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Molecular dynamics simulations of the adsorption of proteins on clay mineral surfaces

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Dedicated to Professor Lou Allinger in recognition of his significant contributions to the field of molecular mechanics

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Abstract

Some initial results of molecular dynamics simulations of the adsorption of proteins on clay mineral surfaces are being reported. Specifically, the interactions of pyrophyllite surfaces with crambin, rubredoxin, and several oligopeptides were investigated. It is found that clay mineral surfaces can have a denaturing effect on adsorbed proteins for two reasons: (1) they are dehydrating agents, because they perturb the random environment of water molecules that globular proteins need to maintain their native structure; and (2) clay surfaces can establish non-bonded interactions with proteins which compete effectively with the interactions inside a peptide chain. The changes in secondary and tertiary protein structure induced by adsorption to pristine surfaces, or surfaces coated with water, lead to backbone torsions away from the most populated regions of ϕ,ψ-space, to regions which are not frequently populated in unperturbed proteins. β- and α_R-conformations, specifically, are not stable on pyrophyllite but undergo transitions, with some preference, to an area close to C_7^{eq}. Because of the size of the adsorbed systems, unit cells with adsorbed peptides may be distorted, bulging at the site of adsorption, and displaying a continuously varying interlayer space between the empty parts and those that are occupied by an adsorbate. As a result, warped, or S-shaped basal planes are found.

Keywords: Molecular dynamics; Amino acids; Clay mineral surface

1. Introduction

Nearly all of the nitrogen in most soils is organically bound and can, to a large extent, be released hydrolytically in the form of α-amino acids [1]. From this observation derives the hypothesis that peptides and proteins represent an important component of the organic matter in soils, much of which is believed to be attached to soil colloids [1] and thus plays an important role in the mobility and transport of organic agrochemicals in soils. For this reason, since the early work by Mattson [2] and Ensminger and Gieseking [3–5], the physical properties of clay/protein complexes have been investigated in countless studies [1]; numerous more recent investigations [6–21] testify to the continued importance of this area of research.

In some of the current work there is particular interest in the properties of enzymes adsorbed on clay mineral surfaces [15–21] and in practical

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applications of clay/protein complexes, such as in specialized filtration processes [9], in purification protocols devised for processing high-value proteins [10], or in dealing with the problem of pesticide contaminated groundwater, where the soil fraction and its complexed organic components have been shown [6] to be responsible for the sorption of many soil-applied pesticides.

As a further example of the utility of clay/protein complexes, it has recently been proposed [8,11,12] to use clays as a means for remediating contaminated animal feed. A significant percentage of the world grain and oilseed supplies is contaminated with toxic proteins. Several researchers [8,11,12] have investigated the preference of protoxin and toxin proteins for binding on different clay mineral surfaces. It is now believed [7] that, as feed additives, clay minerals can reduce the bioavailability of such contaminants in the gastrointestinal tract by adsorption, and protect the animal species from their deleterious effects.

In spite of a large number of experimental investigations [1–21] with a variety of techniques, such as X-ray diffraction, electron microscopy, electrophoretic mobility and various spectroscopic techniques; the mechanisms of adsorption and binding of proteins on clay minerals are still only partially understood. The recent development of an empirical force field for molecular dynamics (MD) simulations of aluminosilicates [22] now makes it possible to take a new look at how proteins interact with clays. For the current paper, MD simulations were performed of various clay/peptide systems, choosing the dioctahedral, unsubstituted, 2:1-type clay mineral pyrophyllite (half unit cell composition $Si_4Al_2O_{10}(OH)_2$) as a representative mineral. Interactions of several peptides and proteins with pristine pyrophyllite surfaces, and mineral surfaces coated with water, are described below. These results are preliminary in the sense that the chosen systems involve vacuum states, or near-vacuum states, which are not really encountered in soils. However, the chosen systems represent a logical starting point for a long-term project of this kind and the calculations are able to illustrate how clay/water–protein interactions can compete with intra-molecular protein non-bonded interactions.

