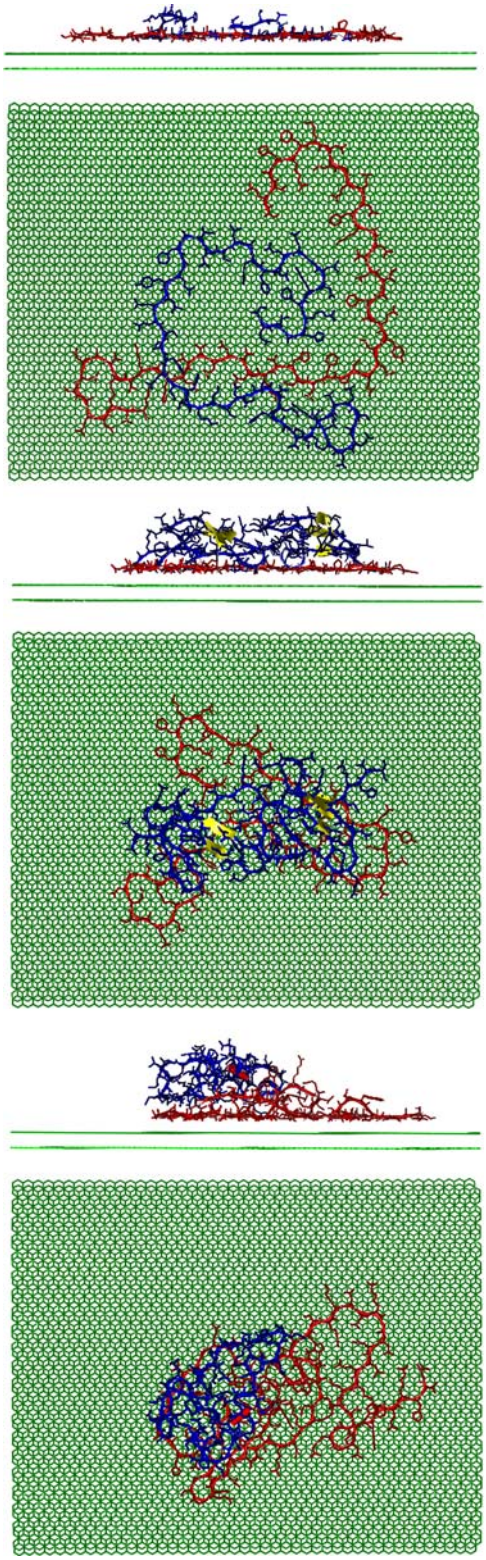

Fig. 3 The final adsorption state obtained for the sequential adsorption of two protein fragments on graphite for the three cases shown in Fig. 1 (in the same order). For each case, the figure shows a lateral view and a view from above (parallel and perpendicular to the surface, respectively)

the principal axes λ_1 and λ_2, obtained by diagonalization of the gyration tensor, reported in Table 1 together with the shortest principal axis, λ_3, perpendicular to the surface. For a comparison, the same Table also reports the radius of gyration and the principal axes of the two protein fragments considered in this paper in their native state, and adsorbed on graphite and on PVA as isolated molecules [7, 8, 11]. Compared to the first, pre-adsorbed albumin subdomain, the second fragment had a smaller radius of gyration of 22.4 Å, and a smaller anisotropy (as shown by the principal axes in Table 1), comparable to that found for the isolated subdomain on hydrophobic graphite (third entry in Table 1), and significantly different from the behavior on hydrophilic PVA (fifth entry in Table 1). The final geometry did correspond again to a monolayer of aminoacids, apart from a minor overlap of the two fragments, with a roughly parallel arrangement of many intra- or inter-molecular strands to form a few intermolecular hydrogen bonds, in addition to the favorable ion-pair, dipolar and dispersive interactions. The interaction energy of the second albumin subdomain with the surface and with the pre-adsorbed one was thus maximized, amounting to 3.03 MJ mol^{-1}, a value rather close to that found for the same isolated fragment on graphite [8, 11] (see Table 1).

A similar trend was shown by the fibronectin module adsorbed on the monolayer of aminoacids formed by a pre-adsorbed albumin subdomain. However, in this case the former fragment could not fully spread because of the disulfide bridges (Fig. 3 at center). Therefore, the interaction of the fibronectin module with graphite was largely hindered, and the pre-adsorbed albumin subdomain showed smaller changes compared to the previous case (eighth entry in Table 1). Consequently, while the center of mass of the backbone of the pre-adsorbed albumin subdomain was again at a distance of 4.4 Å from the graphite surface, such distance amounted to 10.1 Å for the fibronectin module. Moreover, the latter one had a radius of gyration intermediate between the native one (second entry in Table 1) and that realized on graphite as an isolated molecule (fourth entry in Table 1), while the albumin subdomain displayed a relatively minor change, related again with some surface spreading (eight entry in Table 1). As a result, the secondary β-sheet structure of the fibronectin module was partly conserved even in the final adsorption stage. Additionally, the interaction energy was not as large as before, but in any case it amounted to 2.35 MJ mol^{-1}, closer to what found on PVA than on graphite (sixth and the fourth entry in Table 1) due to the interaction with the monolayer of the pre-adsorbed subdomain that makes the graphite surface more hydrophilic.

