

USE OF COMPUTER-GENERATED MODELS IN STUDIES OF MODIFIED COLLAGEN*

by

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ABSTRACT

Production of leather from cattle hide is accomplished by the introduction of crosslinks between collagen triple helices, microfibrils and fibrils. Present tanning technology uses chromium complexes to form crosslinks between acidic amino acid side chains. Pretanning treatments that modify the primary amino groups of collagen have been reported to increase the efficiency of chrome tanning. To aid the effectiveness and efficiency of research leading to improved tanning technology, we have examined a fragment of our previously reported three dimensional model of the collagen microfibril. This 281 residue fragment of the microfibril model contains 15 lysine residues and 28 acidic residues distributed over the microfibril's 15 peptide chains. The effects of reductive alkylation and deamination of the lysine residues were simulated. Five independent binding sites in which carboxyl groups were located 7 to 11Å were identified and a sphere of 8Å radius about the midpoint of each pair was isolated. The sites examined provide for the possibility of inter- and intra- microfibrillar stabilization.

INTRODUCTION

Animal skins are converted into leather through the use of chemical agents to crosslink the polypeptide chains of collagen. This crosslinking of collagen stabilizes the leather, adding properties of strength and resistance to organisms which would otherwise attack and destroy the

hides. The technology using complexes of Cr(III) for tanning has been developed over the past hundred years. Although other tanning agents (vegetable tannins, synthetic organic crosslinking agents and mineral agents) are used to produce leathers for specific markets, trivalent chromium is the most effective reagent for producing high quality leather from bovine skins.¹ Concerns over possible restrictions on the availability, use and disposal of chromium-containing materials have prompted research into alternative tanning methods. Recent research may be categorized as either leading to the development of chrome-free tannages²⁻⁵ or to improvements in the efficiency of chrome tanning.⁶⁻¹¹

Although the detailed mechanism for chrome-tanning is yet to be established, the primary crosslinking reaction is thought to involve the formation of coordination complexes between hydrated basic chromium salts and the carboxyl groups of collagen.¹² Pretanning treatments that modify the primary amino groups of collagen have been found to affect the efficiency of chrome tanning. Those modifications that introduced additional carboxyl groups increased the uptake of chromium, thus decreasing the chrome content of the effluent.⁶ However, if the introduction of additional carboxyl groups involved the loss of the basic charge on lysine amino groups of collagen, the leather produced was of poorer quality than standard. Alternatively, pretanning treatments with aldehydes, phthalates and other reagents that blocked or crosslinked primary amino groups, but did not alter their ionic character improved the utilization of chromium without lowering the quality of leather produced.⁷⁻¹¹

In previous reports¹³⁻¹⁵ we have described the application of molecular modeling to the study of the three-dimensional

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structure of collagen. Molecular modeling techniques were used to better understand the structure-function relationships and interactions of collagen. In the initial study,¹³ templates for triple helical and microfibril models containing the collagen-like repeating sequence (Gly-Pro-Hyp)₁₂ were constructed and refined using an energy minimization algorithm. Thirty-six-residue-long polypeptide segments of the bovine Type I collagen sequence were then substituted into these template structures for the triple helix¹⁴ and microfibril.¹⁵ Clusters of functional amino acid residues were identified in the thirty-six residue segments of both triple helices and the microfibril. Probable interactions between ionized side chains of collagen were then predicted from that model.¹⁶

Methods for the reductive alkylation¹⁷⁻¹⁸ and deamination¹⁹ of lysine side chains in proteins are well established. The goal of the present study is to simulate the effects that these modifications of lysine side chains in collagen might have on the stereochemical character of a chrome (III) binding site composed of carboxyl groups on two different triple helical subunits.

METHODS

COMPUTER HARDWARE AND SOFTWARE

Molecular modeling studies were conducted as described earlier¹³⁻¹⁵ on a Silicon Graphics Indigo workstation[†] with a high resolution graphics monitor. Detailed visualization and real time manipulation of chemical structures were possible on this color graphics system driven by the SYBYL (Tripos Associates, Inc., St. Louis, MO) software (Version 6.0). SYBYL contains computational tools for protein construction, manipulation, energy minimization and analysis.