2. Computational procedures

The clay mineral force field by Teppen et al. [22] was used in all calculations together with the MSI/Discover molecular modeling software suite, version 4.0.0 [23]. The force field parameters chosen were developed specifically for dioctahedral clays [22] and enable full dynamics simulations in which both the mineral surface and the adsorbed phase are dynamic. In the recent past simulations of this kind have successfully reproduced various clay mineral crystal structures, and simulations of the swelling of Na- and Ca-beidellite clays at many different water contents yielded lattice d_{001}-interlayer separations within experimentally observed ranges [22]. In a study of the sorption of trichloroethene [24] it was found that the clay mineral force field parameters can be successfully used in conjunction with existing parameters established for organic molecules.

Several series of simulations were performed for the current paper. In each run a sufficient number of unit cells of pyrophyllite were fused together to produce a surface that was large enough to support the adsorption of the proteins crambin, rubredoxin, and various conformations of the model oligopeptides, *N*-acetyl deca-alanine amide and *N*-acetyl icosa-alanine amide.

For example, to study the sorption of crambin in its native state, an $Si_{480}Al_{240}O_{1200}(OH)_{240}$ $(10 \times 6 \times 1)$ supercell of neutral, idealized 2:1 clay was constructed using the crystal structure of pyrophyllite by Lee and Guggenheim [25]. For the sorption of an artificially denatured, stretched crambin (see below) an $Si_{704}Al_{352}O_{1760}(OH)_{352}$ $(4 \times 22 \times 1)$ supercell was constructed, again using the crystal structure of pyrophyllite [25]. With no water added to the interlayer space, the latter contained 4159 atoms and the former 3039.

Crambin was also studied in a water box with some 920 water molecules, without a clay surface, to test the maintenance of its globular structure in the existing force field, forming a system with 3420 atoms. In all calculations involving crambin, the starting coordinates were taken from the crystal structure [26]. Three sodium ions were then added to neutralize protein charges. The number of water molecules resulted from standard soaking procedures implemented in the MSI software.

In the case of rubredoxin, the crystal structure of pyrophyllite [25] was used to construct an $(8 \times 5 \times 1)$ $Si_{320}Al_{160}O_{800}(OH)_{160}$ supercell. For the starting

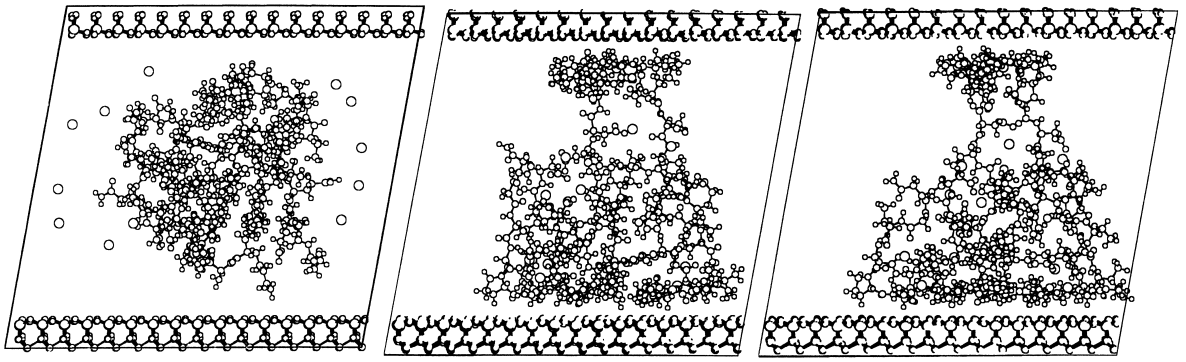

Fig. 1. The adsorption of rubredoxin in the interlayer space of dry pyrophyllite. The crystal structure of rubredoxin (left) was taken from Ref. [27]. The structures of rubredoxin after 5 and 10 ps of NVT dynamics in the pyrophyllite interlayer space are shown in the middle and on the right, respectively.

geometry of rubredoxin, the crystal structure was taken from the Brookhaven protein data bank [27] and 12 sodium ions were added to neutralize the molecule. In the case of rubredoxin, sorption was modeled both on dry clay surfaces and in the presence of water. For the latter, 480 water molecules were added to the $8 \times 5 \times 1$ supercell together with the protein, which was sufficient to produce a monoloayer on each surface. The dry clay/rubredoxin complex contained 2393 atoms; the clay/rubredoxin/water system, 3833 atoms.