Finally, the rearrangement of the pre-adsorbed fibronectin module upon further adsorption of a new protein fragment was again found to be hindered by the intramolecular disulfide bridges. Accordingly, the subsequently adsorbed

albumin subdomain could not interact significantly with the graphite surface, with only very few residues in contact with it. Therefore, the albumin subdomain retained the globular shape (Fig. 3 at bottom and last entry in Table 1), with a large number of intramolecular hydrogen bonds and an extensive, though incomplete, denaturation, as shown by the surviving α-helix. Thus, its radius of gyration remained about the same as in the native state (first entry in Table 1), being almost identical to that assumed on hydrophilic PVA (fifth entry in Table 1). Because of the limited surface spreading, the interaction energy with the modified surface was relatively small, amounting only to 1.28 MJ mol^{-1}, close to the value found as an isolated molecule on PVA. Moreover, the pre-adsorbed fibronectin module was weakly affected by the attractive interaction between the albumin subdomain and the graphite surface. Therefore, it only showed some minor flattening and the distance between the center of mass of its backbone from the graphite surface decreased from 5.8 to 5.5 Å, while the analogous distance for the albumin subdomain changed from 20.5 to 13.4 Å.

As a general conclusion, when the pre-adsorbed fragment could not spread the second fragment did not interact with the bare hydrophobic surface of graphite, and therefore it was adsorbed on a modified surface basically in the same way as on a hydrophilic one (such as PVA, in our case), as pointed out earlier, in keeping with experiments [16, 18]. Therefore, the surface behavior of a biomaterial can effectively be controlled and modulated by coating with appropriate polypeptides.

4 Concluding remarks

This paper reports a molecular dynamics study of the sequential adsorption on graphite of two protein fragments of the same or of an unlike nature, and shows that computer simulations can give significant insights and new information about the surface modification of a biomaterial induced by physisorbed oligo- or polypeptides and the subsequent protein adhesion. In particular, the results did confirm a general picture previously obtained with the hydrophilic surface of a glassy polymer, PVA, indicating that in general protein adsorption is weaker on surfaces that are intrinsically hydrophilic, or that are made more hydrophilic through pre-adsorption of appropriate polypeptides. In these cases, the subsequent adsorption was mainly driven by dipolar and dispersion interactions, with few hydrogen bonds being formed between the incoming fragments and the pre-adsorbed ones. In fact, the new protein fragments could show an extensive and often an almost complete denaturation, but they retained the globular shape to optimize their intramolecular interactions (mainly hydrogen bonds, but also ion-pair, dipolar and dispersive interactions). Correspondingly, the pre-adsorbed fragment or the hydrophilic polymer (PVA) did maximize their own intramolecular interactions (or those among the polymer chains in PVA [12]), and therefore they did not display large interactions with new proteins.

Concerning the hydration effects on the adsorption of the protein fragments, it should be pointed out that they can affect the kinetics of adhesion to the surface and also, to some extent, the time-scale of the subsequent rearrangement and/or spreading of the adsorbate, even though the latter process is largely dictated by the interaction within the fragment and with the modified surface. On the other hand, the final geometries obtained in the dielectric medium correspond in general to the thermodynamically preferred conformation, apart from some possible readjustments of the side groups in explicit water [7–9, 12]. Unfortunately, in the present case due to the very large size of the systems no MD runs in explicit water could be performed, and therefore the hydration of the adsorbed protein fragments could not be fully assessed. However, Fig. 3 shows that even though some fragments may keep a globular shape, possibly with some residual secondary structure, and one side of the fragments is not solvated being in contact with the modified surface, the exposed sides can be efficiently hydrated, forming a large number of hydrogen bonds with water. Therefore, the hydration of the aminoacids comprised within the α-helices and the β-sheets of the native secondary structure is somewhat enhanced, a feature that may largely compensate the net loss of hydration of the outer residues in contact with the surface. Accordingly, the total number of hydrogen bonds the fragments form with water undergoes a relatively small decrease upon adsorption [7, 11, 12], and thus the change in solvation energy can be estimated to amount to a few percent only of the total interaction energy. However, as a word of caution it should be noted that this conclusion might be unwarranted with hard proteins showing minor or no denaturation at all and/or charged surfaces, which may therefore require a detailed analysis of solvation effects.

Finally, it should be added that at present the atomistic computer simulations are not yet capable to describe the replacement of an adsorbed protein or polypeptide in general by a second one more affine to the surface due to the huge computer resources that would be required, both in terms of the number of atoms that must be explicitly included in the simulation, and in terms of the length of the MD runs modeling a relatively slow process.

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