

# MONITORING OF AVAILABLE DECORIN IN DIFFERENT PARTS OF BOVINE HIDE DURING ITS PROCESSING INTO LEATHER\*

by

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## ABSTRACT

During conversion of hides into leather, some hide constituents undergo changes and removal. Among those are decorin, biglycan, sulfated glycosaminoglycan (SGAG) and collagen. Properly monitoring the removal of the predominant and best understood proteoglycan of skin, decorin, was the focus of this work. An ELISA method was improved by dialyzing the guanidine-HCl-extracted proteins in the presence of collagenase, allowing us to obtain a more manageable sample with uniform background and in turn more reliable analytical data. ELISA results on the depletion of decorin in intact hide samples were evaluated and compared among the different parts of bovine hide before and after dialysis. There was a clear difference between undialyzed and dialyzed samples of raw intact hide, whereas after the tanning treatments, the available decorin content was significantly the same from different parts. The amount of decorin that was removed from each area of the hide (shoulder > butt > belly), after processing them using the standard USDA tanning procedure, was directly proportional to the initial amounts. The final available decorin remaining per gram of intact hide in leather (bated samples) was significantly the same in all parts. Based on the dialyzed samples, there was about a 70-78% reduction of available decorin content from raw hide to bated hide samples compared to ~90% in undialyzed samples. The results followed more closely the trend of the SGAG (carbohydrate part of decorin) content determination previously reported by this group, where about a 75% drop was observed from the initial available SGAG content in raw hide to bated hide samples.

## INTRODUCTION

It is generally acceptable and beneficial for leather quality, in particular for softness and flexibility,<sup>1</sup> to remove proteoglycans efficiently from the hide when it is processed into leather.<sup>2</sup> Currently, our efforts are directed toward the removal of the predominant and best understood proteoglycan of skin, decorin.<sup>3</sup> Decorin is a small extracellular matrix proteoglycan involved in several fundamental biological functions typically in “decorating” or in organization of collagen fibrils.<sup>4</sup> It consists of a core-protein and a dermatan sulfate side chain. The core protein has a molecular weight of ca. 40 kDa and the side chain has a molecular weight that varies between 20-50 kDa depending on species and tissue. The single molecules appear as horseshoe-shaped bodies (protein) attached to thin tails (side chain), as seen under the electron microscope. Most of the protein comprises a chain of ten “leucine-rich repeat” segments that tend to aggregate with each other and bind probably at two sites at each end of the collagen molecule.<sup>2</sup>

Kronick and coworkers<sup>2</sup> concluded that the protein part of the proteoglycan decorin survives the beamhouse even under conditions that remove the GAG (glycosaminoglycan) chain. However, a satisfactory leather product could not be produced from a hide of a decorin-free animal as decorin is needed to organize the collagen, as shown with knock-out mice.<sup>5</sup> If all of the decorin were removed from the hide, the collagen framework might collapse. Removal of the glycan during tanning treatments was shown<sup>1</sup> to be correlated with “opening-up” of the collagen fiber structure of the hide to produce softer leather.

## MATERIALS AND METHODS

### Extraction of Decorin from Powdered Hides

Intact hide samples were subjected to a standard tanning procedure of dehairing, reliming, delimiting and bating. A sample was taken at every step of the treatment and was then powdered

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under liquid nitrogen. A gram of each powdered hide was taken for protein extraction by adding 5 ml of Ca buffer solution followed by 5 ml of 8M guanidine-HCl (to final concentration of 4M) and 100 µl of Protease Inhibitor Cocktail (Sigma). The resulting mixture was stirred overnight and centrifuged the following day. The undissolved precipitate was discarded. Half of the supernatant was saved and taken as undialyzed samples. The other half (~3.0 ml) was treated with 24 µl of collagenase (20U) and dialyzed in PBS buffer (with stirring) overnight in the dialysis cassette (Pierce), bringing down the guanidine concentration to ~40 mM. The optimum amount of 20U collagenase was found to effectively (unreported results) remove the high viscosity of the samples due to collagen.