SIMULATED MODIFICATION OF TYPE I COLLAGEN MICROFIBRIL

For this study, a 281 residue segment was extracted from the complete microfibril model recently described by King et al.²⁰ In that model, an entire microfibrillar collagen molecule is represented as a bundle of five 315-residue triple helices with a relative staggering of 234 residues. For convenience, the triple helical subunits included in this fragment are designated A, B, C, D and E. This segment contains 15 lysine residues and 28 acidic residues. To simulate reductive methylation, the ϵ -amino group of each

lysine was replaced with a N,N-dimethyl group to form N,N-dimethyl lysine (Dmk). Reductive alkylation with acetone was simulated by the conversion of lysyl side chains to the N-isopropyllysyl (Lyp) form. Deamination reactions were simulated by converting lysyl side chains to δ - and ϵ -hydroxy-norleucyl side chains (Hld and Hle). 'Native' and modified structures were refined by energy minimization and subjected to 10ps low temperature molecular dynamics simulation.

DATA ANALYSIS

An array (56 x 56) of charge-to-charge distances between carboxyl groups was generated for the 'native' model and each of the lysine modified models. Pairs of acidic side chains for which the charge-to-charge distance was between 7 and 11Å were considered to be possible binding sites for the bi- or trinuclear Cr(III) complexes studied by Gotsis et al.²¹ A set of five inter-helical binding sites that met the criterium of a 7 to 11Å spacing between carboxyl groups in all five models was selected for further study. The aspartate or glutamate residues comprising these sites are designated A₁-B₁, A₂-E₂, B₃-D₃, B₄-E₄ and D₅-E₅, with the letters signifying the triple-helical subunits on which the acidic residue is located. Each of these sites was isolated by removing all atoms of the model except for a sphere, 8Å radius, around the atom most nearly midway between the two acidic groups. It was assumed that residues located more than twice the distance from the center of the sphere to the carboxyl group under consideration would have limited influence on binding at that site. The isolated spheres were then examined to evaluate the effects of lysine modification on the number and type of other side chains impinging on the site.

RESULTS AND DISCUSSION

In Table Ia, the composition of this microfibril fragment is compared with that of the complete type I collagen molecule. Because ionic and hydrophobic side chains tend to be clustered in different regions along the collagen microfibril,¹⁵ it is not surprising that a fragment selected to be rich in charged groups would be correspondingly poorer in hydrophobic residues than is the complete molecule. Table Ib contains the sequences of each of the triple-helical subunits of the microfibril. The presentation of the sequences emphasizes the stagger in their positions at the

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TABLE Ia
Comparison of the Composition of the Model Fragment and Whole Collagen

Residue	A ^a	B	C	D	E	Mole% Model	Mole% Type I Collagen
Ala	6	9	0	6	9	10.68	11.37
Arg	3	2	0	4	1	3.56	5.39
Asn	1	0	0	1	1	1.07	1.97
Asp	0	1	0	2	6	3.20	2.56
Gln	2	0	0	0	3	1.78	2.40
Glu	7	5	0	3	4	6.76	4.60
Gly	19	20	18	19	20	34.16	33.50
His	0	1	0	0	0	0.36	0.56
Hly	0	0	0	0	1	0.36	0.56
Hpr	1	7	18	3	5	11.39	10.19
Ile	0	0	0	0	0	0	1.28
Leu	1	1	0	0	0	0.71	2.63
Lys	1	6	0	3	5	5.34	2.50
Met	0	0	0	0	0	0	0.55
Phe	0	0	0	0	0	0	1.18
Pro	8	3	18	7	2	14.23	11.28
Ser	2	1	0	2	0	1.78	3.16
Thr	0	0	0	2	1	1.07	1.87
Val	3	2	0	4	1	3.56	2.43

a The five triple helices of the model are designated A – E, helix C represents the helix nucleating region that contains only the Gly-Pro-Hpr repeat.

level of the triple helix. Residues shown in bold type are defining residues for the potential binding sites to be described later. Triple helix C, representing the amino terminal portion of the helical sequence, contains only Gly-Pro-Hpr tripeptides and thus does not contribute either acidic or lysyl side chains. Figure 1a shows the arrangement of the triple helical subunits in the microfibril model. Figure 1b shows the locations of the five binding spheres described above in the microfibril segment.

