ɣ STUDIES OF A COLLAGEN MATERIAL FOR LEATHER PRODUCTION TREATED WITH CHROMIUM SALT

by

Clark A. Maxwell[1*], Krzysztof Smiechowski[2], Jan Zarlok[2], Alina Sionkowska[3], Timothy J. Wess[1]

[1]*Structural Biophysics Group*
School of Optometry and Vision Sciences
CARDIFF UNIVERSITY, CARDIFF, UK

[2]*Faculty of Materials Science and Footwear Technology*
Radom Technical University
CHROBREGO 27, 26-600 RADOM, POLAND

[3]*Faculty of Chemistry*
Nicolaus Copernicus University
GAGARIN 7, 87-100 TORUN, POLAND

ABSTRACT

have been made to investigate a treatment that produces minimum effluents (i.e., mentally friendly), and yet significantly es the quality and heat resistance of a collagen material for the leather industry. uctural properties of collagen fibrils in tanned bovine leather have been examined hromium salts with different concentrations plied to treat bovine skin collagen to obtain er product. The treated samples were dried X-ray diffraction study was carried out to the influence of chromium ion tration on collagen fibres.

ations showed that molecular arrangement gen depends on concentration of chromium ed in the tanning process. Bovine collagen with tanning agents at Chrome trations of (1,3,5,7,9,11%), resulted in s in the molecular packing within the n fibrils. The shrinkage temperature of cross-linked with chromium salt increases red to the control.

ABSTRACTOS

zos se han efectuado para investigar los os de tratamiento que producen mínimos es (v. gr. amistosos al medio ambiente), y a que considerablemente aumenten la calidad stencia térmica de material colagénico para la industria del cuero. Las dades estructurales de las fibrillas en cuero al cromo bovino han sido examinadas cuando sales de cromo en diferentes concentraciones fueron ofrecidas al tratar colágeno proveniente de piel bovina para obtener un producto de cuero. Las muestras tratadas fueron secadas y un estudio por medio de difracción de rayos x fue efectuado para evaluar la influencia de la concentración del ión de cromo sobre las fibras colagénicas.

Las observaciones demostraron que la estructura molecular depende de la concentración de los iónes cromo utilizados en el proceso de curtición. Colágeno bovino tratado con agentes curtientes de cromo a [varias] concentraciones (1,3,5,7,9,11%) resultaron en cambios de la estructuración molecular al interior de las fibrillas colagénicas. La temperatura de encogimiento de las fibras reticuladas por la sal de cromo aumentó en comparación al del control.

INTRODUCTION

Collagen products are components in a great variety of important goods: parchment, leather, gelatin for pharmaceutical and food applications, hydrolysates and glues. Collagen is a natural polymer, the main component of skin and connective tissue. It has great tensile strength, and is the primary constituent of ligaments and tendons. It is responsible for skin strength, and its degradation leads to wrinkles that accompany aging. The native tropocollagen molecule (molecular mass 300 kDa) consists of three polypeptide chains of about 1000 amino acid residues each, wound round one another to form a strong triple helix.[1] The atoms in the individual chains are held together with

ding author: maxwellca@cf.ac.uk

covalent bonds, while the helix is stabilised by weaker bonds.[2] Collagen is a fibrous protein composed mostly of glycine, alanine, proline, and hydroxyproline. The spatial configurations of repeated triple amino acid sequences are responsible for the helical conformation between the strands. Water molecules are intimately connected with hydrogen bonding, holding the triple helix together. The collagen molecules align together forming microfibrils, which pack together forming fibrils and then fibres.