**Sandwich ELISA (Indirect) Procedure**

The Sandwich ELISA (indirect) procedure had been developed and optimized previously in this laboratory.<sup>6,8</sup> The assay procedure as well as calculations are detailed in that same reference. The general principle behind that procedure is that the adsorption antibody (PK-1, a rabbit anti bovine decorin) is first introduced into the wells. PK-1 (prepared by Alpha Diagnostics International, San Antonio, Texas) recognizes the 16-amino acid sequence at the N-terminus of bovine decorin. The wells are washed with PBS buffer and then blocked with 0.1 % Tween 20 in PBS buffer. The blocking solution is removed and the sample containing decorin is added. The wells are washed and then the primary antibody, 6D6 (courtesy of Dr. Paul Scott, University of Alberta), which recognizes a sequence of 4 amino acids at C-terminus (residues 241-244) of decorin, is added. After washing the wells, the secondary antibody, goat anti mouse IgG peroxidase, is added. Finally, a peroxidase reaction is induced by adding *o*-phenylenediamine dihydrochloride (OPD, Sigma Product No. P-9187) to all the wells. After incubating the samples for 20 minutes in the dark, a yellow color should appear in the wells. Adding 50 µl of 3 M H<sub>2</sub>SO<sub>4</sub> to all the wells stops the reaction, changing the yellow color to a stable orange color. The amount of decorin is directly proportional to the intensity of orange color and the absorbance is read at 492 nm.

**Calculations**

A standard curve is generated by plotting net absorbance readings at 492 nm from known series of standard concentrations (after averaged absorbance readings from the blank is subtract-

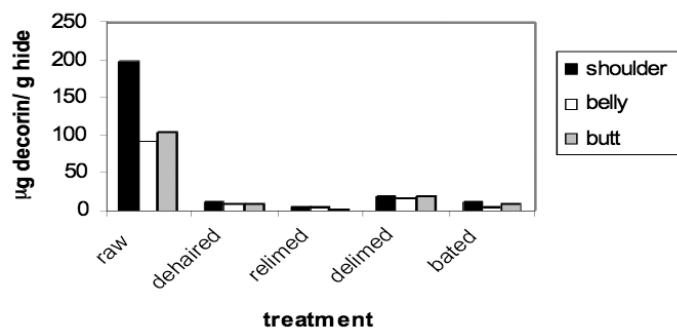


Figure 1: Comparison of available decorin content (µg decorin/g hide) in three hide parts as monitored during tanning of hide to leather before dialysis.

ed) in Y axis versus the known concentrations in µg standard decorin as X-axis. The slope of the curve is then derived. The unknown sample concentration could then be interpolated from the standard curve or be calculated by using the derived slope with corresponding standard deviations applied accordingly (Equation 1).

$$A = (P/M) * Df \tag{1}$$

$$A = [(net\ Absorbance\ of\ Sample) / slope\ of\ curve] * [dilution\ factors]$$

**A** = Available decorin in g/g hide

**P** = (Abs of Sample - Abs of blank) + standard deviations

**M** = slope of standard curve (abs/µg decorin)

**Df** = dilution factors = [Total V of Extract (or 15.1ml) /gram hide] \* [V after dialysis/ V before dialysis] \* [1/V aliquot taken for analysis (or 0.05ml)].

**Statistical Analysis**

The data on decorin have been analyzed by analysis of variance (ANOVA) to determine the effects of treatment, (raw, dehaired, bated, etc.), sample (shoulder, butt and belly), and extraction type (dialyzed and undialyzed ) and their interactions. Means were separated using the experiment-wise Bonferroni LSD technique.<sup>9</sup>

**RESULTS AND DISCUSSION**

The amount of available decorin found in undialyzed raw hide samples from shoulder (200 µg decorin/g hide) is almost double those from belly (93.1 µg /g) and butt (105.4 µg /g), as

