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# Inseparable iduronic acid-containing proteoglycan $PG_{(IdoA)}$ preparations of human skin and post-burn scar tissues: evidence for elevated levels of $PG_{(IdoA)}$-I in hypertrophic scar by *N*-terminal sequencing [1]

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**Abstract**

Hypertrophic scarring is characterized by disordered collagen fibrils. In order to determine whether this is, in part, a result of changes in the population of proteoglycans that are thought to be involved in regulation of collagen fibril formation, we have compared PGs from post-burn normal and hypertrophic scar tissue, as well as from human dermis and epidermis. Efforts to separate the two major iduronic acid-containing proteoglycans, decorin [$PG_{(IdoA)}$-II] and biglycan [$PG_{(IdoA)}$-I], for quantitation were not successful. The different *N*-terminal sequences of these two iduronic acid-containing proteoglycans $PG_{(IdoA)}$-I and -II were utilized to estimate the relative amounts in the above $PG_{(IdoA)}$ preparations. Normal scar, dermis and epidermis were all found to contain primarily decorin with low (< 10%) levels of biglycan relative to decorin. In contrast, iduronic acid-containing proteoglycans from hypertrophic scar were found to be approximately

Abbreviations: GAG, glycosaminoglycan; $PG_{(IdoA)}$, iduronic acid-containing proteoglycan; $PG_{(IdoA)}$-I, biglycan; $PG_{(IdoA)}$-II, decorin.

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30% biglycan [$PG_{(IdoA)}$-I]. This may be a proximal cause of altered collagen fibrils, or may result in alterations in the sequestration of growth factors, which then results in changes in collagen that effect the appearance of the scar. © 1996 Elsevier Science Ltd.

*Keywords:* Skin; Proteoglycan, iduronic acid-containing; Scar, hypertrophic

## 1. Introduction

Scarring, in particular the hypertrophic type, is a common result of the healing of burn wounds. It is characterized by overgrowth of connective tissue beyond the confines of the original wound. Histologically, it is characterized by rather disordered collagen fibrils, as opposed to normal scarring, in which the collagen fibrils lie parallel to each other. Little work has been performed on the biochemical changes associated with hypertrophic scarring. This investigation was performed with a view to extending the previous finding [1,2] that the small, iduronic acid-containing proteoglycans [$PG_{(IdoA)}$s] from scar tissues were different from those from normal skin. In those studies, we found subtle differences in PG post-translational modifications present in human skin and scar tissues. These included: (a) type and length of glysoaminoglycan (GAG), (b) their hexuronic acid content, (c) the ratio of 0-, 4-, and 6-sulfation, and (d) properties of protein core released after treatment with CS ABCase. These modifications appear responsible for relevant differences in normal and scarred skin. It is known that the small, iduronic acid-containing PGs are involved in the regulation of collagen fibrillogenesis [3]. They may, therefore, be a factor in causing the abnormal disorganization of collagen in hypertrophic scar. Iduronic acid-containing proteoglycans [$PG_{(IdoA)}$s] containing one or two glycosaminoglycan (GAG) chains have been reported in bovine [4], pig [5,6], rat [7], and human skin and post-burn scar tissue [1,2]. There are two types of small, iduronic acid-containing PGs, I and II, in fetal calf skin [8]. These have also been called biglycan and decorin, respectively. The $NH_2$-terminal amino acid sequences of the two bovine skin-derived $PG_{(IdoA)}$s were identical to those of bovine cartilage $PG_{(IdoA)}$-I and $PG_{(IdoA)}$-II but different, although clearly closely related, to those from human bone $PG_{(IdoA)}$-I [9] and bone, placenta and post-burn scar $PG_{(IdoA)}$-II [1,9]. The two human $PG_{(IdoA)}$s can readily be differentiated by *N*-terminal sequence analysis; biglycan [$PG_{(IdoA)}$-I] has Ala at position 7, while decorin [$PG_{(IdoA)}$-II] has Ile at position 6. This investigation was initiated to determine whether $PG_{(IdoA)}$s from human skin and post-burn scar contain both biglycan and decorin. The isolated $PG_{(IdoA)}$s were identified and approximately quantitated by *N*-terminal amino acid sequencing.

## 2. Experimental

*Materials.*—Mature scar tissue (i.e., that harvested five years or more after burn injury [10] was obtained from the Shriners Burn Institute, Boston unit; human skin from the abdomen and thigh of an 11-year-old male was obtained from the Massachusetts General Hospital one hour after surgery. Epidermal tissue ($< 0.1$ mm thickness from cadavers) came from the Shriners Burns Institute Skin Bank.

