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Post Mortem Changes of Glycoconjugates in Meat

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SUMMARY: In the present study proteoglycans were extracted from *M.semimembranosus* of young bulls after 0, 7, 14 and 21 days of *post mortem* storage by use of 4M guanidin-HCl in 0.05 M acetate buffer pH 6. The extracts were applied to ultracentrifugation in a gradient of CsCl and the fractions obtained studied by SDS polyacrylamide gel electrophoresis. Two different types of proteoglycans were present; one of high molecular weight which appeared in the high density fractions and another with an appearant molecular weight above 100.000 which appeared in the low density fractions. A reduction in the size of the high molecular weight proteoglycans was observed as a result of *post mortem* storage.

INTRODUCTION: Several studies have been performed on *post mortem* degradation of meat proteins using gel electrophoresis. It has been suggested that *post mortem* degradation of meat involved breakdown of glycoconjugates (McIntosh, 1967, Dutson and Lawrie, 1974). We have reported previously that perimysium from *M.semimembranosus* contained chondroitin-4-sulfate and dermatan-sulfate as a part of the proteoglycan molecules (Eggen and Waddington, 1989). The methods used were gelfiltration, cellulose acetate electrophoresis and ELISA. The present study was undertaken in order to establish methods of obtaining glycoconjugates from whole meat in a way that made the study of proteoglycans by use of conventional gel electrophoretic techniques possible. Proteoglycan breakdown *post mortem* was then studied by use of SDS polyacrylamide gel electrophoresis.

MATERIALS and METHODS: Extraction: *M. semimembranosus* from young bulls were obtained from the local slaughterhouse immediately after slaughter and brought to the laboratory. After the epimysium had been removed, the muscle was minced, divided randomly, vacuum packed and stored for 0, 7, 14 and 21 days respectively at a temperature of 15 °C. At the end of the storage period the portions were frozen at -80 °C. The samples were homogenized in liquid nitrogen and incubated in an extraction buffer consisting of 4 M guanidin-HCl and 0.05 M sodium acetate of pH 6 (10 ml buffer to 1 g tissue). The solution contained furthermore protease inhibitors (for review: Heinegård and Sommarin, 1987). The suspensions were left for 16 hours at 4 °C under gentle stirring, centrifuged and the sediments reincubated with fresh extraction buffer. The supernatants were clarified by centrifugation and filtration through cheese clothes. Samples of the supernatants were dialysed against distilled water, freeze dried and used for SDS polyacrylamide gel electrophoresis.

Ultracentrifugation: Before ultracentrifugation the supernatants were concentrated to 1/3 of the original volume in an Amicon ultrafiltration cell on a PM 30 filter under the influx of nitrogen. CsCl was added to the concentrates to a density of 1.37 g/ml, and ultracentrifugation was carried out at 140.000 g in a Beckman Ultracentrifuge, Optima L-80 for 92 hours. The gradients were harvested by puncturing the bottom of the tubes. The eluates were divided into 5 fractions called D1, D2, D3, D4 and D5. D1 represented the bottom fraction. Each fraction were monitored for density, the contents of uronic acid and

protein. The top of the tube consisted of a red, sticky surface layer which was discarded. Analytical methods: Densities were measured with a 200 ul pipette as pycnometer. The protein content was estimated by the Bio-Rad assay based on the method of Bradford (1976), whereas the amount of uronic acid was estimated by dimethylmethylene blue (DMB) (Farndale *et al.*, 1982). SDS polyacrylamide gel electrophoresis of the freeze dried supernatant samples as well as the gradient fractions after ultracentrifugation was carried out on gradient gels ranging from 3 -12 % by use of the discontinuous buffer system described by Laemmli (1970). Proteoglycans from the gradient fractions were precipitated with ethanol and dissolved in SDS-buffer prior to electrophoresis. Bands were visualized by commassie or silver staining (Merrill *et al.*, 1981). Scanning of the gels was performed by use of LKB Ultroscan XL Laser Densitometer.