All simulations with crambin and rubredoxin were started with an artificially enlarged interlayer space, expanding the natural spacing to 40–70 Å, in order to create a volume that was sufficient to install the various peptides in their undistorted crystal structures. All initial MD simulations were then performed for 10–25 ps under NVT conditions (constant mass, volume and temperature) to allow the protein to interact freely with the clay surface. In some cases the calculations were then continued imposing NPT conditions (constant mass, pressure and temperature).

A series of simulations on smaller, dry, supercells were performed with various conformations of the model peptides *N*-acetyl deca-alanine amide (deca-ala) and *N*-acetyl icosa-alanine amide (icosa-ala) in order to study how different peptide conformations interact with clay surfaces. Three conformations were chosen for this part of the investigation, including a β-extended structure, an α-helical structure, and a β-bend, all of which represent conformational energy minima found in previous ab initio studies [28] for oligopeptides of this kind.

All simulations were performed with a stepsize of 0.5 fs, using periodic boundary conditions. The Ewald summation scheme available in the MSI software was used for both electrostatic and non-bonded interactions.

3. Results and discussion

The results of the calculations are presented in Figs. (1–7). Figs. 1 and 2 show the evolution of interactions of rubredoxin on dry and wetted clay surfaces, respectively, in NVT simulations. Fig. 3 shows the structure of rubredoxin in the pyrophyllite interlayer space after 30 ps of NPT dynamics. Fig. 4 shows the corresponding MD results obtained for crambin. Fig. 5 shows distributions of the ϕ(N–C(α)) and ψ(C(α)–C′) protein backbone torsional angles of rubredoxin and crambin in their respective native (crystal) states and after sorption. Fig. 6 shows the evolution of the ϕ(N–C(α)) and ψ(C(α)–C′) protein backbone torsional angles of deca–ala and icosa–ala from initial positions in the β-extended and α_R-helical states in the free molecules to the values attained on the clay surface. Fig. 7 shows the warping of an elongated supercell that resulted when a denatured, i.e. stretched, crambin molecule was sorbed on the crystal surface. The stretched form of crambin was produced from the crystal structure by forcing all of its backbone torsions, by hand, into the β-region,

