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The thickness of 18-MEA on an ultra-high-sulfur protein surface by molecular modeling

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Synopsis

The use of computational chemistry techniques via molecular modeling software provides additional support to the hair surface model by Negri *et al.* (1) and refines the thickness of the 18-methyl eicosanoic acid (18-MEA) lipid layer attached by thioester linkages to an ultra-high-sulfur protein (UHSP) at 1.08 ± 0.2 nm. This value compares favorably to the thickness of that same layer from X-ray photoelectron spectroscopy (XPS) measurements by Ward *et al.* (2) at 1.00 ± 0.5 nm on Soxhlet-extracted wool. The model clarifies that the results of Ward *et al.* via XPS are not an artifact of high vacuum (3), but due to relaxation of the 18-MEA structure onto the wool protein backbone as suggested by Zahn *et al.* (4). In this molecular model, 18-MEA is attached to beta sheets of an UHSP via thioester linkages as suggested by Negri *et al.* in their 1993 study (15) and by earlier work by Evans *et al.* (5). The beta sheets of this model provide an intersheet spacing of 0.7 nm and a beta sheet density of 1.42 g/cm^3 compared with Allworden membrane fractions that varied from 1.39 to 1.54 g/cm^3 (6).

INTRODUCTION

It is of utmost practical importance to cosmetic science that we understand the true "virgin" surface of hair fibers so that we can determine changes to that surface by cosmetic treatments and be able to reconstruct that surface. This paper is part of an attempt to help clarify the actual structure of the virgin hair fiber surface.

The surface of mammalian hairs is covered with a thin covalently bound lipid layer of 18-MEA that is bonded to a proteinaceous cell membrane called epicuticle (1,7) (see Figure 1). Negri *et al.* (1) have shown that the outer surface of hair fibers consists of about 75% of a heavily cross-linked protein and about 25% of a fatty acid that is predominantly 18-MEA. These scientists have proposed a model wherein the fatty acid layer (attached lipid layer) is connected to the underlying fibrous protein layer through thioester linkages involving the cysteine residues of the underlying epicuticle proteins (1,4) (see Figure 1). These scientists concluded that the attachment of 18-MEA is through thioester linkages because chlorine water would not remove this lipid layer if

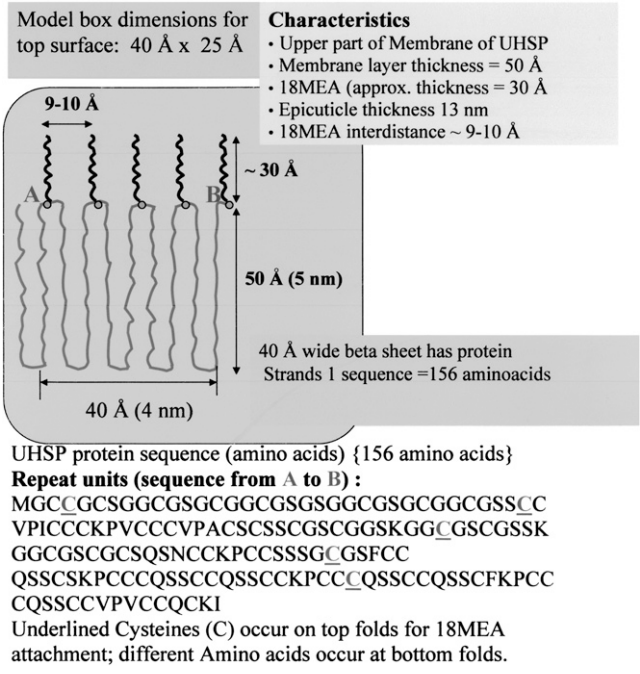

Figure 1. Schematic representation of the beta sheets setup for the keratin fiber surface molecular model.

it were attached through an ester or amide linkage; however, chlorine water will readily cleave thioester bonds.

Jones and Rivett (7) have stated that secondary ion mass spectrometry (SIMS) and XPS "indicate the surface of wool fibers is almost exclusively hydrocarbon" consisting of 18-MEA and free lipids (solvent-extractable lipids), as described later in this paper. The protein membrane (epicuticle) is approximately 13-nm thick (8).