Leather is made from animal skins or hides which have been chemically treated to preserve quality and natural beauty. During tanning the collagen in the rawhide is cross-linked to make it stronger, more durable, and to keep it from rotting. Raw animal skins go through several steps during the process. Dependent on the type of hide used and the desired end-product, the steps taken during tanning can vary greatly. Tanning is essentially the reaction of collagen fibres in the hide with the tanning agent. The use of chromium salts as the tanning agent has dominated the tanning industry in recent decades and this remains the most widespread method of tanning today, although there is growing pressure in many countries for replacement by materials with a lower environmental impact.[3,4] Leather waste usually contains 3-7% Cr(III), obtained in the chromic tanning and production of shoes and leather goods.

Research has been dedicated to the improvement of leather quality, and to extend leather durability by developing new technology for the tanning of collagenous materials. This can be achieved by judicious selection of parameters for leather drying to promote retention of the proper content of water, by improving the UV and heat resistance of automotive upholstery leather, and by developing in-line monitoring of the mechanical properties of leather during its manufacture. Research programs are aiming to minimize the environmental impact of leather production, through the development of new tanning processes and the utilization of solid tannery waste.[4,5,6] Central to the success of leather production is the degree of modification to the hierarchical structures formed by collagen molecules, fibrils and fibres during the conversion from rawhide to leather.[7,8,9]

Processed leather consists of an interwoven matrix of fibres composed of a chemically cross-linked form of the triple-helical protein collagen. A study by Maeser[10] indicated that intra-hide variability of tensile stiffness depended upon the preferred direction of fibres in different regions of the hide. Kronick and Beuchler[11] showed that stretching untanned hide can produce changes in the orientation and distribution of its collagen fibres.

Washing procedure:
Soaking:
110% - water, temp. 22°C
0.05% - Rokafenol N8
0.05% - Biocid 40,
0.2% - Na_2CO_3, stir 90 min, drain float.
Main soaking:
110% - water temp. 26°C
0.05% - Biocid 40
0.15% - Rokafenol N8
.,1% - Na_2CO_3,
+150% - water, temp. 20°C, (14 hours)
Liming:
80% - water, temp. 28°C
0,1% - Rokafenol N8
0,9% - Erhavit EF
0.5% - calcium hydroxide, 60min.
0.7% - NaHS, 20min.
0.6% - Na_2S, 60min.
2% - calcium hydroxide, 60min.
100% - water, temp. 30°C, 30min.
1.5% - calcium hydroxide, 18 hours, drain float
Washing:
200% - water, temp. 25°C, 15min, drain float
Washing:
200% - water, temp. 30°C, 15min, drain float.
Deliming):
100% - water, temp. 30°C,
0.5% - sodium metabisulphite $Na_2S_2O_5$,
30min, drain float.
Main deliming:
100% - water, temp. 30°C, 15min.
+0.5% - Adisin N
+1.5% - $(NH_4)_2SO_4$, 90min.
+0.5% - Oropon OR, 20min, drain float.
Washing:
200% - water, temp. 30°C, 15min, drain float.
200% -water, temp. 25°C, 15minm, drain float.
Pickling (3 hours together)
80% - water, temp. 20°C
7% - NaCl, 15min.
1% - HCOONa
+1.2% - H_2SO_4, 1:10, three times per 10 min.
Tanning:
80% -water, temp. 20°C, 24 hours
1,3,5,7,9,11 % of Chromal. (tanning agent)
pH controlled using the reagent based on MgO.

Figure 1. - Steps of leather chrome tanning process (general flow diagram). Chemicals used are described in Table I.

TABLE I
Process Chemicals, Sources and Characteristics

Chemical Name and Source	Characteristic
Rokafenol N8, Rokita S.A.Poland	Nonionic agent (detergent) based on ether. polyoxyethylene(9)nonylphenol ether (99%); water (1%).
Biocid 40, Biokimica Italy	Bacteriocide Concentration 40-42%.
Erhavit EF, TFL Germany	Agent for increasing the rate of calcium diffusion into the hide. Regulation of swelling.
Adisin N, Adipol Chorzow, Poland	Mixture of weak aliphatic acids, for removal of calcium.
Oropon OR, TFL Germany	Agent based on pancreatin (enzymatic) 650-790LVE).Used at pH 6-8