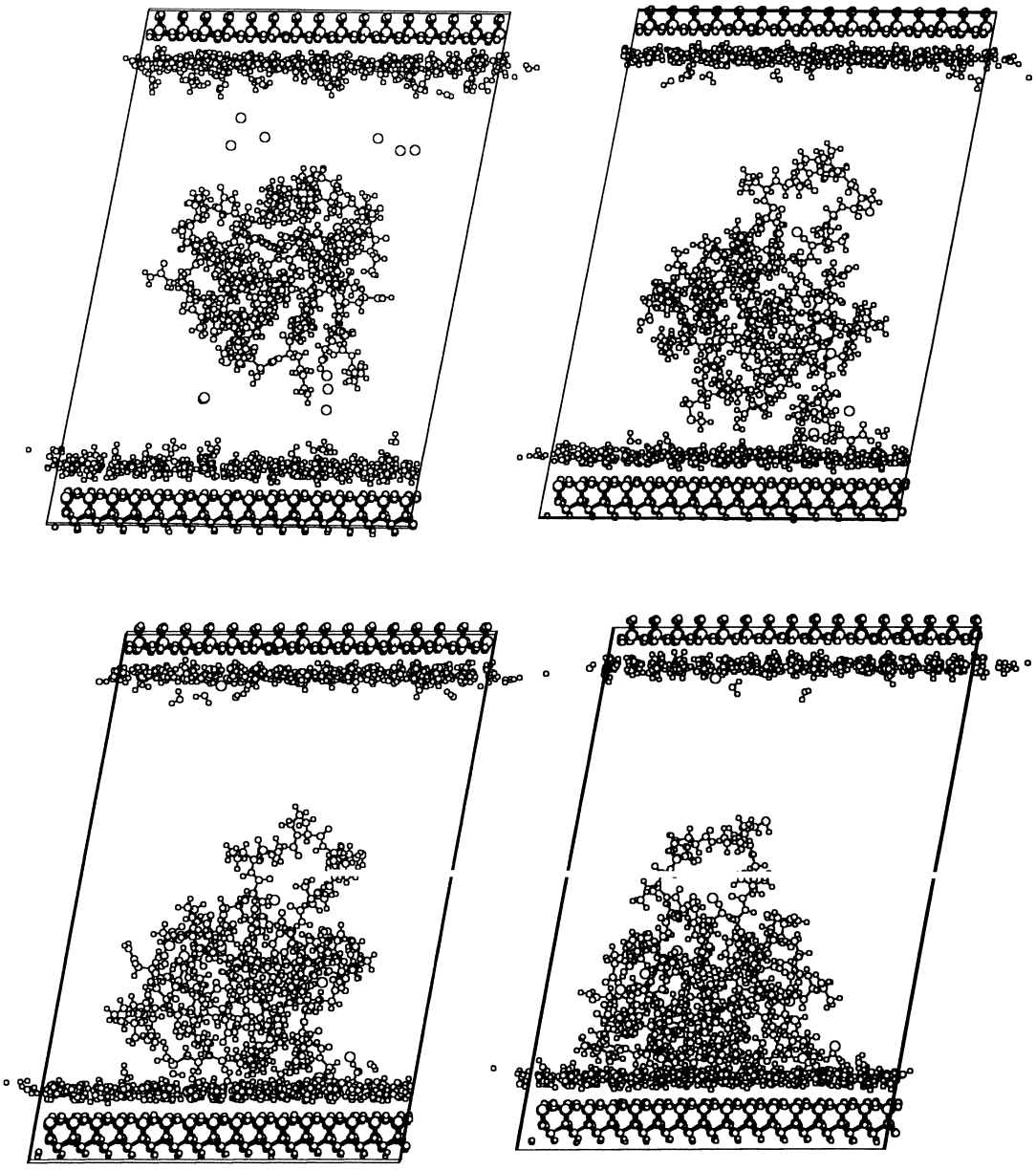

Fig. 2. The adsorption of rubredoxin on a monolayer of water in the interlayer space of pyrophyllite. Top left: rubredoxin in its crystal structure [27]. Top right, bottom left, and bottom right: the structures of rubredoxin after 5, 10, and 25 ps, respectively, of NVT dynamics in the pyrophyllite/water interlayer space.

regardless of the enormous strain introduced in this way.

Expanded 2:1 type layer silicate minerals are ideal model systems for investigations of this kind because they are able to intercalate proteins of any type. When charged clay minerals are chosen as substrates, protein interlayer penetration can, under certain conditions, involve cation exchange mechanisms [1]. In order not to complicate the matter, the current, initial study was performed with the neutral 2:1 clay

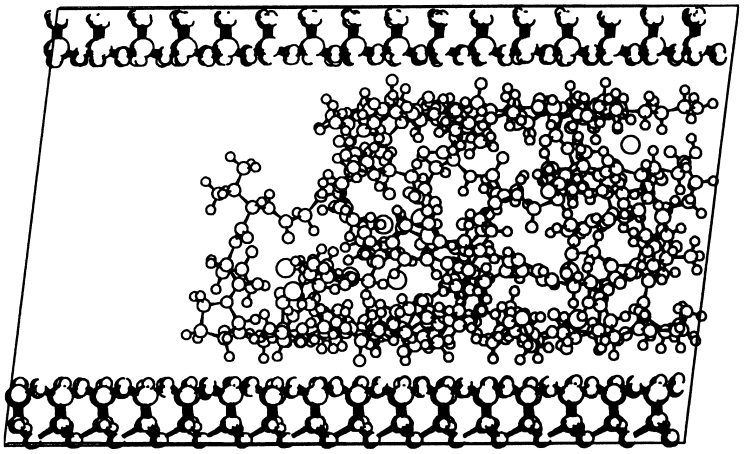

Fig. 3. The structure of rubredoxin in the pyrophyllite interlayer space after 30 ps of NPT dynamics.

pyrophyllite and with neutral proteins in which all protein charges were compensated with Na^+ ions. The latter condition also entails that pH effects on macromolecular conformation are not considered, even though there is experimental evidence [18,20,21] demonstrating their importance. Questions of this kind, as well as studies with more realistic distributions of water are being addressed in continuing investigations.

