Molecular Modeling Approach to Vegetable Tanning: Preliminary Results for Gallotannin Interactions with the Collagen Microfibril

by

Eleanor M. Brown* And Dennis C. Shelly[1]
*U. S. Department of Agriculture**, *Agricultural Research Service, Eastern Regional Research Center,*
600 E. Mermaid Lane, Wyndmoor, Pennsylvania 19038
[1]*Engineered Protein Materials Lab And Leather Research Institute*
Texas Tech University, Lubbock, Tx 79409

Abstract

Tanning of animal hides produces leather, a durable, flexible material that is stabilized against putrefaction. Chrome-tanned wet blue, aldehyde crosslinked wet white, and vegetable tanned hides are major contributors to current leather production. Although the chemistries involved are significantly different, the product in each case is leather. Vegetable tanning, the oldest of these technologies is, from a mechanistic perspective, the least well understood. To explore possible vegetable tanning mechanisms, we have applied molecular modeling techniques to the study of interactions of gallotannin, a component of chestnut tannins, with the collagen microfibril. A model gallotannin molecule was constructed and energy minimized. This model was docked into several energetically favorable positions on a fragment of the ERRC collagen microfibril model, generally with a good fit at a proline or hydroxyproline residue. The alpha carbon backbone of collagen was kept immobile during molecular dynamics simulations at 400 - 800K with and without an added layer of water of solvation to identify possibly more favorable interactions sites for the gallotannin molecules. Both inter and intra chain interactions were possible, and several potential sites for hydrogen bonding via arginine residues or hydrophobic interactions with alanine or isoleucine residues were identified. The information gained from this model study is an early step in the development of a vegetable-tanning model.

Resumen

El curtido de las pieles de animales produce el cuero, un material flexible y durable, que es estable a la putrefacción. Wet blue curtido al cromo, wet white reticulado por aldehído, y pieles curtidas al vegetal son los procesos principales contribuyentes hoy en día a la producción del cuero. Aunque las químicas involucradas sean significativamente distintas, el producto en cada caso es cuero. Curtido al vegetal, siendo la más vieja de estas tecnologías, es desde la perspectiva de la comprensión del mecanismo químico, la menos comprendida. Para la exploración de los mecanismos más probables para la curtición al vegetal, hemos aplicado técnicas de modelado molecular al estudio de las interacciones con tanino pirogálico, un componente de curtientes derivados del Castaño [*Castanea Vesca*], con un microfibrilo colagénico. Un modelo de la molécula del tanino pirogálico fue construido y su energía minimizada. Este modelo fue introducido entre varias posiciones favorables en un fragmento del modelo ERRC del microfibrilo colágenico, generalmente encajando donde hubiese un residuo de prolina o hidróxiprolina. La columna conformada por los alfa carbonos del colágeno, se mantuvo inmóvil durante las simulaciones moleculares dinámicas entre 400-800°K, con y sin una capa añadida de agua de solventación para así lograr identificar los sitios más favorables de interacción para las moléculas de tanino pirogálico. Tanto interacciones inter como intra con las cadenas colagenicas se encontraron factibles, y varios sitios aptos para formar puentes de hidrógeno vía residuos de arginina, así como también sitios para posibles interacciones hidrofóbicas con residuos de alanina o isoleucina, fueron identificados. La información obtenida de este estudio por modelación es un primer paso en el desarrollo de un modelo describiendo el curtido vegetal.

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*Corresponding Author - E-mail: eleanor.brown@ars.usda.gov
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INTRODUCTION

The hides of slaughtered cattle are the most valuable byproduct stream of the U.S. meat industry. Tanning of animal hides produces leather, a durable, flexible material that is stabilized against putrefaction. Chrome-tanned wet blue, aldehyde-crosslinked wet white, and vegetable tanned hides are major contributors to current leather production. Although the chemistries involved are significantly different, the product in each case is leather. The leathers produced by these different types of tanning agents have both similar and unique properties. From the perspective of the early twenty-first century tanner, who must be concerned with issues of safety and sustainability, none is ideal. An understanding of the mechanisms by which different types of leather are produced would be an asset in the design of more sustainable processes. Although the considerable data available from experiments directed toward the elucidation of tanning mechanisms provides insight into some tanning mechanisms, none of the currently practical tanning processes is fully understood.