CHARACTERIZATION OF MODIFIED RESIDUES

Figure 2a shows the structures of the lysine side chain and each of its modified forms as incorporated into a collagen-like tripeptide Gly-X-Hpr. In Table II, the sizes of the modified lysine residues are compared with lysine to show the effects on molecular volume of the microfibril segment. The lysine modifications simulating reductive alkylation

increase the size of the side chain by 13 percent (Dmk) or 25 percent (Lyp) but do not affect the charge. The simulation of deamination replaces the ε-amino group with a hydroxyl group thus removing the positive charge and decreasing the size of the side chain by 7 percent. When these side chains were incorporated into the microfibril, the overall volume effects on the collagen fragment were essentially negligible (-1 percent to +3 percent). Figure 2b shows probable structures²¹⁻²³ for a binuclear or a trinuclear Cr(III) tanning complex. Recent studies²¹⁻²³ of these complexes suggest that the span of either the binuclear or trinuclear chrome complex is about 8 to 10Å. To locate possible binding sites in our static model, all negative-charge to negative-charge distances between 7Å and 11Å were identified. In the modified microfibril models, the total number of potential Cr(III) binding sites (including sites within a single helical subunit) is 85 percent to 98 percent of that in

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TABLE Ib
Sequence^a of Model Microfibril Fragment

A			B			C			D			E		
α -1	α -2	α -1	α -1	α -2	α -1	α -1	α -2	α -1	α -1	α -2	α -1	α -1	α -2	α -1
		<i>Lys</i>			Pro			Hpr			Hpr			Ala
Gly	Gly	Gly		<i>Lys</i>	<i>Lys</i>	Gly		Gly	Gly		Gly		Asp	Asp ²
Glue	Glu ²	Glu	Gly	Gly	Gly	Pro	Pro	Pro	Pro	Ala	Pro	Gly	Gly	Gly
Ala	Leu	Ala	Ala	Pro	Ala	Hpr	Hpr	Hpr	Val	Val	Val	Gln	Gln	Gln
Gly	Gly	Gly	Ala	Ser	Ala	Gly	Gly	Gly	Gly	Gly	Gly	Hpr	Hpr	Hpr
Pro	Pro	Pro	Gly	Gly	Gly	Pro	Pro	Pro	Pro	Pro	Pro	Gly	Gly	Gly
Glu ¹	Val	Glu	Glu	Asp	Glu	Hpr	Hpr	Hpr	Ala	Val	Ala	Ala	Ala	Ala
Gly	Gly	Gly	Hpr	Hpr	Hpr	Gly	Gly	Gly	Gly	Gly	Gly	<i>Lys</i>	Hly	<i>Lys</i>
Ala	Asn	Ala	Gly	Gly	Gly	Pro	Pro	Pro	<i>Lys</i>	<i>Lys</i>	<i>Lys</i>	Gly	Gly	Gly
Arg	Hpr	Arg	<i>Lys</i>	<i>Lys</i>	<i>Lys</i>	Hpr	Hpr	Hpr	Ser	Arg	Ser	Glu ⁵	Glu	Glu
Gly	Gly	Gly	Ala	Ala	Ala	Gly	Gly	Gly	Gly	Gly	Gly	Hpr	Arg	Hpr
Ser	Pro	Ser	Gly	Gly	Gly	Pro	Pro	Pro	Asp ⁵	Asn	Asp	Gly	Gly	Gly
Glu	Ala	Glu	Glu ³	Glu ¹	Glu ⁴	Hpr	Hpr	Hpr	Arg	Arg	Arg	Asp	Thr	Asp
Gly	Gly	Gly	Arg	<i>Lys</i>	Arg	Gly	Gly	Gly	Gly	Gly	Gly	Ala	<i>Lys</i>	Ala
Pro	Pro	Pro	Gly	Gly	Gly	Pro	Pro	Pro	Glu ³	Glu	Glu	Gly	Gly	Gly
Gln	Ala	Gln	Val	His	Val	Hpr	Hpr	Hpr	Thr	Hpr	Thr	Ala	Pro	Ala
Gly	Gly	Gly	Hpr	Ala	Hpr	Gly	Gly	Gly	Gly	Gly	Gly	<i>Lys</i>	Val	<i>Lys</i>
Val	Pro	Val	Gly	Gly	Gly	Pro	Pro	Pro	Pro	Pro	Pro	Gly	Gly	Gly
	Arg		Pro	Leu	Pro	Hpr	Hpr		Ala	Ala	Asn	Asp	Glu ⁴	Asp
	Gly		Hpr	Ala		Gly			Gly	Gly		Ala	Asn	
			Gly	Gly						Ala		Gly	Gly	Pro

a Superscripts on residues in bold type indicate the binding site designated by that residue. Lysine residues are in italics for visibility.