3.1. The denaturization of proteins by adsorption on mineral surfaces

On the basis of various experimental observations [1,16,20,21] it is possible to conclude that adsorption on clay mineral surfaces induces alterations in the native conformations of proteins, affecting tertiary protein structure (i.e. the folding of the extended parts of a peptide chain) as well as secondary structure (i.e. the ϕ_i, ψ_i values of individual residues). This hypothesis is entirely consistent with the results of our simulations.

In Fig. 1 it is seen that, when rubredoxin, in its native state, is brought close to a pristine silicate surface, within a few picoseconds its outer residues begin to unwind, moving away from the peptide chain, reaching for the mineral surface and spreading out over it. The same result is obtained for a wetted clay (Fig. 2), but in this case the non-bonded interactions within the peptide are replaced with interactions involving the monolayer of water deposited on the mineral surface. Similar aspects of crambin are shown in Fig. 4. In contrast to this process, crambin maintained its native globular shape in NPT test simulations in a box of randomly placed water molecules, when the MSI force field [23] was applied. Thus, part of the effect of a clay surface on a protein is indirect; i.e. due to the fact that the random water environment is perturbed such that a globular protein needs to maintain its structure.

It has been suggested [1] that, when sorbing to clay minerals, some proteins may collapse mainly to an extended β-structure. Fig. 5 is an analysis of the ϕ_i, ψ_i torsional angles of rubredoxin and crambin before and after adsorption on pyrophyllite. It is seen from Fig. 5 that, in their native states, the ϕ_i, ψ_i-values are found mainly in those regions of ϕ, ψ-space that are usually highly populated in unperturbed proteins; i.e. in the right-handed α_R-helical state ($\phi = -75°$, $\psi = -45°$), the bridge region ($\phi = -90°$, $\psi = 0°$), and the β region ($\phi = -165°$, $\psi = +165°$). In contrast (Fig. 5), after adsorption the ϕ_i, ψ_i-torsional angles cover the entire ϕ, ψ-conformational space, including regions which are normally sparsely populated. No preference for a single region of ϕ, ψ-space, such as the β-region, is apparent in the values presented in Fig. 5.

The drastic changes in torsional angles indicated by Fig. 5 are the result of systems interacting in a vacuum state that will hardly be found in real soils. It is expected that more realistic water distributions will mitigate the denaturing effects that mineral surfaces

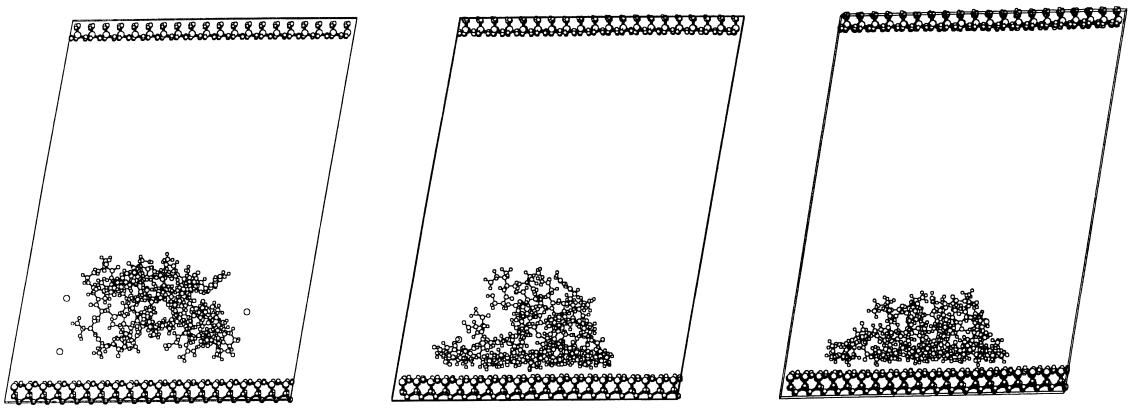

Fig. 4. The adsorption of crambin in the dry interlayer space of pyrophyllite. The crystal structure of crambin was taken from Ref. [26]. The structures of crambin after 5 and 15 ps of NVT dynamics in the pyrophyllite interlayer space are shown in the middle and on the right.

have on peptides and proteins. The current results are nevertheless interesting to consider, because they illustrate to what extent the interactions between a protein and a mineral surface can effectively overpower non-bonded intramolecular protein interactions.

Further information on the distribution of torsional angles in adsorbed peptides is obtained from Fig. 6.