On the most fundamental level, any tanning agent must interact with and stabilize the extracellular matrix of the hide. Covalent crosslinks, hydrophobic and electrostatic interactions, hydrogen bonds, and water activity are among the contributing factors in a stabilization mechanism. The elucidation of detailed mechanisms is hampered by the complex nature of both the hide matrix and the tanning molecules. Molecular modeling is an excellent approach to the study of the effects of these factors, individually and collectively, on collagen structure. Although it is impractical to simulate all aspects of a tanning process, several groups have made progress in the area of tanning-relevant modeling. Madhan and coworkers[1,2] used a small collagen-like polypeptide and several vegetable tannin models to estimate binding energies as a function of tannin structure and peptide configuration. A simulated tanning study by Fennen[3] modeled the bridging interactions of a binuclear chromium complex with two triple helices. Monti and coworkers[4,5] modeled the effects of adding a few formaldehyde molecules to short segments of microfibril in water, as a basis for studies of the structural and binding properties of supramolecular collagen. Siggel and coworkers[6-8] modeled the behavior of a microfibril under conditions of pickling, and evaluated interactions with calcium cations, sulfate anions, and polymeric retanning agents.

Vegetable tanning, the oldest of the currently used technologies is, from a mechanistic perspective, the least well understood, some combination of hydrophobic interactions and hydrogen bonds are likely[9] as is a filling action in the gap portion of the collagen fiber.[10] To explore possible vegetable tanning mechanisms, we have applied molecular modeling techniques to the study of interactions of gallotannin, a component of chestnut tannins, with the collagen microfibril. This work differs from our previous study[11] in that the tannin molecule is larger than catechin, and thus more representative of tannins in general, at least minimal hydration of the collagen microfibril is included, and both gap and overlap segments of the microfibril are examined.

METHODS

Models

The ERRC collagen microfibril model initially proposed by Chen,[12-14] refined by King,[15] and extended by the addition of the short, nonhelical telopeptide sequences at the N- and C-termini of the triple helix[16,17] was the starting protein structure. To make the study computationally manageable, two segments of the microfibril model were isolated and examined separately. An overlap segment comprised of 5 triple helices 85 residues long, and a gap segment that included the entire gap domain with telopeptides, plus a 10 residue long stretch from the overlap at each end to provide stability, were extracted from the complete microfibril. The gallotannin model was extracted from file 1JJQ of the Protein Data Bank,[18] and modified by the addition of a gallic acid moiety to one arm of the structure as shown by Leng.[19] Six copies of the gallotannin model were docked at separate sites along the each of the microfibril segments. Because of the documented preference of tannins for proline-rich proteins,[20,21] the gallotannin models were initially positioned near proline or hydroxyproline residues. To begin to explore the role of water in tannin-collagen interactions, calculations were performed in the absence (no added water molecules) and the presence of explicit solvation (a two-water-molecule layer, ~5000 water molecules), applied to the tannin-collagen models.

Calculations

Molecular modeling was conducted on a Silicon Graphics Fuel workstation (Fremont, CA) with the SYBYL 8.0 software package (Tripos, St. Louis MO). To prepare the model for energy or dynamics calculations, essential hydrogens and lone pairs were added, Kollman charges[22] were loaded on to protein and water components, and Gasteiger-Marsili charges[23] on to the gallotannin ligands. The generic Tripos forcefield that is compatible with both proteins and small molecules was used for all calculations.[24] Energy minimizations were performed before and after dynamics simulations. The energy minimized 300 K structures provided the starting conditions for molecular dynamics. To allow the system to equilibrate and observe the mobility of the gallotannin molecules as a function of temperature, dynamics simulations were performed in 10000 fs (1×10^{-11} s) intervals at 400 K, 600 K and 800 K. The A atoms of the collagen segments were immobilized to prevent the chains from separating. Gallotannin and water molecules were allowed to move freely.