RESULTS and DISCUSSION: Previously it has been shown that about 90 % of the proteoglycans in perimysium dissected from bovine *M.semimembranosus* was extracted by use of 4M guanidin-HCL based on measurements of the content of uronic acid in the supernatant and the sediment (Eggen and Waddington, 1989). In the present study whole muscle preparations homogenized in liquid nitrogen were preferred as the source for proteoglycans. The same buffer solution was, however, used for extraction. The use of whole muscle preparations made it possible to obtain the total proteoglycan population extractable by 4M guanidin HCl and not only the proteoglycans present in the pieces of dissected perimysium. The results from ultracentrifugation are shown in Table 1. The fractions of densities around 1.5 showed the highest content of uronic acid, whereas the major proportion of the proteins were concentrated in the top fractions of lower densities. SDS polyacrylamide gel electrophoresis of the uronic acid containing fractions after ultracentrifugation showed high molecular weight proteoglycans in the area of the gel representing molecular sizes above 200.000, Figure 1. This material was only detectable by the silver staining technique. The results showed furthermore that the high molecular weight proteoglycans obtained from samples stored for 7, 14 and 21 days penetrated deeper into the gel. The migration distance increased with increasing time of storage *post mortem*, Figure 1 and 2. *Post mortem* degradation of *M.semimembranosus* thus involved breakdown of glycoconjugates of high molecular size.

Table 1: The table shows the density of the fractions from CsCl-ultracentrifugation and the distribution of uronic acid and protein in the fractions prepared from meat samples after a period of storage of 0, 7, 14 or 21 days

	0 days			7 days			14 days			21 days		
	density g/ml	uronic acid µg/ml	protein mg/ml	density g/ml	uronic acid µg/ml	protein mg/ml	density g/ml	uronic acid µg/ml	protein mg/ml	density g/ml	uronic acid µ/ml	protein mg/ml
D1	1.51	34	0.5	1.49	35	0.4	1.50	42	0.6	1.49	36	0.2
D2	1.42	36	0.4	1.42	35	0.6	1.43	40	1.2	1.40	40	0.3
D3	1.39	26	0.5	1.39	33.4	1.3	1.39	30	1.6	1.38	26	0.1
D4	1.37	24	0.9	1.34	12	2.1	1.37	23	3.0	1.34	23	1.0
D5	1.31	6	2.9	1.32	0.6	4.0	1.31	3.4	3.5	1.31	4	4.2

Figure 1: The figure shows the pattern obtained by SDS polyacrylamide gel electrophoresis of the fractions with densities above 1.4 g/ml. LMW represents a protein standard preparation of low molecular weights whereas HMW represents a standard preparation of low molecular weights

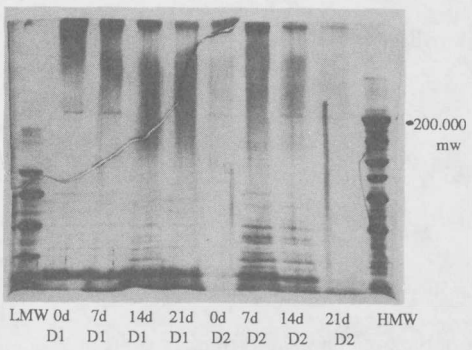


Figure 2: The figure shows the densitometric scans of gels with the high molecular weight proteoglycans

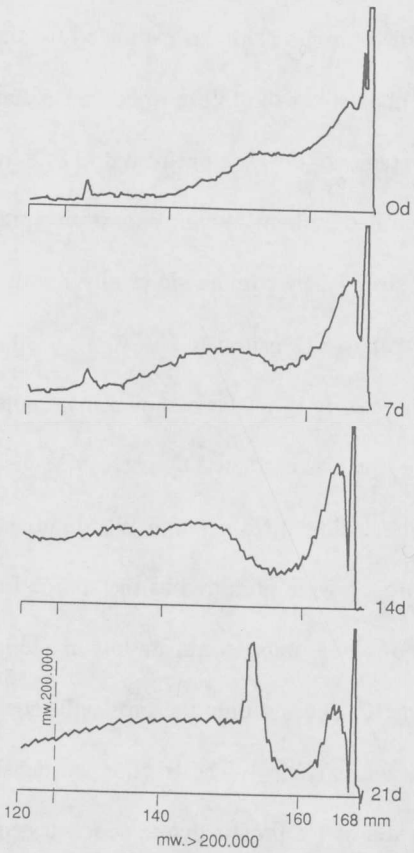
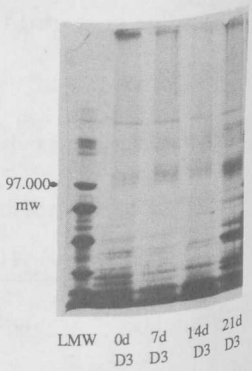