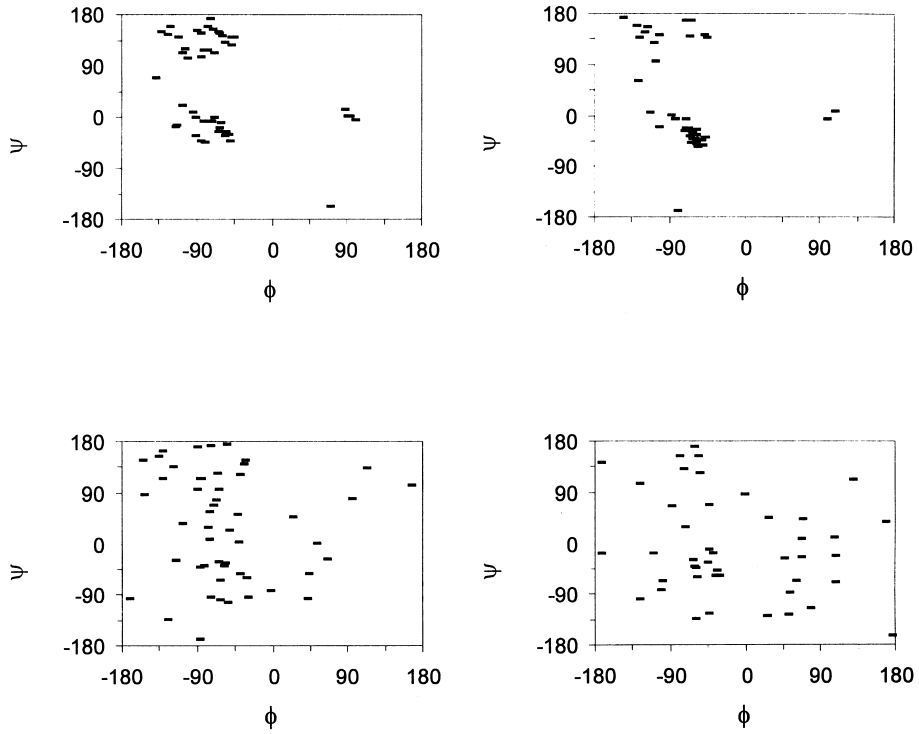

Fig. 5. The distributions of ϕ, ψ-torsional angles of rubredoxin in the crystal structure [27] (top left) and after 10 ps of NVT adsorption to a pyrophyllite/water surface (bottom left). The corresponding distributions for crambin are shown on the right.

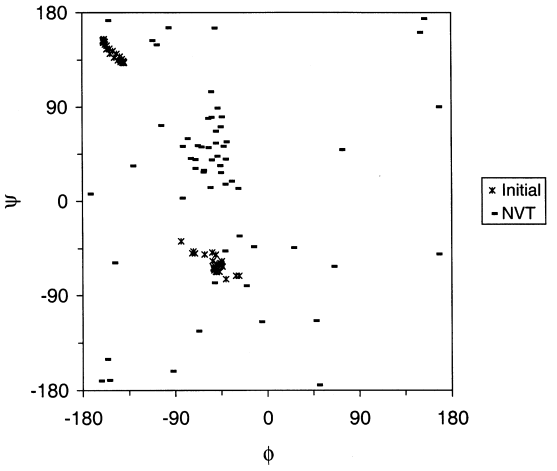

Fig. 6. The distributions of ϕ, ψ-torsional angles in the model peptides *N*-acetyl deca-alanine amide and *N*-acetyl icosa-alanine amide after, respectively, 15 and 10 ps of NVT adsorption to a surface of pyrophyllite. The initial torsional angles were all either in the β- or α_R-helical region of ϕ, ψ-space and are indicated by the cross-marks. The positions resulting from NVT simulations are given by the dash-marks.

When the β-extended conformations and α_R-helical conformations of deca–ala and icosa–ala, obtained from NVT simulations of the gas phase molecules with the MSI [23] force field, were adsorbed to pyrophyllite, neither of them was stable on the mineral surface but the torsional angles changed spontaneously with some preference into an area near the C_7^{eq} region, which is normally situated at approximately $\phi = -80°$, and $\psi = +70°$. It is seen from Fig. 6 that roughly one half of the torsional angles which were initially either α_R or β are clustered close to C_7^{eq}. The latter corresponds to the most stable conformation of oligopeptides, but it is relatively rarely populated in proteins.