Although there have been studies on the structural order of collagen fibres, relatively few have investigated the structural changes of collagen molecular packing within a fibril that are central to the success of the tanning process. A broad objective of research in leather technology is to provide knowledge of collagen fibril and molecular integrity necessary for the development of effective low chromium tanning systems. Approaches are generally through a correlation of biophysical experiments[7,8] of bovine skin treatment with different concentrations of chromium salt. X-ray diffraction studies covering length scales from ~100 to 0.2nm spacing enables rapid assessment of interactions between amino acid residues in the helix and inter-helical interactions within the fibrils[12]. The synchrotron EXAFS study of Reich and co-workers[13] into binuclear chromium-collagen complexesaddresses the nearest neighbour atomic environment of chromium in leather. Our work is complementary to this as we are examining how chromium binding alters collagen structure at different hierarchical levels from the atomic to the nanoscopic.

EXPERIMENTAL

Sample preparation:[3,14,15]
The excess flesh and fatty tissue under the hide was removed by a fleshing machine before the hides were loaded into drums. The hides were washed to remove dirt and blood from the surface. After fleshing, the hides were weighed and washed. The washing procedure and the leather tanning protocol are outlined in figure 1. The chemicals used in these processes are characterized in Table I. The percentages in figure 1 express the amount of additive in comparison with the weight of hide (200% of water means that for 100 kg of hides, 200 kg of water was used, etc). The soaking process, which has a pH of 8-9 due to the addition of Na_2CO_3, restored lost moisture to hides that had been salted and stored for long periods before processing. Hair removal using lime was followed by washing and deliming cycles before pickling. In the pickling process: water, sulphuric acid and sodium chloride are added. The acidic environment of this solution made the hides ready to accept the tanning chemicals. The addition of sodium chloride prevented any swelling of the hide.

The 24 hour tanning process converts the hide into a stable material which will not putrefy and be less susceptible to attack by bacteria. The tanning agent Chromal (containing about 25% of Cr_2O_3) was added and the Cr_2O_3 penetrated (24 hours at 18 - 20 °C) into the hide structure and cross-linked with the collagen. Different amounts of Chromal were used (1,3,5,7,9,11%) and after tanning, the amount of Cr_2O_3 in the leather was measured by titration with $KMnO_4$. Once adequate penetration of the chrome had occurred (2 hours), the hides were basified. A slightly alkaline chemical such as magnesium oxide was added and the pH was slowly raised to ~4. The parameters of the process are presented in Table II. The samples were dried at room temperature for 24 hours and then submitted for analysis.

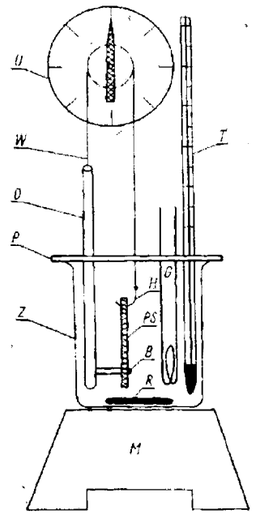

Figure 2. - The experimental set up for shrinkage temperature measurements (M- magnetic stirrer, R- magnetic rod, Z- beaker, P- cover, G- heater, T- thermometer, B- handler, H- hook, O- pipe (tube), PS- sample of leather, W- rope (fibre), U- indicating device). Temperature was measured at the moment when the sample began to contract (shrink). During the measurement the sample of leather was soaked in a beaker containing water and glycerol (1:1). The heating increment was 1° per minute (starting from 20°C).