TABLE II
Effects of Lysine Modification

Residue ^a	Vol ^b RES	Vol, MCF	No. Binding Sites	No. Bridging Sites
Lys	110.6	21328.7	118	63
Dmk	125.7	21864.4	109	60
Lyp	138.6	22075.9	116	60
Hld	103.9	21263.1	100	56
Hle	103.1	21260.9	114	63

a Abbreviations for modified residues are dimethyl lysine (Dmk), isopropyllysine (Lyp), δ - and ϵ - hydroxynorleucine (Hld and Hle).

b Volumes in \AA^3 of residue and microfibril segment containing the residue were computed based on the van der Waals surface of the molecule.

c Total number of pairs of carboxyl groups with 7 to 11 \AA spacing.

d Number of pairs of carboxyl groups where a crosslink would bridge two triple helical subunits.

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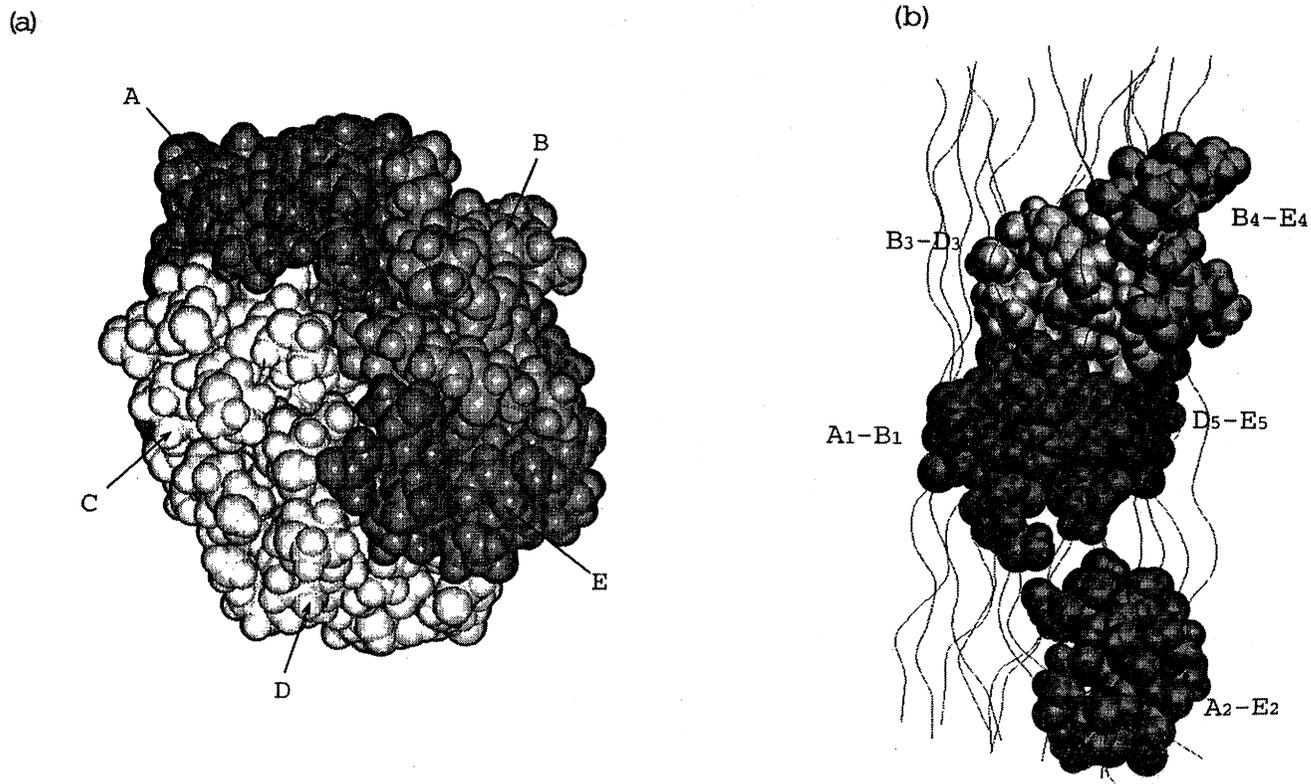


FIGURE 1. — (a) End-on view of the microfibril segment showing the relative positions of triple helical subunits. Clockwise from the top are A-B-E-D-C-A. (b) Lateral view of the microfibril segment using ribbon strands to represent the peptide backbone, the binding sites A_1-B_1 , A_2-E_2 , B_3-D_3 , B_4-E_4 and D_5-E_5 located on the microfibril.

the 'native' microfibril. The number of sites that bridge two different helical subunits is 88 percent in the case of the Hld side chain and 93 percent or more for the other modifications. These results suggest that one beneficial effect of a lysine modifying pretanning step might be to reduce the amount of intra-helical Cr(III) binding.