Analysis

Data analysis primarily focused on a 10 Å sphere surrounding a central atom in the gallotannin molecule. Spheres selected in the starting configuration were compared with those based on the same central atom after dynamics at 800 K with and without added water. The distance and direction of motion by the individual gallotannin molecules during the molecular dynamics simulation, in the presence and absence of added water, was monitored. The compositions of the starting and final spheres of interest were compared. Increases or decreases in the number of individual chains and triple helices in proximity to the tannin molecules were compared. The chemical characteristics of the starting and final spheres were compared with respect to the proximity to proline or hydroxyproline residues, the potential for hydrogen bond formation or hydrophobic interactions between gallotannin molecules collagen, and differences between the overlap and gap segments.

RESULTS AND DISCUSSION

This gallotannin model (Figure 1a), although smaller than a typical vegetable tannin complex, has a span between non-hydrogen atoms of 22.5 Å, slightly larger than the 20 Å backbone to backbone diameter of the microfibril. Thus in this model system, a single gallotannin molecule would have the potential to interact with any or all of the atoms in a cross section of the microfibril. The overlap segment (Figure 1b), 15-chains, containing 1290 residues was extracted from one end of the ERRC microfibril model. The gap segment (Figure 1c) is larger, 2275 residues, encompassing the entire 12-chain gap with telopeptides, plus a ten-residue long 15-chain overlap segment at either end to give stability to the model. A ribbon representation of the backbone of the entire ERRC collagen microfibril model (Figure 1d) is included to lend perspective the isolated segments. The distribution of amino acid residues with ionizable or hydrophobic side chains is not uniform along the collagen peptide chain, and although the sizes of the two segments are significantly different, the overall compositions of the gap and overlap segments used for this study, are quite similar (Table 1).

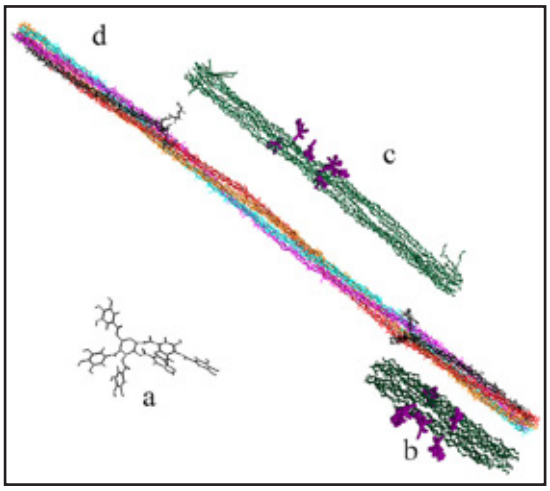

Figure 1. Starting model configurations: (a) gallotannin model, (b) overlap segment of the collagen microfibril model, with six gallotannin molecules in their initial positions, (c) gap segment with telopeptides and gallotannin molecules, overlap and gap segments are aligned on either side of the (d) complete microfibril. Collagen models are represented as ribbons on the protein backbone, with space filling gallotannin models superimposed, each triple helix in the complete microfibril model is shown in a different color.

A primary objective of this research was to develop a rationale for identifying preferred interaction sites for tannins with collagen. Minimal requirements for such a site were that the collagen structure not be disrupted, and that the total energy of the combined microfibril-tannin model after energy minimization be no greater than that of the microfibril alone. Six positions, or docking sites, on the overlap and gap segments were selected for study. In contrast to our earlier research to identify possible binding sites for catechin in the gap domain,[11] molecular dynamics simulations on this system were performed at temperatures significantly higher than would be experienced in a tanning process, in an attempt to dislodge gallotannin models from their docking sites and allow them to find more optimal sites.

TABLE I

Comparison of the Overlap and Gap Segments

	Overlap	Gap
number of residues	1290	2275
% Ionizable[a]	15.4	16.0
% Hydrophobic[b]	40.6	42.0
% Hydrophilic	25.5	24.9

[a]Ionizable residues are Asp, Glu, Arg, His, Lys, and Hly
[b]Hydrophobic residues are Ala, Hpr, Ile, Leu, Met, Phe, Pro, Val, and Tyr
[b]Hydrophilic residues are Asn, Gln, Ser,Thr and the ionizable residues

Models were examined after 10000fs simulations at 400 K, 600 K and 800 K. To explore the effect of solvating water on the mobility of the tannin molecules, simulations were performed with and without a two-molecule layer of water on the microfibril. During these simulations, gallotannin and water molecules were able to move freely, the A backbone of collagen was kept immobile to prevent the individual chains of the collagen model, which are not crosslinked, from separating.