Since the attachment of 18-methyl eicosanoic acid to keratin fibers is through thioester linkages and the cell membrane protein is cross-linked through cysteine linkages, the 18-MEA must be attached to a UHSP (1). Concerns have been expressed in the literature (3,4) about the actual thickness of the 18-MEA layer, based on the fact that Ward *et al*. (2) used XPS to estimate the thickness of this surface lipid layer at 1.0 ± 0.5 nm, but this estimate is less than half of the length of a fully stretched 18-MEA molecule as calculated by Jones and Rivett (7). Zahn *et al.* (4) suggested that the difference could be explained by 18-MEA folding back on itself and onto the wool surface, while Peet *et al.* (3) suggested that the measurement by Ward *et al*. was likely an artifact created by the high vacuum conditions of XPS.

To resolve these issues about the thickness of 18-MEA attached by thioester linkages to a UHSP surface and to test the Negri *et al*. model and learn more about the keratin fiber surface, we employed molecular modeling software to construct and test a skeleton model (Figure 1) of 18-MEA attached by thioester linkages to beta sheets of a keratin-associated protein-5 (KAP-5) from a family of UHSP proteins found in the cuticle of keratin fibers.

EXPERIMENTAL

CONSTRUCTION OF AN EPICUTICLE MOLECULAR MODEL

The software used in this work was Cerius[2] modeling software for molecular simulation by Accelrys, Inc. Based on the work of Negri *et al.* (1), we used the beta sheet configuration for the UHSP in the uppermost part of the epicuticle. The conformation of protein in a model beta sheet, containing two folds (top and bottom) was taken from the Protein Data Bank (PDB) for polyalanine. This strand was reproduced in the molecular modeling graphical package, and the multiple strands thus created were successively connected to form a beta sheet of eight strands with four top and bottom folds in the periodic cell, as shown schematically in Figure 2. The number of strands per periodic cell was such that exactly one polymeric UHSP sequence was fitted (see Figure 1).

The top folds contained cysteine amino acid residues, which are essential for the thioester linkages with 18-MEA. Although the exact protein that 18-MEA is attached to is not

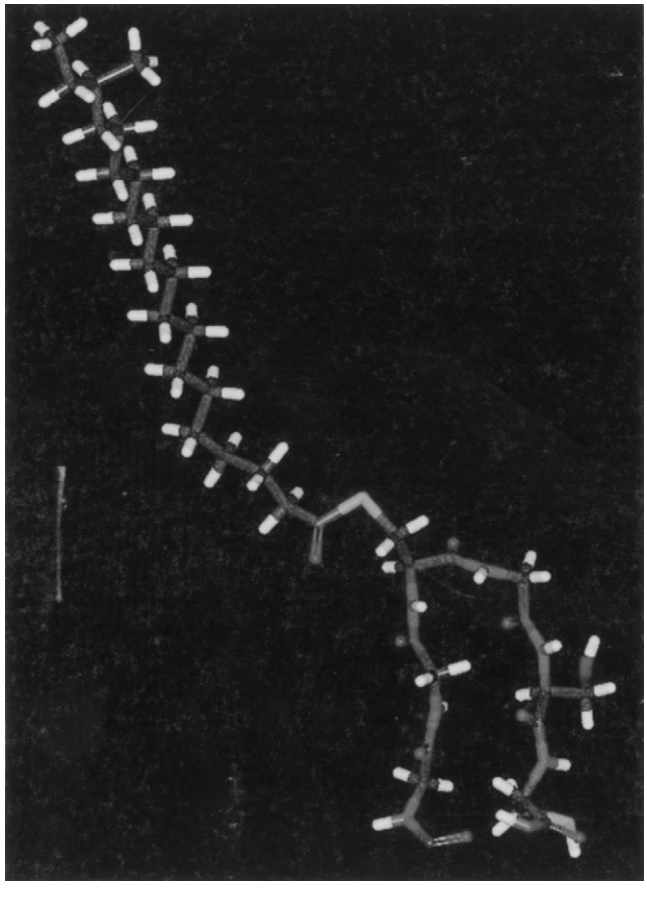

Figure 2. Stick model of 18-methyl eicosanoic acid attached to a protein backbone at the angle indicated by the modeling software. The sticks, in varying shades of gray, represent oxygen, hydrogen, nitrogen, the backbone carbons, and the cysteine sulfur group.