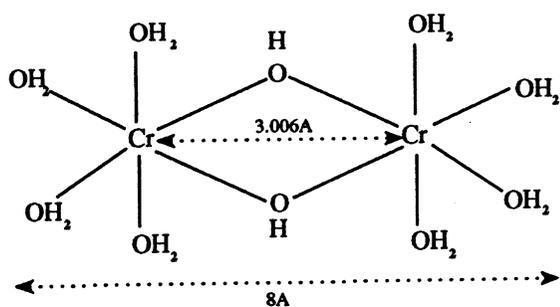
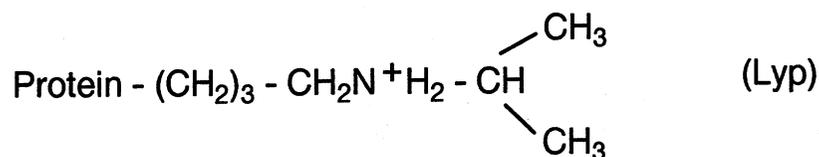
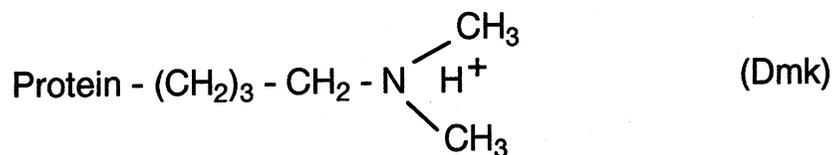
CHARACTERIZATION OF POTENTIAL BINDING SITES

A set of five potential binding sites was selected for further characterization. In these sites, the carboxyl groups, 7 to 11Å apart, are on different triple helical subunits and each site occurs in the 'native' fragment, and in all of the modified microfibril segments. In order to focus on each of these sites individually, a sphere with an 8Å radius was constructed around that atom in the 'native' microfibril fragment located most nearly midway between the two carboxyl groups. This central atom was identified by comparing the coordinates of each atom in the 'native' microfibril with the average of the coordinates of the carboxyl carbons for each site. The choice of an 8Å radius was made with the assumptions that in the modified microfibril models both carboxyl groups would be likely to be found within the sphere and that most of the other

residues likely to have local effects on these groups would likewise appear at least partially within the sphere. Although the processes of energy minimization and molecular dynamics are designed to relieve stresses induced in the molecule by the various modifications, the overall final structures were nearly superimposable. Therefore, the same atom was used as the center of the sphere in each of the models. All atoms outside this sphere were then removed. Those residues, from each of the models, having atoms located within the volume of the sphere were compared to illustrate the effects of lysine modification on the environment of the binding site. The compositions of these spheres are described in Table III.

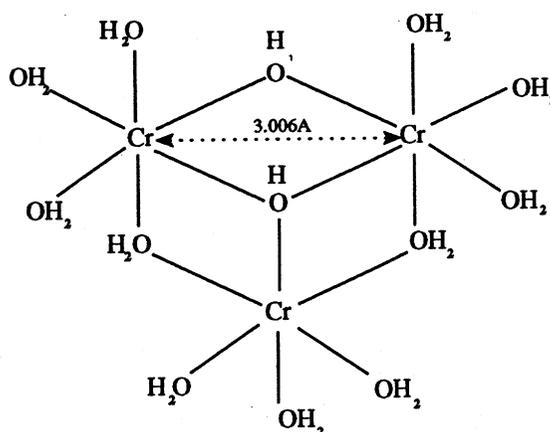
Variations in the number of residues contributing atoms to the 8Å sphere may be caused by changes in the volume of the lysyl side chain that could force distortion in the conformation to either allow other sidechains to approach more closely or prevent their approach. Differences in the electrostatic attraction or repulsion may also contribute to final selection of residues with the sphere. The selected sites fall roughly into two classes. Residues defining specific binding sites are labeled in Table Ib, lysine residues are in italics.