TABLE II
Parameters of the Tanning Process and Shrinkage Temperature of Obtained Leather

Sample No.	Chromal [%]	% Cr_2O_3 in leather	pH before tanning	pH after tanning	Shrinkage temperature[°C]
0	Sample without tanning agent (control sample)				68
1	1	0.9	3.7	4.6	83
2	3	2.0	3.5	4.6	93
3	5	3.4	3.2	4.4	97
4	7	3.7	3.0	4.2	107
5	9	4.3	3.1	4.2	119
6	11	5.2	3.0	4.1	119

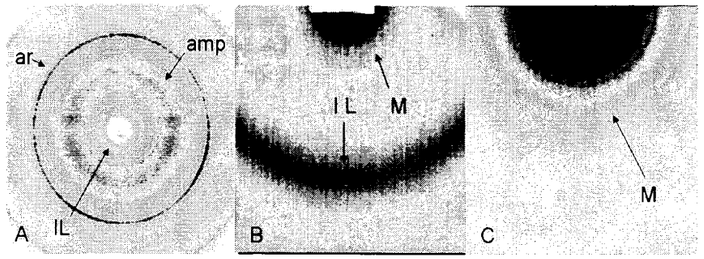

Figure 3. - X - ray diffraction pattern of isotropic collagen from bovine hide at different sample to detector distances representing packing characteristics of A) Axial rise per residue (ar) and amorphous region (amp), B) Intermolecular lateral packing (IL), C) Meridional diffraction series (M) of collagen structure corresponding to the 65nm repeat.

X-ray diffraction studies

High-angle X-ray diffraction patterns were obtained using a Bruker AXS Nanostar small angle scattering station with a camera length of 4.4cm, which allowed the wide angle diffraction features of collagen to be observed to a resolution of 0.2nm. Details of the scattering system and data reduction can be obtained in.[16]

Small angle X-ray diffraction studies were made at the SRS Daresbury on beamline 2.1. This allowed long range features of collagen in the tanned material to be investigated over length scales of up to 100nm. (For full details of the experimental set up see Kennedy *et al.*[17] All diffraction patterns were converted using in-house software into linear profiles of scattered intensity (I) vs scattering angle d (where $d = (2/\lambda)Sin\theta$), where λ is the x-ray wavelength and θ is half of the scattering angle. The linear profiles were further analysed using XFIT the one-dimensional peak - fitting program (Collaborative Computational Project 13)[18] In all cases the samples were examined at ambient temperature and humidity.

Fibril shrinkage temperature experiments

The shrinkage temperature of leather was measured according to the Polish Norm PN-92/P-22152. Temperature was measured at the moment when the sample started to shrink in water/glycerol liquid (1:1) during heating with the rate 1°C per minute. The experimental setup used to determine shrinkage temperature is shown in figure 2.

RESULTS

Thermal stability

Collagen fibres exhibit a sudden shrinkage in length when heated. Fibres in leather have had chemical cross-links introduced which raise the temperature at which the fibres shrink. If wet leather is heated, there is a point when it begins to change radically. This is the shrinkage temperature which for vegetable tanned leather this occurs between 75 and 85°C. The amount of shrinkage can range from 5% to 40%, depending on the characteristics of the leather. The shrinkage temperature is a test that identifies hydrothermal stability, and therefore requires the presence of excess water (and occasionally added glycerine) for it to be carried out. A high shrinkage temperature is one of the parameters demanded of good leather. The thermal behaviour of fibrillar collagens is rather complex and depends on amongst other factors, water content, age of collagen, and sample preparation.[19-23] The shrinkage temperature of raw hide is between 50-60 °C, while for tanned leather, it is much higher. The complex cross-links between Cr(III) salt and collagen makes leather more stable thermally.[24,25] The shrinkage temperature for leather that contains different % of chromium was investigated, and the results are presented in Table II. Shrinkage temperatures of cross-linked fibres with chromium salt, shows increases when compared to the control sample. Shrinkage temperature for hide without tanning agent (control sample) was 68 °C, hide with 2% of Cr_2O_3 was 93 °C, and 5% was 119 °C.