Adsorption onto clay mineral surfaces does not necessarily lead to the denaturing of the entire native structure of a protein [1]. Modification of tertiary structure can be expected to be restricted to those parts of a molecule that are in direct contact with the mineral surface. For this reason, enzymes adsorbed on mineral surfaces may retain their activity [1,15–21], and if inhibited, this effect may be due to other factors than structural changes. For the two proteins chosen for this study, the conformational changes following adsorption are total, involving every residue, because they are so small. For larger systems, with a molecular mass of tens of thousands or even hundreds of thousands of Daltons, it is reasonable to assume that denaturing by adsorption will be partial, involving only those parts that are in contact with the attracting surface. Because of their size, such systems cannot currently be simulated using the dynamics techniques chosen for this study.

The changes in torsional angles incurred when a protein is adsorbed to a mineral surface depend on how non-bonded interactions can be established most effectively. Inspecting the closest non-bonded distances that exist between either crambin or rubredoxin and the pyrophyllite surface, one finds that hydrogen bonding, involving N–H and O–H groups in the protein and basal oxygen atoms in the clay, is an important factor in stabilizing the clay/protein complexes. Numerous bonds of this type are found in the 2.2–3.0 Å range. Apart from this, a large number of non-bonded distances are found in an attractive part of the van der Waals potential at ~3.5–4.0 Å.

3.2. Bent clays: the warping of mineral basal planes due to a variable interlayer space

In addition to the structural aspects of adsorbed proteins, the interlayer spacing of the clay minerals involved is also of great interest. For the sorption of lysozyme on montmorillonite, interlayer separations of ~15 and ~39 Å were measured [1] suggestive of monolayers and bilayers of lysozyme, respectively. Because of the artificial water distribution, the current calculations cannot be directly compared with existing experimental data. Nevertheless, some NPT simulations were performed on the systems described above to get an estimate of the range of separations that can be expected.

For example, for the NPT simulation of rubredoxin on dry pyrophyllite (Fig. 3) an interlayer spacing of 26.9 Å was obtained. When crambin was denatured into a fully extended chain and into a second, U-shaped extended conformation, NPT simulations led to an interlayer separation of 14.2 Å at the site of adsorption. Thus, depending on the molecule adsorbed and the conditions of adsorption, a large range of interlayer separations can be expected for clay/protein complexes.

In the case of the artificially extended crambin, the simulations yielded an additional, interesting effect (Fig. 7), involving a variable interlayer space. To create a surface that was long enough for the extended form of crambin, an elongated $4 \times 22 \times 1$ supercell was constructed and the protein aligned with the long axis on the surface. However, during the dynamics simulations, the elongated molecule rotated away from the initial orientation to that shown in Fig. 7. From this motion, a unit cell resulted that was empty in large parts and occupied by an adsorbed protein only in its center. This arrangement led to a warped or bent clay structure in which the interlayer space was different in the empty part of the supercell compared with the occupied part; that is, the interlayer space varied continuously from 14.2 Å at the center of the protein to ~9.5 Å at the empty ends of the cell.

It is possible that this result is an artifact of the calculations due to the vacuum conditions applied. There are no reports on abnormalities in the X-ray crystallographic data used to measure clay–protein interlayer spacings [1,21]. At the same time, we obtained similar results in the simulations of the adsorption of deca–ala and icosa–ala. In each case the dynamics simulations of the fully periodic systems produced unit cells which were bulging at the site of adsorption.

The absence of experimental reports on such phenomena in the case of protein adsorption may be due to the fact that the warping may occur merely at especially low loadings or under conditions, which have not yet been explored experimentally. In view of these considerations it seems worthwhile to further investigate this matter with new simulations and experiments.