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O-Cr-O : 78.2 deg
Cr-O-Cr : 101.8 deg
Cr - O : 1.936 A
Cr - OH₂ : 1.98 A

Spiccia et al (1987) Inorganic Chemistry



Gotsis et al (1993) J.Soc. Leather Tech. & Chem.

FIGURE 2. — (a) Structures of lysine and modified lysine side chains: lysine (Lys), N,N-dimethyllysine (Dmk), N-isopropyllysine (Lyp), δ -hydroxynorleucine (Hld), ϵ -hydroxynorleucine (Hle) and binuclear or trinuclear Cr(III) complexes for crosslinking collagen.

TABLE III
Characteristics of An 8Å Sphere About the Midpoint Between
Carboxyl Groups Separated by 7 to 11Å and suitable for Binding a Cr(III) Complex

	Site	No. Atoms	No. Res.	No. Acid	No. Lys	A	B	C	D	E
lys	A ₁ - B ₁	165	14	2	1	X	X			
lyp	A ₁ - B ₁	185	20	3	2	X	X			
dmk	A ₁ - B ₁	173	16	2	1	X	X			
hld	A ₁ - B ₁	161	14	2	1	X	X			
hle	A ₁ - B ₁	170	16	2	1	X	X			
lys	A ₂ - E ₂	186	22	4	1	X	X			X
lyp	A ₂ - E ₂	192	21	3	1	X	X	X		X
dmk	A ₂ - E ₂	193	25	4	1	X	X			X
hld	A ₂ - E ₂	195	23	3	1	X	X		X	X
hle	A ₂ - E ₂	213	26	3	1	X	X	X	X	X
lys	B ₃ - D ₃	213	16	4	2		X	X	X	X
lyp	B ₃ - D ₃	214	15	3	2		X	X	X	X
dmk	B ₃ - D ₃	210	14	3	1		X	X	X	X
hld	B ₃ - D ₃	209	17	3	2		X	X	X	X
hle	B ₃ - D ₃	212	15	3	1		X	X	X	X
lys	B ₄ - E ₄	157	16	2	2		X			X
lyp	B ₄ - E ₄	157	17	1	2		X			X
dmk	B ₄ - E ₄	159	16	2	2		X			X
hld	B ₄ - E ₄	170	17	2	2		X			X
hle	B ₄ - E ₄	172	17	1	2		X			X
lys	D ₅ - E ₅	217	23	4	1			X	X	X
lyp	D ₅ - E ₅	214	20	4	0			X	X	X
dmk	D ₅ - E ₅	219	22	5	0			X	X	X
hld	D ₅ - E ₅	213	22	5	0			X	X	X
hle	D ₅ - E ₅	216	20	4	0			X	X	X

SITE A₁ - B₁

GluA₁ and GluB₁ (Table Ib) define a site (Figure 3a) on this microfibril model that bridges two adjacent helical subunits A and B (Figure 1a,b) and contains residues only from those two subunits. In the 'native' microfibril, all or parts of 10 residues from helix A and 4 from B are located within the sphere. This site would appear to be essentially neutral due to the lysine residue immediately following B₁ and the arginine residue at GluA₁ plus 3. The various modifications do affect the numbers of residues within the sphere identified

for the site (Table III, Figure 3a). The Dmk modification brought in one more residue from each helical subunit. The Lyp modification brought in parts of six additional residues (including a glutamate and a Lyp on helical subunit B, thus maintaining the electrostatic character of the site) apparently by pushing the binding residues further away from the surface of the model. The Hld side chain had minimal effect on the binding site but when the hydroxyl group was in the ε-position (Hle) it behaved more like Dmk. Intuitively one would have expected the bulkier side chains to allow fewer residues access to the site. However, this site