**TABLE I**  
**Available Decorin Content (µg Decorin/G Hide) in Undialyzed Extracts from Samples Taken During the Tanning of Intact Hide to Leather**

	Shoulder	Belly	Butt
<b>Raw</b>	200.4	93.1	105.4
<b>Dehaired</b>	12.0	10.4	10.3
<b>Relimed</b>	6.0	4.6	4.0
<b>Delimed</b>	18.7	16.9	18.9
<b>Bated</b>	12.6	4.4	9.8

**TABLE II**  
**Comparison of Decorin Content ( $\mu\text{G}/\text{G}$  Hide) Before and After Dialysis of Extracts (From Shoulder Tissue)**

Shoulder Hide	Extracted Solutions Before Dialysis	Extracted Solution After Dialysis
Raw	200.4	56.6
Dehaired	12.0	12.8
Relimed	6.0	10.3
Delimed	18.7	18.1
Bated	12.6	12.3

**TABLE III**  
**Available Decorin Content ( $\mu\text{g}/\text{g}$  hide) in Three Different Hide Parts during Tanning Treatments after Dialysis**

	Raw	Dehaired	Relimed	Delimed	Bated
<b>Shoulder</b>	56.6	12.8	10.3	18.1	12.3
<b>Belly</b>	25.5	11.3	10.3	17.4	10.6
<b>Butt</b>	51.7	10.5	10.2	17.1	11.6

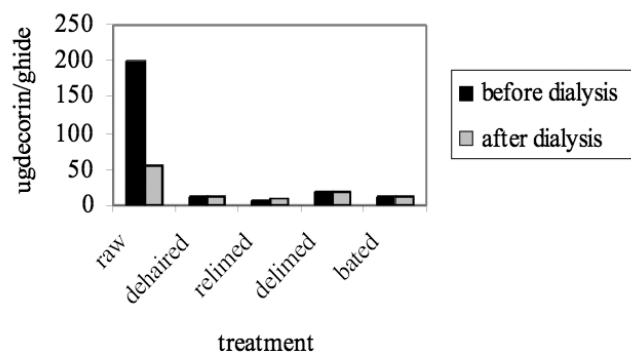


Figure 2: Direct comparison of results before and after dialysis of available decorin content during tanning treatments of hide (from shoulder) to leather.

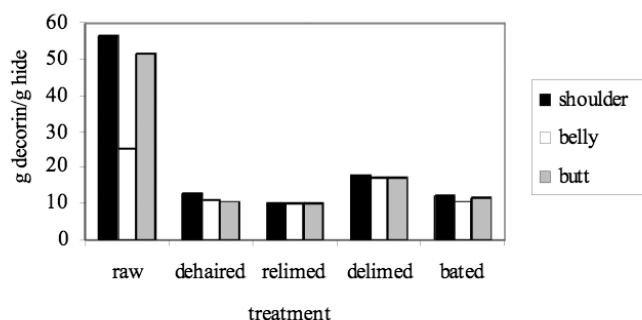


Figure 3: Comparison of available decorin content ( $\mu\text{g}/\text{g}$  hide) in three hide parts as monitored during tanning of hide to leather after dialysis.

shown in Table I and Figure 1. After the dehairing step, ~94% of available decorin from shoulder, ~89% from belly and ~90% from butt samples are unaccounted for. During the reliming step, about half more of decorin left after dehairing is gone, but surprisingly, during deliming, the amount of

available decorin is somewhat recovered back to the dehairing step values.

A possible cause would be incompatibility or inhibition of reagents in dehairing and reliming but enhancement from reagents present in the deliming step. Alternatively, deliming may open up more decorin that had been unextractable during the prior steps. Since decorin is so tightly bound to collagen, there is going to be some decorin that cannot be removed from the hide. At the end, only about  $10 \pm 3\%$  of the original available decorin in raw hide is left after bating the sample to leather.