The 400 K dynamics simulation produced only small amplitude oscillations of gallotannin molecules around the docking sites, and water molecules remained near the microfibril. At 600 K, water molecules and some gallotannin molecules began to move away from the docking site, but not dramatically. At the conclusion of 800 K molecular dynamics simulation, the most interesting observation was that in the solvated model, at least 20% of the water molecules, had moved at least 25 Å away from the microfibril, far enough to free them from its environment. Under the same conditions, the distance moved by a gallotannin molecule was 4.5 ± 3.2 Å generally toward the microfibril. When simulations were performed without solvation, the distances moved by individual gallotannin molecules were 5.8 ± 2.7 Å toward the microfibril. Under these conditions, the average distance that a gallotannin molecule moved into the overlap segment was 4.9 Å, and 6.6 Å into the gap segment, in the presence of water these average distances were reduced to 3.7 Å for the overlap segment and 5.3 Å for the gap segment. A separate feature of the motion of gallotannin molecules under molecular dynamics simulation was the tendency for distortion of the gallotannin structure to "flatten" itself against the microfibril as seen in Figure 2.

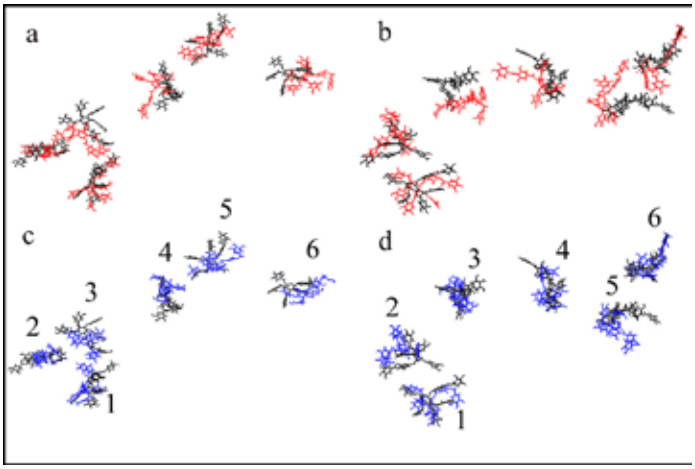

Figure 2. Configurations of gallotannin molecules at the completion of 800 K molecular dynamics are superimposed on the starting configurations at 300 K, in black. Final configurations on the solvated overlap (a) and gap (b) segments are shown in red, and on the non-solvated overlap (c) and gap (d) in blue. GT molecules are numbered 1 – 6 as shown in (c and d).

To compare potential interaction sites in the gap and overlap segments of the microfibril structure, attention was focused on a sphere with a 10 Å radius surrounding a central atom in each gallotannin molecule. Such a sphere around any non-hydrogen atom of a gallotannin molecule is large enough to impinge upon those collagen side chains that might provide stabilizing interactions. In order to explore the potentials for hydrophobic or electrostatic interactions and hydrogen bond formation, the number and type of collagen side chains in close proximity to the initial docking sites for gallotannin molecules was varied. For the most part, the rather limited motion of the gallotannin molecules even during 800 K simulations resulted in similar environments for the gallotannin molecules before and after dynamics. Differences in numbers of residues and/or chains within the 10 Å sphere appear to result from the characteristics of the initial docking sites, and do not represent trends (Tables II and III).

Because of reported interactions of tannins with proline-rich salivary proteins,[21] an effort was made to locate docking sites that would contain one or more proline or hydroxyproline residue. The compositions of the 10 Å spherical sites where gallotannin were docked, as well as the compositions of equivalent spheres after 800 K molecular dynamics with and without solvation of the collagen are compared in Tables II and III. The following observations with respect to the overlap segment are summarized from the data in Table II. Although the number of side chains in the docking sites for gallotannin molecules on the overlap segment was varied (7 – 18), the individual collagen chains (2 – 5) represent (1 – 2) triple helices. Thus, each of the gallotannin molecules associated with the overlap segment of the microfibril has the potential to interact across collagen chains and generally across triple helices. The degree of association of gallotannin molecules with collagen chains and helices in the overlap segment was little changed by the molecular dynamics simulations.