known at this time, the amino acid sequence used here is shown in Figure 1 and is for the Yahagi UHSP (9), a protein from the KAP-5 family, a protein family that has been shown to be in the outermost A-layer of wool fiber by Bringans *et al.* (10). Rogers and Koike (11) describe its presence in the exocuticle and A-layer of human hair. Once the beta sheet was created using the model protein sequence from polyalanine, the amino acid chemistry was changed to fit the amino acid sequence of the Yahagi UHSP into the model. The fully constructed sheet was then reproduced (duplicated) to several other sheets and these sheets were stacked parallel to each other in the Y direction (the direction of stacking is perpendicular to the original beta sheet), as shown in Figure 2. Each periodic simulation model cell contained three beta sheets. The overall density of the system (per periodic simulation cell) was set at a value of 1.47 gm/cm^3, which is within the range provided by the Allworden membrane isolated by Allen *et al.* (6) of 1.39 to 1.54 gm/cm^3 and close to that of whole fiber, 1.32 gm/cm^3 (12). The length of each strand (top fold to bottom fold, Z direction) was nicely fitted to 50 Å. The thickness of the epicuticle as measured by Swift and Smith (8) is 13 nm. Since (at this time) we do not use other proteins in our model, we constructed only the uppermost portion of the epicuticle, using only the UHSP as a single layer of 5 nm. We also have a constraint of not exceeding 10,000 atoms in our modeling software. This issue was important in determining the number of sheets and the length of the strands in the beta sheet of our model.

The initial model showed numerous interatomic steric hindrances from the amino acid side groups within the single strands and in-between strands in different neighboring or adjacent strands in different folds. To alleviate these non-bonded interactions, the side groups were allowed to move and relax in an energy-minimization procedure, keeping the main backbones of the strands fixed in space. Backbone atoms of only three amino acid residues on each of the top and bottom folds were allowed to fully relax and move during the molecular simulation (energy minimization and, later on, the molecular dynamics (MD) simulations with the 18-MEA attached). This procedure allowed relaxation of realistic arrangements while maintaining the essential backbone-ordered structure of the beta sheet.

All bonded and non-bonded interatomic interactions were calculated using DreidingII force field, including van der Waals and electrostatic interactions (wherein hydrogen bonding is included). The dielectric constant was set to a value of 1.0 (because at present we do not have the correct value for hair protein systems). Bonded terms such as bond stretching and bond-angle bending, as well as torsion terms, were included. The simulation box cells (each structure thus called) were subjected to an initial energy minimization of 50 steps with the steepest descent method and 750 steps with the conjugate gradient method. Interatomic interactions during energy minimization were calculated until a radius of 13–15 Å (cut-in to cut-off) and interactions were terminated at a cut-off radius using a fifth order spline function. Convergence of the minimization was noted by total potential energy gradients being less than 0.1 kcal/mol. The energy-minimized structure was then subjected to MD in the NVT ensemble at a temperature of 300 K (room temperature). Interatomic interactions during MD simulations were evaluated within a radius of 9 Å (terminated using a cut-off radius of 11 Å with spline function). A total of 120 ps of MD simulation were run to relax the 18-MEA chains. The final configurations of the 18-MEA chains of virgin hair were coiled. This structure is further discussed in the Results section.

Having thus obtained the well-relaxed hair protein model, the 18-MEA molecules were chemically attached to the cysteine groups on the top folds of each beta sheet. The initial conformations of the 18-MEA molecules were all-trans (straight and perpendicular to the

top surface plane XY of the beta sheet folds). The 18-MEA molecules were then allowed to move and relax using an NVT ensemble MD simulation for a 120-ps time period. The result of this simulation, followed by energy minimization, was the well-relaxed virgin hair surface, and is shown in molecular graphics views in Figures 3–5. The snapshot shown in Figure 4 is at the end of 120 ps of MD simulation wherein the 18-MEA chains are quite coiled conformationally and packed nicely into a film or monolayer without any significant van der Waals gaps. The final thickness of the 18-MEA layer came to be 1.08 nm.