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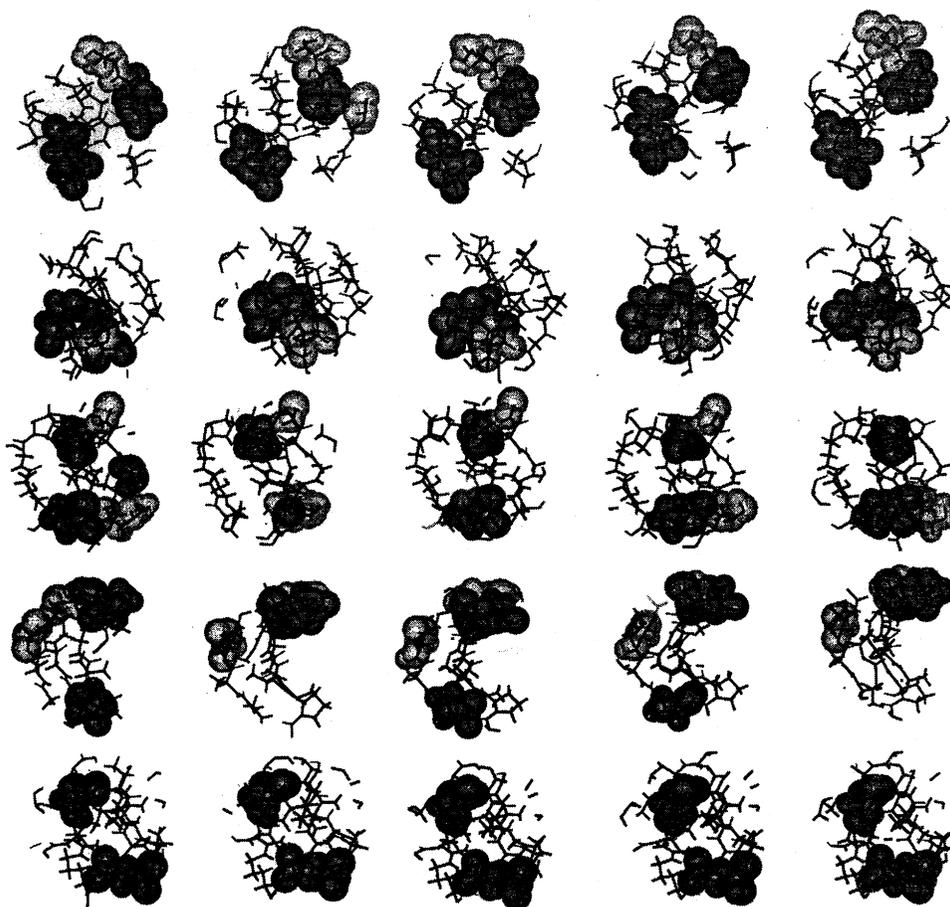


FIGURE 3. — Ball and stick representations of the spheres described around each of the potential binding sites. Binding sites are top to bottom A_1-B_1 , A_2-E_2 , B_3-D_3 , B_4-E_4 and D_5-E_5 . Right to left are sites extracted from models containing lysine or modified lysine side chains (a – e of Figure 2). The darkest shading is used for the acidic side chains, that would interact directly with chrome complexes; the middle level of shading is used to show the lysine or modified lysine side chains and the lightest shading is used for all other atoms within the 8Å sphere described by the selected acidic groups.

is on the surface of the microfibril (Fig. 1b) so that in the 'native' model the sphere is less densely packed than for an internal site. This external location makes a site of this type more useful for crosslinks to other microfibrillar structures.

SITE $A_2 - E_2$

Glu A_2 and Asp E_2 (Table Ib) define a site bridging two non-adjacent helical structures (Figure 1a). The sphere described around this site (Figure 3b) is more densely packed than that around $A_1 - B_1$. All or part of 21 to 26 residues are found in this sphere with a total of 186 to 213 atoms (Table III). A single lysine or modified lysine residue is found within the sphere, without other basic side chains, leaving this binding site with more negative charge than the $A_1 - B_1$ site. In the 'native' microfibril model, the sphere contains 22 residues (including a lysine, glutamate and aspartate) on helix B in addition to Glu A_2 and Asp E_2 and four other residues on E. In each of the modified models,

atoms are more densely packed than in the native model. The largest effects on this site occur when the bulky Lyp side chain increases the atomic density of the site while decreasing the number of residues, and when the neutral, unbranched Hle side chain allows other residues to pack more closely. As may be seen in Figure 3b, with Lyp and Dmk a larger portion of the side chain is within the sphere than for Lys, suggesting a greater electrostatic effect to compete with Cr(III) for the acidic groups.

SITE $B_3 - D_3$

Glutamate residues Glu B_3 and Glu D_3 (Table Ib) define a highly charged site (Figure 3c) bridging the nonadjacent helical subunits B and D (Figure 1a). In the 'native' form, the sphere described for this site contains portions of helical subunits B, C, D and E, four acidic residues, two lysines and an arginine. In each of the modified forms the second Glu on B is moved out of the binding sphere. In the Lyp

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modified form GluB₃ has moved toward the edge of the sphere. Although the presence of an arginine at the center of this sphere maintains a level of positive charge on all forms, the movement of other residues out of the sphere along with the neutralizing affects of H1d and H1e tends to make the modified forms generally less positively charged than the 'native.'