To determine if the trend is realistic or if there are some nonspecific interactions with the ELISA technique, a more reliable and more decorin-specific method of analysis is essential by removing any matrix interferences from the samples. Our current results in Tables II and III (and Figures 2 and 3) show that after dialyzing the samples with collagenase, the initial available decorin in raw hides has diminished by 70% in shoulder tissue (from 200.4 to 56.6  $\mu\text{g}$  decorin/g hide) and belly (from 93.1 to 25.5), while about 50% in the butt tissue (from 105.4 to 51.7).

The dehairing treatment showed that the amount of decorin in  $\mu\text{g}$  per g of hide from shoulder in undialyzed (12.0) and dialyzed (12.8) samples (Table II; Figure 2) are significantly the same ( $p < 0.05$ ). There is only a slight increase of values from deliming over the preceding reliming step (from 10.3 to 18.1) in dialyzed compared to a noticeable increase (from 6 to 18.7) in undialyzed samples. Dialysis could have eliminated some anomaly due to matrix interferences, which can manifest itself as either a false increase or false decrease in true analyte levels.

It is also shown that the same values for dehaired and bated samples are obtained before and after dialysis. Could this be

that the dehairing step has eliminated most of the interfering elements present in the raw hide? The dehaired results are almost recovered during deliming. There is no remarkable increase of values as previously observed during deliming. In reliming, the pH of the sample is extremely increased, a lowering of decorin content detectable by the ELISA method may have occurred. The high pH (very basic) and high lime concentration could be masking and interfering with the ELISA method, so that after removing the alkali by dialysis, the true higher values similar to the dehaired and bated samples are revealed.

When bated samples were dialyzed, all hide parts gave almost the same amount of decorin ( $\sim 11.5 \pm 0.9 \mu\text{g}$ ) per gram of hide. Dialyzing with collagenase removed the varying amounts of collagen, keratin, salts and other ingredients in raw hides. Keratin from hair and fatty membrane of the skin must also have brought additional enhancing contaminants to ELISA determinations. This can be inferred by the fact that the skins from shoulder and butt are thicker and have higher amounts of interfering contaminants from collagen and fats that were removed by dialysis.

### CONCLUSION

There is a clear variation of initial decorin content in the three major sampling parts of the raw hides—shoulder, belly, and butt. After dialyzing the samples in the presence of collagenase, the variation was still observed except that the values decreased to only about 28% from shoulder (200 to 56.6) in  $\mu\text{g}$  decorin/g hide and belly (93.1 to 25.5), while from the butt, the value decreased to just half the undialyzed value (from 105 to 51.7). The relative increase of decorin content from the relimed hide to the delimed hide could probably be from the “opening-up effect” on the hide, which makes more decorin available to analyze.<sup>1</sup> When the hide is bated, however, the same lower amount found in dehaired and relimed steps is recovered. Since decorin is so tightly bound to collagen, there is going to be some decorin that cannot be removed from the hide. Overall, the amount that is being removed from each area of the hide (shoulder > butt > belly) is directly proportional to the initial amount of decorin in order to attain almost the same final amount of  $\sim 11.5 \pm 0.9 \mu\text{g}$  decorin per gram of hide in the bated samples.

The same trend in amount of decorin removed in every step during tanning procedure is observed whether the hide is first powdered<sup>8</sup> or still intact. In essence, based on the dialyzed samples, there is about a 70-78% reduction of available decorin content from raw hide to bated hide samples compared to  $\sim 90\%$  in undialyzed samples. The dialyzed results follow more closely the same trend of the SGAG content determination,<sup>7</sup> where about a 75% drop was observed from the initial available SGAG content in raw hide to bated hide samples. This shows that almost all of the matrix contaminants or interferences have been removed during the tanning treatments and dialysis.

The ultimate goal is to develop a respectable correlation between the amount of decorin and the physical characteristics of a given leather sample. The same sample can be used to study and determine tensile strength, stretchability and softness. Since the interaction between collagen and decorin is very strong, we need to find something else that will break that bond between the two molecules, thereby letting us expose more decorin. We are currently tanning hides in the presence of alkaline protease and bating with pepsin to see if this will help us expose more decorin.

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