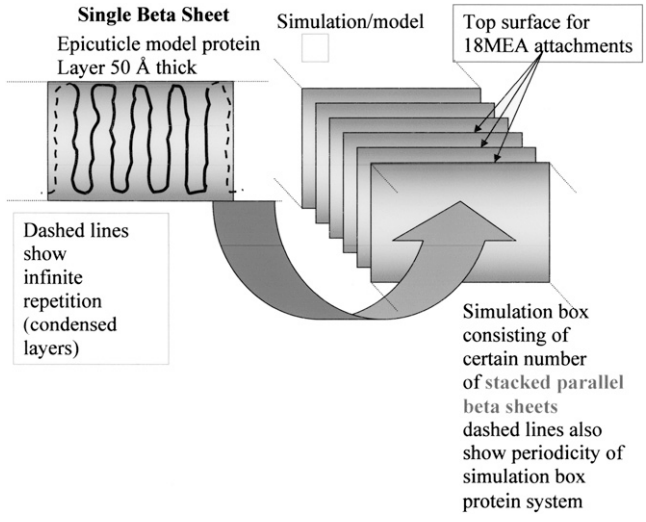

Figure 3. Schematic representation of the beta sheets setup of the epicuticle molecular model.

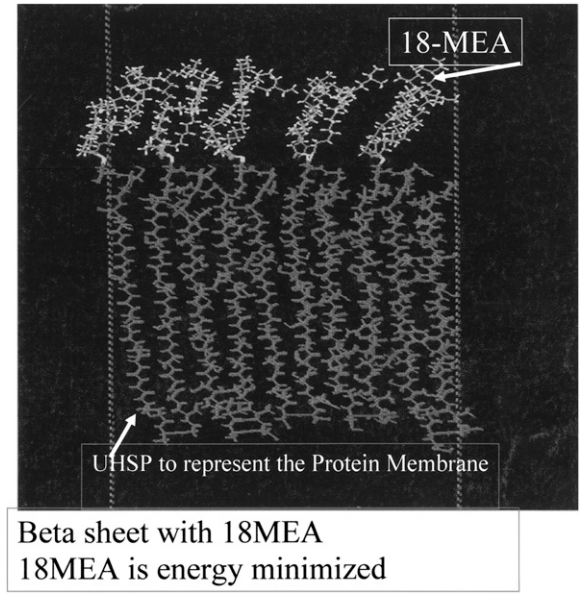

Figure 4. Energy-minimized structure of 18-MEA attached to a beta sheet of a KAP-5 protein to represent the lipid layer attached to the backbone of the cuticle cell membrane of a keratin fiber.