TABLE III
Helical Rise per Residue and Peak Characteristics as Measured Using XFIT Program

Bovine hide Samples (0.02nm resolution)	Axial rise per residue (nm)	Peak Height (Intensity)	Full Width half maxima d (nm^{-1})	Integrated Intensity
0 % Cr_2O_3 (Control)	0.276	0.01334	0.733	0.065
0.9 % Cr_2O_3	0.276	0.00931	0.787	0.049
2.0 % Cr_2O_3	0.274	0.00619	0.873	0.036
3.4 % Cr_2O_3	0.278	0.00729	0.820	0.040
3.7 % Cr_2O_3	0.277	0.00311	1.585	0.033
4.3 % Cr_2O_3	0.276	0.00473	0.951	0.030
5.2 % Cr_2O_3	0.275	0.00395	0.902	0.024

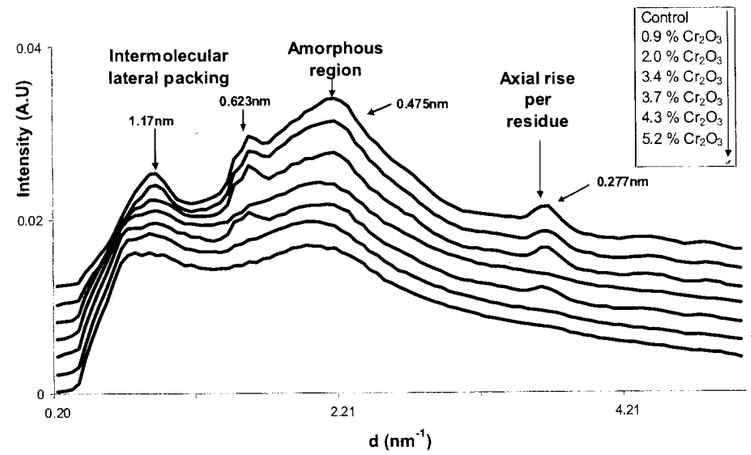

Figure 4. - Linear X-ray scattering profile of bovine hide treated with chromium. At such high angles the diffraction results mainly from the helical properties of the collagen molecules. The amorphous halo present in most diffraction data is also observable here as is the characteristic reflection due to the side by side packing of collagen molecules. The major trend observable is the contribution of diffraction corresponding to the axial rise per residue, this diminishes with chrome tanning. (All linear traces have been normalised and offset).

X-ray diffraction

Although the interaction of X-rays with each collagen triplex will produce its own fibre diagram, the isotropy of the felt like arrangement of the collagen fibres within bovine hide samples usually result in an isotropic fibre diagram. It is possible that preferential alignment of collagen in skin can be seen, but this is most likely due to the location that the sample was taken from rather than the processing. Collagen fibres taken from the neck and spinal areas are known to be preferentially aligned.[26] The typical isotropy of collagen X-ray diffraction patterns from bovine hide are shown in figure 3.

High angle X-ray diffraction

High angle X-ray fibre diagrams were obtained and used to analyse the effects of chromium tanning on collagen at the intra and inter molecular level by measuring quantitatively, the changes in the axial rise per residue reflection position of the collagen helix, and the contribution of diffuse and helical scattering to the fibre diagram. The term helical rise per residue represents the mean axial step between the amino acid residues of collagen chains within the molecules. Linear traces of the radially averaged fibre diagrams are shown in figure 4. The data obtained for the linear profiles of figure 4 is represented in table III.