SITE B₄ – E₄

GluB₄ and GluE₄ (Table Ib) define a site that bridges the adjacent helical subunits B and E and is clearly at the edge of the microfibril (Figure 1a,b). The volume described by this site (Figure 3d) is the least densely packed of those considered here. Because it is located at the edge of the microfibril, only about two-thirds of the sphere is in the microfibril model. One would expect a greater mobility and the movement of more groups into the sphere when modified side chains are introduced. However, except for the exclusion from the sphere of GluB₄ in the Lyp and H1e containing models, the modification caused relatively little change. As was seen in the A₁ – B₁ site, that also bridges adjacent helices, only residues on the structures that define the site were within the limits of the sphere. Overall, the site appears neutral except where one of the defining residues has been moved out of the sphere, which would of course make it likely that a bound Cr(III) complex could form a crosslink with a different microfibril.

SITE D₅ – E₅

AspD₅ and GluE₅ (Table Ib) define a site that bridges the adjacent helical subunits D and E (Figure 1a). In the 'native' model one lysine residue projects into the sphere, but none of the modified 'lysyl' residues are in the sphere described for the binding site (Figure 3e), thus this site is more similar across the various modifications than are any of the others. It is a highly charged site containing four arginine residues and four or five acidic groups. The ability of these groups to act as counter ions may make this the least likely for Cr(III) binding. The sites chosen for study represent the possibility of a variety of binding scenarios. In the 'native' form, their characteristics vary as to the number and character of closely surrounding residues, the density of atoms within a specified distance, and the degree to which nearby basic groups may neutralize the acidic groups. Those sites where one or both acidic residues are on the surface of the microfibril model offer the potential of linking residues on some other microfibril to help stabilize the larger collagen matrix. The more internal sites could provide for effective stabilization within a microfibril. Electrostatic interactions between the residues on collagen

and other components of the hide system undoubtedly affect the binding characteristics.

On the basis of size, either the bi- or tri-nuclear Cr(III) complex described by Gotsis et al.²¹ could be accommodated in any of the five sites considered here. The net charges on these Cr(III) complexes are +4 and +5 respectively. A single Cr(III) complex would electrostatically bridge two acidic side chains and counter ions (sulfate, acetate, hydroxyl) would neutralize the remaining +2 and +3 charge.

The examination of isolated binding sites in an isolated microfibril model cannot provide information as to how the binding of a Cr(III) complex across one site might restrict the availability of another site, but it can provide a starting point for further experimental and modeling studies.

CONCLUSIONS

This study describes the use of a fragment of the Type I collagen microfibril to examine potential binding sites for chromium crosslinks. The model presented here is static, like a snap shot of a single frame in a dynamic system. Therefore, the interactions described above are potential, but not unique. Interactions of the structural residues of collagen (glycine, proline, hydroxyproline) and of nonionized side chains (polar, hydrophobic) in this fragment, and the rest of the collagen matrix will affect the binding of Cr(III) and other crosslinking agents. Examination of the effects of molecular dynamics calculations to simulate ambient temperature, the inclusion of a layer of water to hydrate the model, consideration of hydrophobic interactions and inclusion of larger fragments of the amino acid sequence should enhance the value of the predictions made from this type of model. Although amino acid side chains are flexible and can adopt different stabilizing conformations, our static models are effective in allowing studies to identify a set of possible interaction sites. A full collagen microfibril is about 13 times as large as this model, and a single collagen fiber consists of many such microfibrils. Therefore, the ability to study and identify specific regions in this very complex environment will greatly assist in the designing of potential tanning agents. The parameters estimated from this simplified model provide a reasonable start to the process of understanding how pretanning steps affect potential binding sites. In the real world, one cannot isolate a single binding site from the rest of the supermolecular assembly of which it is a part. Work is presently underway on a laboratory-scale experimental study of the systems simulated for this paper. The use of the large

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computer model allows us to focus on a single site and begin to see some of the effects of more distant parts of the molecule. Interaction between laboratory experiments and the computer experiments should allow us to refine both models, and ultimately develop a rationale for more efficient utilization of chromium in the tanning process.

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