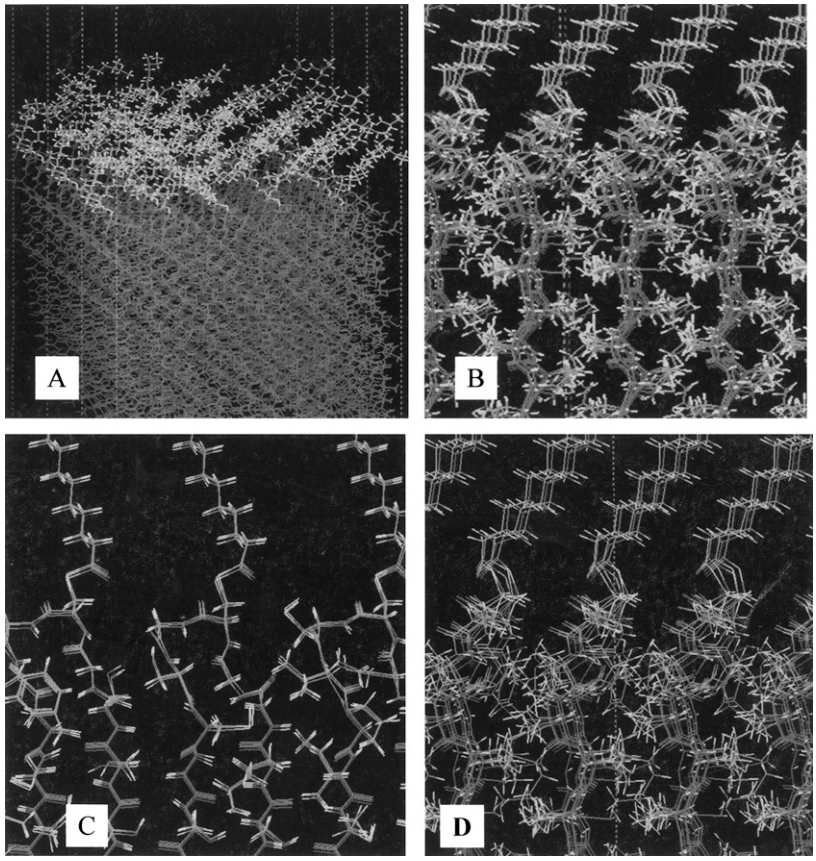

Figure 5. Views of the virgin hair surface from molecular modeling: (A) Diagonal view of 18-MEA on the UHSP surface (spacing is 1 nm × 0.7 nm (lattice)). (B) Schematic: Initial atomic structure. 18-MEA not relaxed to equilibrium. (C) Close-up view of top fold and MEA. (D) Close-up of top fold and 18-MEA. Side view looking between the beta sheets.

Various models (up to five) of the protein sequencing in the beta sheet were arrived at by a specific choice of particular cysteines that can take part in the linking to 18-MEA at the top folds. Of these representative, and possibly stereochemically different, arrangements of amino acids with respect to the top folds (i.e. with respect to the top plane of the hair surface), one model was chosen, which is depicted in Figure 1 for its protein sequence as well as the cysteines that take part in linkage with 18-MEA on the top folds. All studies were performed with this uppermost model of the epicuticle hair surface.

RESULTS AND DISCUSSION

The exact UHSP that 18-MEA is attached to is not known at this time, but a likely possibility is from the KAP-5 family of cuticle proteins (10,11). Human KAP-5 sequences that we considered for modeling are the one from Yahagi *et al.* (9) and the one by McKinnon *et al.* (13). The Yahagi *et al.* UHSP provided the composition as cystine (35%),

glycine (19%), serine (22.4%), and lysine (5%). The UHSP by McKinnon *et al.* gives cysteine (36%), glycine (16%), serine (24%), and lysine (5%). These two UHSPs are similar and we could in principle use either sequence for our model. For this initial model we chose the one by Yahagi *et al.* (9) because it is the smaller of the two (156 vs 168 total amino acids).

As described in detail in the Experimental section, a beta sheet model of polyalanine was first constructed based on the conformation obtained from the Protein Data Bank. Next, the amino acid sequence was changed to fit the amino acid sequence of the Yahagi UHSP (see Figure 1). The fully constructed beta sheet was then duplicated to several beta sheets that were stacked parallel to each other in the Y direction, as shown in Figure 2.

The Yahagi UHSP sequence provides cysteine residues at the top of each fold, and in that manner it provides an anchor for 18-MEA at a distance along the chain of approximately 0.9 nm, with an intersheet spacing of about 0.7 nm, thus providing an inter-attachment distance along the UHSP of about 0.9 nm. This intersheet distance of 0.7 nm provides spacings relatively close to the calculation of 0.936 nm in a "pseudo-hexagonal array," as described by Swift (14), and a beta sheet density of 1.42 gm/cm^3 compared with Allworden membrane fractions, as described by Allen *et al.* (6), that varied from 1.39 to 1.54 g/cm^3.

This outer layer of the epicuticle after relaxation and energy minimization (see Experimental section and Figure 1) was approximately 5-nm thick. The relaxation procedure allowed energy minimization while retaining cysteine residues on the top folds and maintained the essential backbone of the beta sheets. The 18-MEA molecules were then attached, and after 120 ps of MD simulation followed by energy minimization, the average thickness of 18-MEA was found to be 1.08 ± 0.2 nm, in good agreement with the XPS results {1 ± 0.5 nm} of Ward *et al.* (2).