Although not completely correlating with chromium salt levels, there is a decrease in peak height and an increase in half width maxima, as concentrations of chromium salt increase. The peak height and half width maxima values can be used to determine the integrated intensity, which is an indication of order within the collagen structure. The integrated intensity decreases with the exception of sample

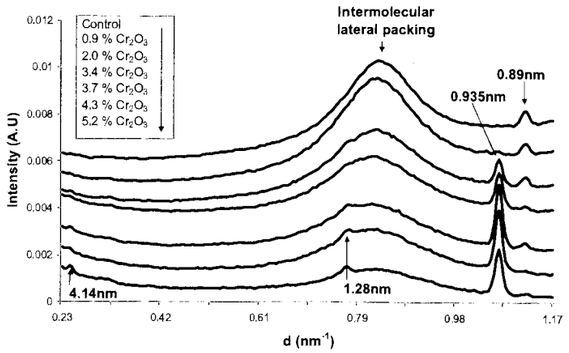

Figure 5 - X-ray scattering data obtained at station 2.1 SRS Daresbury at a sample to detector distance of 1.25m of bovine hide treated with chromium was analysed using XFIT to obtain a linear trace profile. (All linear traces have been normalised and offset).

3 (although still decreasing) linearly as chromium salt concentration increases. Therefore chromium salt seems to cause a loss of order within the collagen molecules at the molecular and helical level dependent on its concentration. The increase in half width maxima can be interpreted as the distribution of axial rise per residue values in the sample. There is a link between chromium salt concentration and increasing helical rise per residue distribution, but a direct link with chromium salt concentration is not clear. The change in the mean value of the axial rise per residue as a function of chromium treatment is probably negligible.

Wide angle x-ray diffraction with a resolution of 0.9nm

X-ray diffraction to a real space resolution of 0.9nm allows a more accurate evaluation of the intermolecular lateral packing of the collagen molecules, which occurs in the region of ~1 nm for the dehydrated state. The treatment of bovine skin with chromium has an effect on the peak profiles (figure 5), which seems to be directly related to concentration. Although not a completely linear relationship, increasing chromium salt concentration relates to a reduction in height of the peak that represents the characteristic intermolecular lateral packing of the collagen molecules. There is also a reduction of the peak occurring at d = 1.07 nm^{-1} (0.935nm real lattice space), with increasing chromium salt concentration and also an appearance of another peak at d = 1.12 nm^{-1} (0.89nm real lattice space) possibly a diffraction order of lipid bilayer interactions with Cr^{3+} ions. The linear traces of figure 5 were further analysed using XFIT to give values concerning peak profiles (tables IV, V, and VI). The intermolecular

TABLE IV

Intermolecular Lateral Packing and Peak Characteristics as Measured Using XFIT Program

Bovine hide Samples (0.9nm resolution)	Intermolecular lateral packing (nm)	Peak Height (Intensity)	Full Width half maxima d (nm^{-1})	Integrated Intensity
0 % Cr_2O_3 (Control)	1.19	0.003044	5.379	0.10949
0.9 % Cr_2O_3	1.20	0.003414	5.893	0.13458
2.0 % Cr_2O_3	1.20	0.002377	6.331	0.10068
3.4 % Cr_2O_3	1.21	0.001927	6.076	0.07831
3.7 % Cr_2O_3	1.23	0.001612	5.990	0.06461
4.3 % Cr_2O_3	1.22	0.001545	5.936	0.06137
5.2 % Cr_2O_3	1.22	0.000864	5.703	0.03297

TABLE V

Unknown Peak (0.89nm) Characteristics as Measured Using XFIT Program

Bovine hide Samples (0.9nm Resolution)	Peak at 1.13 d Real space (nm)	Peak Height (Intensity)	Full Width half maxima d (nm^{-1})	Integrated Intensity
0 % Cr_2O_3 (Control)	0.889	0.00057	0.614	0.00232
0.9 % Cr_2O_3	0.889	0.00044	0.562	0.00167
2.0 % Cr_2O_3	0.889	0.00038	0.527	0.00134
3.4 % Cr_2O_3	0.888	0.00012	0.451	0.00037
3.7 % Cr_2O_3	0.889	0.00021	0.486	0.00070
4.3 % Cr_2O_3	0.889	0.00005	0.244	0.00009
5.2 % Cr_2O_3	0.887	0.00009	0.508	0.00031

TABLE VI

Unknown Peak (0.93nm) Characteristics as Measured Using XFIT Program.