Figure 3: The figure shows the pattern from SDS polyacrylamide gel electrophoresis of uronic acid containing fractions with lower densities



A decomposition of the glycoconjugate matrix will most likely influence the mechanical stability of the tissue. Another population of proteoglycans of lower bouyant density with a molecular size around 100.000 was observed by gel electrophoresis of the fractions of densities around 1.34, Figure 3. The gel pattern was like that obtained by electrophoresis of small proteoglycans isolated from sclera, Figure 4. Treatment of the component by chondroitinase ABC resulted in a band around 40.000 whereas the original band disappeared. Figure 5 a and b show the results obtained by electrophoresis of freeze dried samples of the supernatants before ultracentrifugation.

Figure 4: The figure shows the results from electrophoresis of small proteoglycans from sclera before and after chondroitinase ABC treatment. Lane a) represents the protein standard preparation, b) represents the enzyme, c) represents small proteoglycans from sclera whereas d) represents enzyme treated proteoglycans from sclera

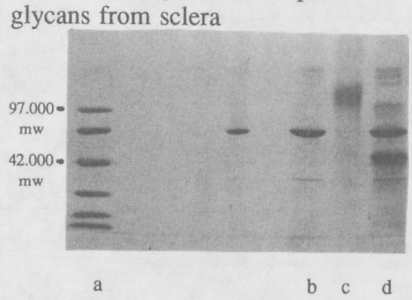
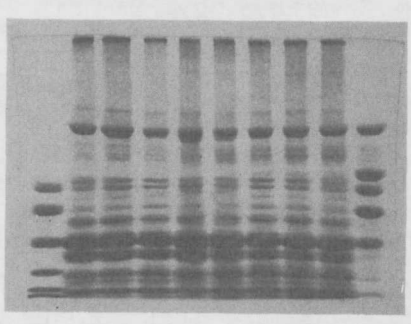
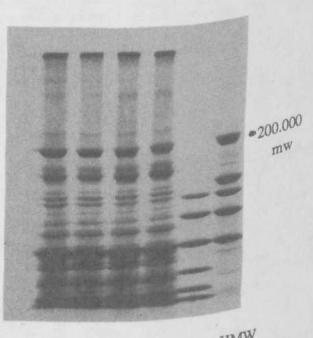


Figure 5 a and b: The figures show the patterns obtained by SDS gel electrophoresis of the supernatants of nitrogen powdered samples after various periods of storage *post mortem*





Comparison of the gels obtained from electrophoresis of the supernatants before and after ultracentrifugation showed that the proteoglycans were hardly visible in the gels before ultracentrifugation. The reason for this is most likely that the proteoglycans are minor components of the muscle and have in contrast to other proteins a weak binding capacity for Coomassie stains.

CONCLUSIONS: Extraction of nitrogen powdered whole meat samples in 4M guanidin-HCl with subsequent ultracentrifugation in a gradient of CsCl was a convenient method for preparation of proteoglycans. SDS polyacrylamide gel electrophoresis on gradient gels ranging from 3 - 12 % with subsequent silver staining of the gels made it possible to study the proteoglycans and *post mortem* degradation of these components. Two types of proteoglycans were detected; one of high molecular weight which appeared in the fractions of the highest densities, and another one which appeared in the fractions of lower densities. The results showed furthermore a reduction in size of the high molecular weight proteoglycans after 14 days of storage.

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