This energy minimization procedure confirms that 18-MEA chains bound by thioester linkages to a UHSP surface at attachment distances of approximately 0.9 will bend back on themselves to the thickness found by Ward *et al.* on Soxhlet-extracted wool fiber, as suggested by Zahn *et al.* (4). The 18-MEA chains interact with each other by van der Waals attractive interactions. The 18-MEA chains are quite mobile, and without any other molecule between them they can adopt different conformations to energetically favor interchain interactions within themselves as well as van der Waals interactions with hair surface amino acids at the top folds of the beta sheets of the epicuticle protein complex.

The preliminary molecular model described in this paper (Figures 3–5) was selected and evaluated from eight different skeleton models, and it satisfies the following requirements better than the other models: A 1.08 ± 0.2-nm thickness of 18-MEA at the surface after equilibration following MD simulations. The variance of this 18-MEA thickness is smaller than that of Ward *et al.* (2), but the actual thickness is consistent with their XPS measurements, and the attachment of 18-MEA to the epicuticle proteins by thioester linkages through the cysteine residues of a UHSP arranged in a beta sheet is consistent with the description by Negri *et al.* (1,15).

This molecular model shows that the beta folds of this UHSP are perpendicular to the surface, as in Figures 1 and 2. Furthermore, this arrangement allows vacant spaces between 18-MEA molecules, as shown in Figure 6B, for free lipid moieties consistent with our conclusions from XPS measurements by Carr *et al.* (16) and others (17,18) described in the next section of this paper.

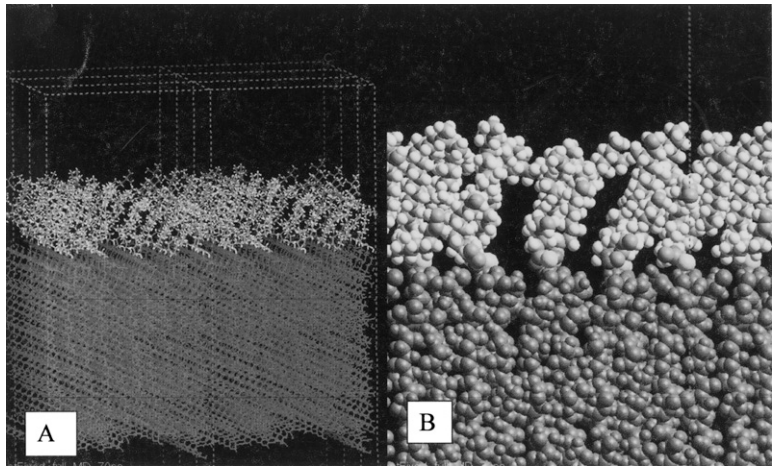

Figure 6. (A) Partially relaxed model to represent "bare-hair" (virgin hair) surface structures. Snapshots were taken after 40 ps of molecular dynamics simulations where top folds and 18-MEA were allowed to move during simulation. (B). Side view with van der Waals spheres for 18-MEA. Vacancies between 18-MEA residues can be seen, possibly available for free lipid deposits/interactions.

FREE LIPID IN THE SURFACE LAYERS

Several different laboratories have analyzed the outer surface of wool and human hair via XPS, examining the outer 2 to 4 nanometers of the hair surface (2, 16–18). As indicated, Ward *et al.* (2) estimated the thickness of the lipid layer of 18-MEA at 1 ± 0.5 nm from carbon/nitrogen analysis, assuming XPS examines the top 3 nm. Carr *et al.* (16) estimated 60% protein and 40% lipid in the top 3 nm of Soxhlet-extracted wool fiber. This estimate provides for 36% 18-MEA (at a 1.1-nm thickness) and 4% free lipid (non-covalently bound) in the top 3 nm of this wool sample. A related estimate using data from Robbins and Bahl (18) on "virgin" hair after shampooing with a sodium laureth-2 sulfate-containing shampoo provided 12% free lipid in the top 3 nm.