Bovine hide Samples (0.9nm Resolution)	Peak at 1.07 d Real space (nm)	Peak Height (Intensity)	Full Width half maxima d (nm^{-1})	Integrated Intensity
0 % Cr_2O_3 (Control)	No peak			
0.9 % Cr_2O_3	No peak			
2.0 % Cr_2O_3	0.931	0.00102	0.527	0.00381
3.4 % Cr_2O_3	0.930	0.00138	0.583	0.00540
3.7 % Cr_2O_3	0.931	0.00253	0.553	0.00937
4.3 % Cr_2O_3	0.931	0.00245	0.565	0.00927
5.2 % Cr_2O_3	0.931	0.00184	0.587	0.00723

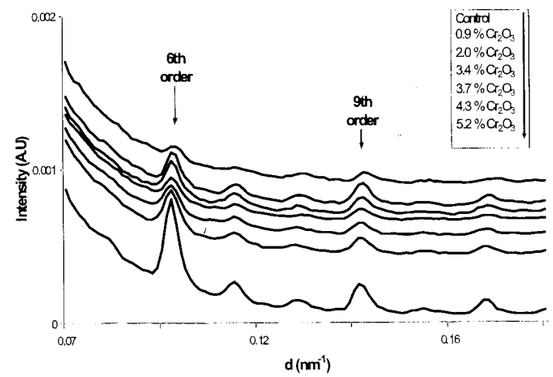

Figure 6. - X-ray scattering data of bovine hide treated with chromium obtained at station 2.1 SRS Daresbury at a sample to detector distance of 4m was used to obtain a linear trace. The intensity of the meridional diffraction peaks increases as a function of chromium treatment, the change in the position of the diffraction peaks corresponds to a lengthening of the D period with chrome tanning. (All linear traces have been normalised and offset).

lateral packing distance between the collagen molecules increases slightly after the addition of chromal to the bovine hide (table IV). The full width half maxima of the peaks increase on the addition of chromal, indicating an increase in the distribution of packing distances represented by the intermolecular lateral packing of collagen molecules. At 3% chromal concentration (2% chromium salt), the increase in distributions of packing distances seems to be at a maximum level with subsequent increases in concentration causing a reduction in packing distribution, possibly due to chemical penetration being more even. The integrated intensity, which represents the level of order within the structure, reduces with increasing chromium salt concentration.

The reduction in order associated with the intermolecular lateral packing distance of collagen seems to correlate with the loss of the peak at 0.89nm (table V). This peak is not due to chromium salt concentration as it is found in the control sample, but its integrated intensity does appear to reduce as chromium salt concentration increases. There is a variation in the distribution of the repeating unit responsible for this peak; this small sample run of chromium salt treated leather indicates that there is a possible link between chromium salt concentration and structural order.

The peak that occurs at d = 1.07 nm^{-1} is not present in the control sample and therefore is a diffraction characteristic

TABLE VII

XFIT Software Used to Determine Peak Profile Data of Bovine Hide Samples Analysed from X-ray Scattering Data Obtained from SRS Daresbury Station 2.1 at a Sample to Detector Distance of 8m (6nm Resolution).

Bovine hide (6nm resolution)	Quarter staggered array spacing (nm)	Peak Height (Intensity)	Full Width half maxima d (nm^{-1})	Integrated Intensity
0 % Cr_2O_3 (Control)	62.7	0.000173	1.192	0.001377
0.9 % Cr_2O_3	64.4	0.000274	0.893	0.001638
2.0 % Cr_2O_3	64.0	0.000276	0.899	0.001659
3.4 % Cr_2O_3	63.8	0.000226	0.945	0.001429
3.7 % Cr_2O_3	64.0	0.000269	0.900	0.001618
4.3 % Cr_2O_3	64.0	0.000356	0.877	0.002086
5.2 % Cr_2O_3	64.3	0.000627	0.680	0.002854

of the addition of chromal. The cumulative effect of the repeating unit at this peak only appears to be significant at chromal concentrations of 3% or more. The peak position does not change with increasing chromium concentration (table VI), but the peak height does. Subsequently there is an increase of order within the structure as chromium salt concentration increases, possibly indicating that this peak is due to the local structure of chromium salt crystallinity and not collagen chromium interactions.