A hydrophobicity scale[25] that includes values for the post-translationally modified amino acid residues hydroxyproline and hydroxylysine, ranks arginine as the most hydrophilic at 0, phenylalanine as most hydrophobic at 1.0 with glycine (0.5) at the midpoint. Using this scale, in the overlap segment, only the docking site for GT4 is essentially hydrophobic in character, and possibly coincidentally, GT4 is the only gallotannin molecule that moved away from the solvated overlap segment under molecular dynamics simulations, the changes in the overlap environment of GT4 are illustrated in Figure 3, where GT4 was docked near 3 proline residues, and moved away from them during molecular dynamcs on the solvated molecule.

GT1, GT2, and GT5 were docked into sites that were only slightly hydrophilic, and these gallotannin molecules showed little tendency to move toward more hydrophobic positions. GT3 and GT6, initially in more strongly hydrophilic sites remained in hydrophilic sites. In terms of the number of available hydrophobic residues, the initial docking sites contained between three and seven hydrophobic residues, numbers that were little affected by molecular dynamics on the solvated model and somewhat reduced in the absence of solvation.

No particular trend either toward or away from specific types of residues was observed when the overlap model, either solvated or not, was subjected to molecular dynamics.

TABLE II

Composition of 10 Å sphere surrounding GT in overlap segment

	residues	chains	helices	Pro+Hpr	Hb[a]	H bond[b]
GT1						
Start	18	5	2	8	0.50	1
D800 Solc	12	3	2	6	0.45	1
D800	20	5	2	8	0.46	1
GT2						
Start	13	4	2	3	0.41	3
D800 Sol	16	4	2	3	0.42	7
D800	10	4	2	2	0.42	3
GT3						
Start	7	3	1	1	0.25	0
D800 Sol	5	4	2	1	0.32	1
D800	11	5	2	1	0.28	1
GT4						
Start	7	2	1	3	0.58	0
D800 Sol	0	0	0	0	NA	0
D800	6	2	1	3	0.67	0
GT5						
Start	14	3	1	2	0.46	1
D800 Sol	7	2	1	1	0.56	1
D800	20	4	2	4	0.47	1
GT6						
Start	15	4	2	2	0.31	1
D800 Sol	11	3	2	0	0.26	2
D800	13	4	2	0	0.25	0

a Average hydrophobicity[25] of the residues in a 10 Å sphere around gallotannin molecule.
b Number of potential H-bonds between GT and collagen.
c Microfibril initially solvated with two layers of water.

Potential hydrogen bonding sites were identified as described earlier,[11] several of the docking sites contained residues with the potential for forming hydrogen bonds with GT molecules. These potential hydrogen bonding positions tended to remain available after the molecular dynamics simulations, and they primarily involved interactions with terminal nitrogen atoms on arginine residues.

On the gap segment of the microfibril (Table III), an effort was made to further diversify the docking sites. The number of side chains in docking sites for gallotannin molecules on the gap segment varied more widely (0 – 43) than those on the overlap segment, as did the number of individual collagen chains (0 – 9) representing (0 – 4) triple helices. GT3 and GT5, initially positioned slightly away from the microfibril moved the greatest distance (8 – 10 Å) of the gallotannin molecules, into positions of greater proximity to the microfibril under molecular dynamics simulations. GT1 and GT6, initially embedded within the microfibril in close proximity to more than 35 collagen sidechains remained embedded throughout the simulations. GT2 and GT4 intially in docking sites more similar to those in the overlap segment moved into slightly more dense sites during the molecular dynamics simulations. At the completion 800 K molecular dynamic simulation, each of the gallotannin molecules associated with the gap segment of the microfibril had moved into a position where interactions between collagen chains were possible.