Capablanca and Watt (19) examined wool fiber that had been washed with detergent and extracted with various solvents using a streaming potential method to measure zeta potentials, from which they estimated the effect of extracted lipid on the isoelectric point of wool fiber. These scientists found an appreciable effect of free lipid (solvent-extractable lipid) on the isoelectric point, with surfactant-scoured wool having an isoelectric point of 3.3, but after extraction of this same wool with the most effective lipid solvent they found an isoelectric point of 4.5. These data show that the true isoelectric point of the hair surface proteins is close to 4.5 and that free lipid, which contains fatty acids, is an important and essential component of the surface (top few nanometers) of animal hairs, especially hair or wool in good condition that has only been scoured or cleaned with surfactants or shampoos. Furthermore, the more free lipid in these surface layers, the lower the isoelectric point of the fibers, suggesting that free lipid is an integral part of the surface of animal hairs after and between normal shampooing and scouring treatments.

We intend to explore the inclusion of free lipid in our keratin fiber surface models with improved software to see if free lipid deposited between 18-MEA molecules affects the thickness of 18-MEA on the hair surface, i.e., if free lipid moieties between 18-MEA

molecules bound to a UHSP cause 18-MEA to straighten out to approach its full length on a protein backbone that we have calculated at more than 2.5 nm, in reasonable agreement with TEM measurements of the upper beta layer of human hair by Swift and Holmes (20) of 2.5 nm and by Swift (14) of 3 nm.

NEXT STEPS IN MOLECULAR MODELING OF HUMAN HAIR

Our next steps involve constructing a molecular model of the virgin keratin fiber surface to build on this molecular model by adding free lipids to the existing model to determine if free lipids can cause 18-MEA to stretch out to its full length and approach the length found for the upper beta layer of keratin fibers. We hope at some time to construct models to simulate the lower layer(s) of the epicuticle by adding a second layer of proteins to provide an overall thickness of the epicuticle layer of approximately 13 nm, as reported by Swift and Smith (8). This will require new software to allow us to increase the number of atoms so that we can test the inclusion of KAP-5 and KAP-10 proteins (10,11) including disulfide and/or isopeptide cross-links in either one or both, or between protein layers. We also intend to develop different damaged hair models, working from our current virgin keratin fiber surface molecular model.

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The authors thank the Polymer Chemistry Division of the National Chemical Laboratory, Pune 411 008, India, where the modeling part of this project was carried out.

GLOSSARY OF TERMS USED FOR MOLECULAR MODELING IN THIS PAPER

Dreiding II force field: A versatile force field that contains the parameters and mathematical functions for describing the interatomic potential energy of interaction between atoms that are either bonded to each other or non-bonded (dispersion, van der Waals, electrostatic, and hydrogen bonding terms). Dreiding II force field is useful for predicting structures and dynamics of organic, biological, and main-group inorganic molecules. Details are given in the following reference: S. L. Mayo, B. D. Olafson, and W. A. Goddard III, Dreiding: A generic force field for molecular simulations, *J. Phys. Chem.,* **94,** 8897–8909 (1990).

Energy minimization procedure: A mathematical procedure for finding minimum values of the interatomic thermodynamic potential energy function that is continuous in spatial dimensions, specifically using gradient-based (derivative) methods. Examples are the Newton-Raphson methods and their variants such as conjugate gradient, Fletcher-Powell, etc. The x,y,z coordinates of atoms taking part in this process is obtained from this procedure.

Molecular dynamics simulations (MD): A theoretical method to track the motion of atoms and molecules in space and time coordinates, by numerical integration of the equations of motion. MD can be used to calculate equilibrium thermodynamic properties.

NVT ensemble: A thermodynamic ensemble for describing the chemical system with a fixed number of moles, volume, and temperature. Under these conditions, an accurate specification of the density is required to be able to predict molecular structure and thermodynamic properties.

Periodic cell: A simulation "box" having x,y,z dimensions containing the atoms and/or molecules representative of the chemical system, and which replicates itself in the three spatial dimensions by periodic continuation conditions, so as to represent the large-scale bulk system without posing any material discontinuities.

Protein data bank (PDB): A large database containing information on coordinates for atoms in crystal structures of protein molecules (such as beta sheets), for various known proteins.

Simulation box cell: Same as "periodic cell" above.

van der Waals gaps: Vacant spaces between atoms. Overlaps between atomic radii are permitted to the extent atoms are approachable with respect to each other, as defined by the minimum energy conditions of the van der Waals equation for interatomic potential.

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