Small angle x-ray diffraction with a resolution of 6nm

Collagen is known to contain a number of long range interactions resulting from axial order and substructures within the fibril. Observation of these features by X-ray diffraction requires long camera geometries in order to resolve closely spaced features in reciprocal space. Meridional peak positions can be determined accurately at a geometry that allows observation of diffraction from structural features to a real space resolution of 6nm. In particular the 6th order up to the 11th order, and a more intense 6th order indicating a lower level of sample hydration. The peak profiles (figure 6) are characteristic diffraction reflections of the gap and overlap regions between the collagen molecules in the axial direction. The relative intensities of the large 6th order in comparison with the 7th and 8th for example is typical of a dry collagen sample. The spacing between the orders is very similar for all the samples, with the exception of the control sample indicating chromium salt effect on the gap and overlap region of the collagen molecules. The XFIT program was used to give a more detailed interpretation of the peak profiles representing the characteristic axial staggered array (d-spacing) of the collagen molecules in table VII. The decrease in the breadth of the meridional diffraction peaks is indicative of the D periodic structure of the collagen fibrils becoming regular or indeed the overall length of the coherent crystallites becoming longer.

Conclusions

The effect of chrome tanning to different extents has been observed here as a function of structural order over a range of length scales. The data shown here suggests that the axial rise per residue of the collagen helix is altered by tanning; however this does not correspond to a concomitant increase in the contribution of the amorphous region of the fibre diagram indicating that the degree of gelatinisation is not increased. It may therefore be possible that significant alterations are made to the collagen helical backbone without total disruption. This will have a cumulative effect on the side by side molecular packing within the collagen fibril that leads to an increase in the intermolecular distances, as well as the variance of helical interactions. This may be due to deviations from the standard helix geometry that allows molecules to pack on a simple quasi-hexagonal lattice with a ~1nm spacing.[27] The nm length scale also reveals structural features that are altered by the tanning process; the evolution and change of sharp Bragg reflections at 4.4, 0.89 and 0.835 nm correspond to reflections observed to result from lipid diffraction, the interaction of Cr(III) ions with lipid bilayers will not only increase fundamental spacing but also increase the contrast between bilayers resulting in enhanced diffraction peaks.

The long range interactions of collagen are dominated by the axial repeating structure due to molecular staggering along the collagen fibril. This showed that the axial periodicity of the stagger between collagen molecules is increased upon tanning. The main change observed in these samples however is the increase in diffraction signal of Bragg peaks above the continuous background. This indicates that the overall long range crystallinity of the collagen molecules and the specificity of the axial order may be increased. It also may result in part from the enhanced electron density contrast made by the addition of Cr(III) ions to the structure. If the latter is the predominant effect, an important feature to note is that the intensity distribution amongst meridional reflections of the enhanced signal is the same as that of an untanned sample. This would imply that the labelling of Cr (III) along the length of the collagen molecule is relatively even and if specific to certain amino acids, must indicate that their distribution is even through the collagen molecule. Specific heavy atom staining by a number of metal species is known to occur and alter the intensity of meridional reflections in both the wet and dry samples.[28]

In relation to the increased measurements of shrinkage temperature as a function of chromal addition, this may be reflected in the broadening of the peak at approximately 1nm spacing which is characteristic of the side by side interactions of collagen molecules. Here the possible changes in conformation brought about by intermolecular cross-links may be producing a less locally ordered lattice structure.

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