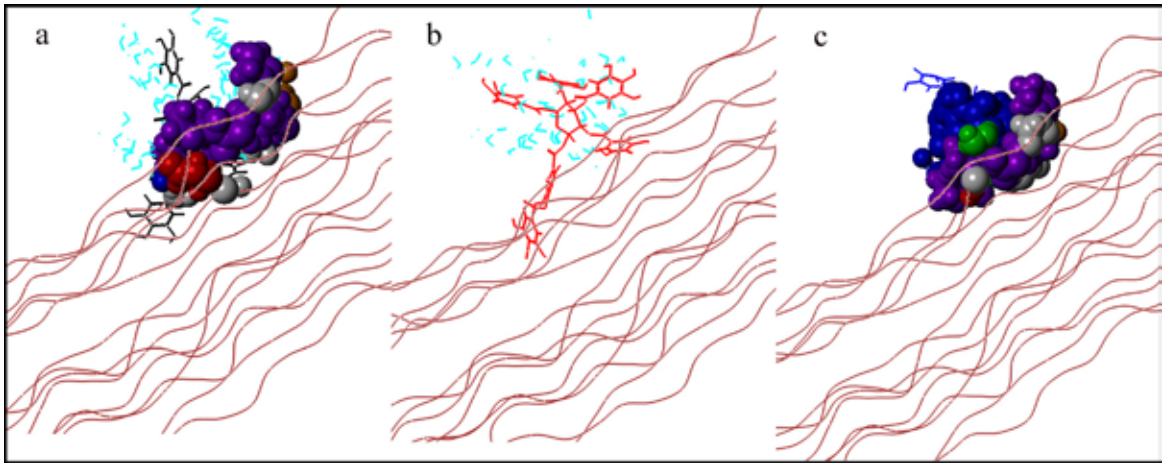

Figure 3. The environment of GT4 in the overlap segment of the collagen microfibril is illustrated with line ribbons to represent the collagen backbone, stick drawings of the GT structure and water molecules and spacefilling representations of the amino acid residues within a 10 Å sphere surrounding GT: (a) the starting configuration of GT4 (black) surrounded by water molecules in cyan, Pro and Hpr in purple, Gly in gray and acidic residues in red; (b) GT4 in red after 800 K dynamics on the solvated system; (c) GT4 in blue after 800 K dynamics without solvation where the sphere contains polar residues in green and basic residues in blue.

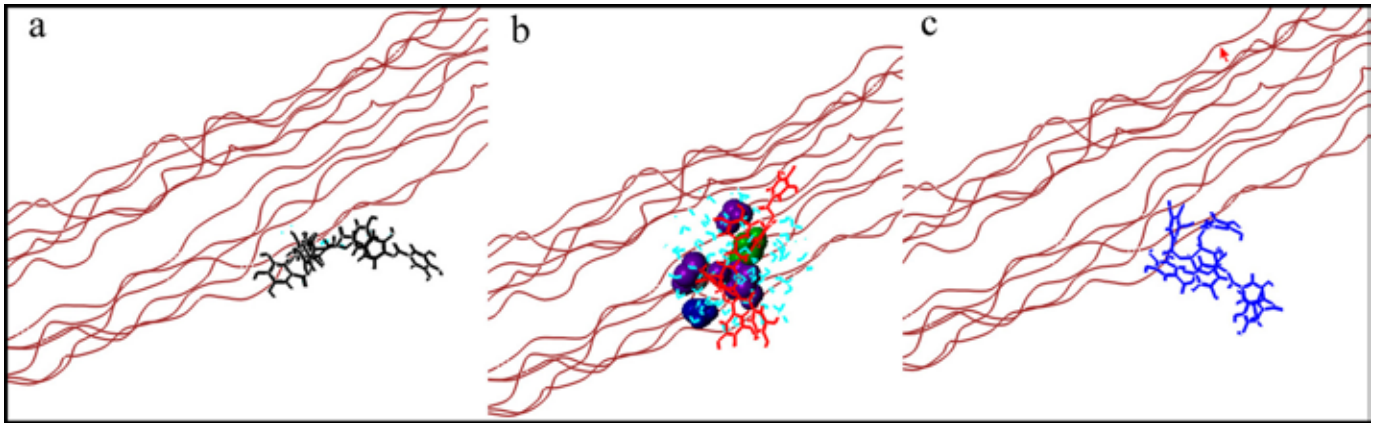

Figure 4. The environment of GT5 in the gap segment of the collagen microfibril is illustrated with line ribbons to represent the collagen backbone, stick drawings of the GT structure and water molecules and spacefilling representations of the amino acid residues within a 10 Å sphere surrounding GT: (a) the starting configuration of GT5 (black) is associated with a very few water molecules in cyan, (b) GT5 in red after 800 K dynamics on the solvated system; (c) GT5 in blue after 800 K dynamics without solvation.