4. Conclusions

The results presented above illustrate that clay mineral surfaces can have a strong denaturing effect on proteins. The adsorption of a protein on a silicate surface can induce drastic changes in its secondary and tertiary structure, leading to backbone torsions

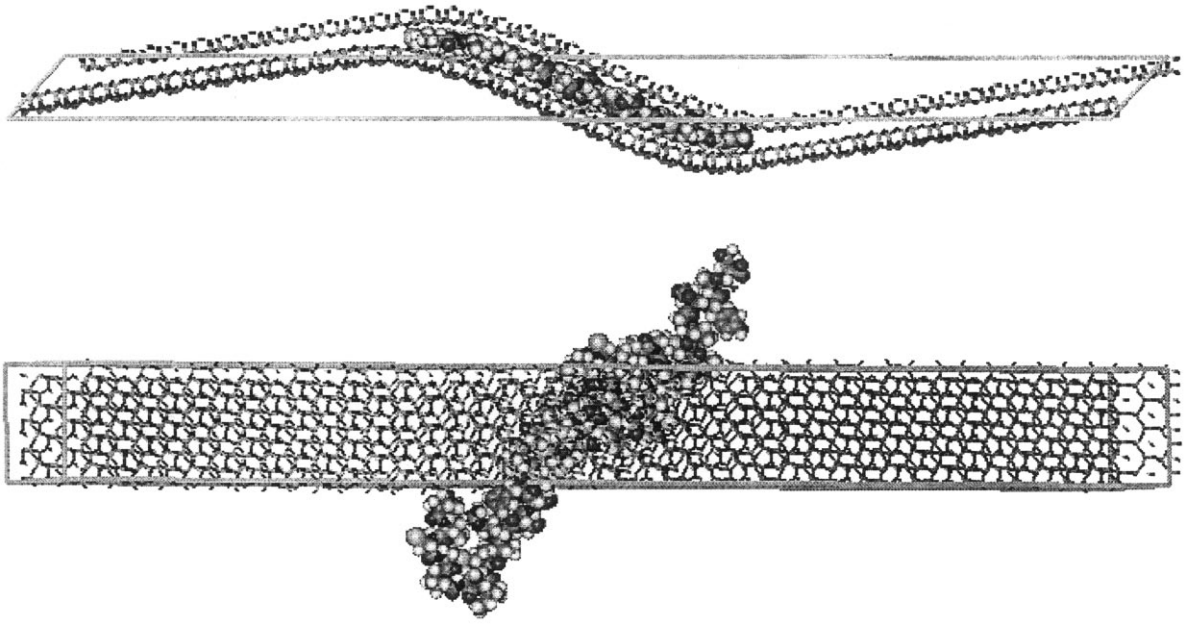

Fig. 7. Results of MD simulations of an artificially extended (denatured) crambin (see text) on pyrophyllite. The stretched form of crambin was produced from the crystal structure by forcing all of its backbone torsions by hand into the β-region. Initially, the extended form of crambin was aligned on a pyrophyllite surface along the long axis of a $4 \times 22 \times 1$ supercell. During the dynamics simulations the molecule rotated away from the initial orientation to that shown. The interlayer space of the resulting unit cell varies from ~9.6 Å, at the empty ends of the cell to 14.2 Å at the center of adsorption. Variations in this parameter led to the warped or bent clay structure displayed in the figure.

in regions of ϕ, ψ-space which are sparsely populated in unperturbed proteins. β- and α_R-conformations of peptides on pristine pyrophyllite, specifically, are not stable but spontaneously undergo conformational transitions, with some preference into an area close to C_7^{eq}.

The denaturing effects of clay minerals on proteins involve two phenomena:

1. Clay surfaces are dehydrating agents. By ordering intercalated water molecules in layers along the mineral surface, the random environment of solvent molecules is perturbed in a way that globular proteins need to maintain their native structure.
2. Pristine clay surfaces can directly establish non-bonded interactions with proteins that effectively compete with the interactions inside the peptide chains. Among these interactions, hydrogen bonding between basal oxygen in the clay and protein N–H and O–H groups is particularly important.

We expect that, in systems with more realistic water distributions than those implemented above, the changes in torsional angles effected by adsorption will be less drastic than those found here. Because of the large number of molecules involved, the corresponding calculations are rather time consuming, but they are part of ongoing research in our group.

A special effect may occur for intercalated peptides or proteins when the surface loading is low. In that case the unit cell may be distorted, bulging at the site of adsorption, and the interlayer space varies continuously between the empty parts of a cell and those that are occupied by an adsorbate. As a consequence of this interlayer variation warped, or S-shaped basal planes may result.

Acknowledgements

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