The average hydrophobicity of gap segment docking sites was higher (0.51 vs 0.42) than those of the overlap segment. Docking sites for GT1 and GT4 were clearly hydrophobic (0.55 and 0.59) and those molecules moved relatively small distances under dynamics simulations, remaining in hydrophobic environments. GT3 and GT5 (Figure 4) initially outside the microfibril moved, under molecular dynamics simulation, into sites where the hydrophobicity was comparable to those on the overlap segment. The initial orientation of GT5 (4a), aligned with the microfibril, but not close enough for hydrogen bonds or other stabilizing interactions with the collagen, was altered by dynamics simulation at 800 K. In the solvated model (4b), GT5 moved into the microfibril structure, coming in close contact with proline and hydroxyproline residues. In the not solvated model (c) the orientation of GT5 with respect to the collagen microfibril was altered, but it did not come into closer contact with the collagen side chains. The initial docking sites contained between zero and 18 hydrophobic residues, numbers that again were little affected by molecular dynamics on the solvated model and somewhat reduced in the absence of solvation.

The proline and hydroxyproline content of docking sites on the gap segment varied (0 – 18) considerably more than in the overlap segment. The numbers of these residues remained relatively constant under molecular dynamics simulation of the solvated model, and decreased when the model was not solvated. Half of the docking sites in the gap segment had potential hydrogen bonding positions. The participating residues in this segment were frequently acidic, with hydrogen bond formation via carboxyl oxygens.

TABLE III

Composition of 10 Å sphere surrounding GT in gap segment

	residues	chains	helices	Pro+Hpr	Hba	H bondsb
GT1						
Start	43	8	3	18	0.55	3
D800 Solc	41	7	3	17	0.57	1
D800	41	9	3	17	0.56	1
GT2						
Start	18	5	2	6	0.4	1
D800 Sol	5	3	2	1	0.25	3
D800	22	5	2	7	0.46	1
GT3						
Start	0	0	0	0		0
D800 Sol	8	1	1	2	0.4	0
D800	1	2	1	0	0	0
GT4						
Start	9	2	1	3	0.59	0
D800 Sol	8	3	2	1	0.51	0
D800	3	2	1	1	0.56	0
GT5						
Start	0	0	0	0	0	0
D800 Sol	8	2	1	2	0.48	0
D800	0	0	0	0	0	0
GT6						
Start	37	9	4	10	0.52	8
D800 Sol	20	6	2	5	0.54	3
D800	37	9	4	11	0.53	3

[a] Average hydrophobicity[25] of the residues in a 10 Å sphere around gallotannin molecule.
[b] Number of potential H-bonds between GT and collagen.
[c] Microfibril initially solvated with two layers of water.

SUMMARY

Gallotannin models were docked into six sites each in an overlap and a gap segment of the ERRC collagen microfibril model. The system consisting of the collagen microfibril with or without a two molecule layer of water and the tannin molecules was subjected to molecular dynamics simulations at 400 K, 600 K and 800 K. The collagen backbone was immobilized, while tannin molecules and water were free to move in any direction during the simulations. Neither moved significantly at 400 K or 600 K. At 800 K, water molecules migrated away from the microfibril while tannin molecules tended to move toward the microfibril. Proline and hydroxyproline residues were selected as targets because they had previously been implicated for the potential to interact with polyphenols.[21] Under the conditions of this study, no particular preference for these residues was observed. Interactions leading to possible hydrophobic or hydrogen bonding with other residues in the docking pockets were observed. Gallotannin molecules docked slightly outside the gap segment of the microfibril were, as would be expected, the most able to move from their docking sites. Some as yet undefined attraction caused them to move into closer proximity with the microfibril. Chemical characterization of the interaction sites after 800 K molecular dynamics simulations show the potential for hydrophobic interactions, via alanine, isoleucine, proline, or hydroxyproline in some

sites. Hydrogen bonding appears most likely with arginine residues in sites other than those where hydrophobic interactions are most probable. This survey of possible gallotannin-collagen interactions suggests a variety of future studies to evaluate specific types of interactions. Notably, the use of shorter lengths of the microfibril will make it feasible to evaluate the effects of solvation of protein and ligands in more detail.

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