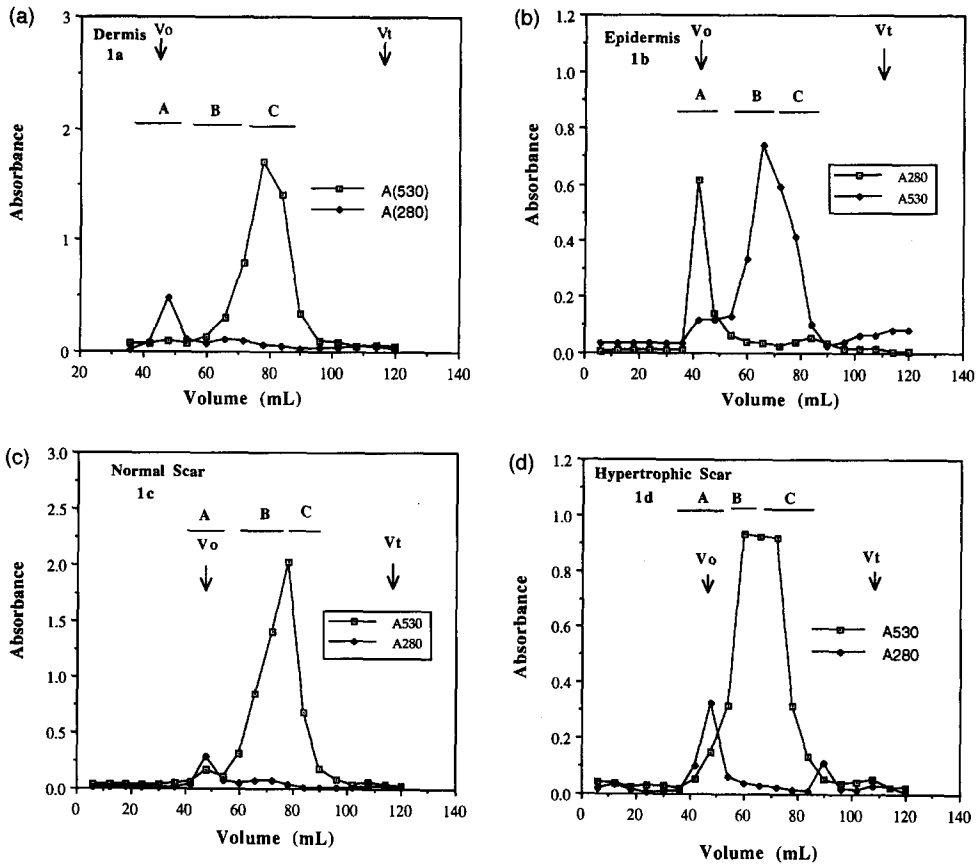


Fig. 1. (a) Fractionation of $PG_{(IdoA)}$s from human dermis, (b) epidermis, (c) normal scar, and (d) hypertrophic scar by Sepharose CL-6B chromatography (1 × 142 cm) with 0.5 M sodium acetate, pH 5.8. The column fractions (3 mL) were analyzed to determine the uronic acid and protein contents. The arrows marked $V_0$ and $V_t$ indicate the elution position of dextran blue ($M_r$ 200 kDa) and glucuronic acid, respectively. Pools A, B, and C were made as shown by bars above the chromatograms.

*Preparation and purification of $PG_{(IdoA)}$s from human skin and post-burn scar tissue.* —Skin and post-burn scar tissue were extracted with 4 M guanidinium · HCl. The iduronic acid-containing proteoglycans were fractionated on a DEAE-cellulose column and were then purified by ethanol precipitation as previously described [1,2]. The $PG_{(IdoA)}$s were further purified by sequential gel-filtration, ion-exchange chromatography and a further gel-filtration step as previously described [11]. The protein and uronic acid content of the column fractions were determined by measuring their absorbance at 280 nm and by the carbazole reaction [12]. Three pools of fractions from the final Sepharose CL-6B gel filtration column were made: a protein-rich pool A (the void volume) and pools B and C (Fig. 1).

N-*terminal amino acid sequencing.*—After chromatography, dialysis, and lyophilization, the *N*-terminal amino acid sequences of different pools of $PG_{(IdoA)}$ fractions from

human skin and post-burn scar tissue were determined by standard Edman chemistry on an Applied Biosystems model 477A gas-phase sequencer [11].

## 3. Results and discussion

Iduronic acid-containing proteoglycans were isolated from skin and different types of scars using standard procedures, and the uronic acid-containing fractions were analyzed by size-exclusion chromatography. Our original intention was to promote the formation of $PG_{(IdoA)}$-I aggregates as described by Choi et al. [8], thus separating biglycan [$PG_{(IdoA)}$-I] from decorin [$PG_{(IdoA)}$-II]. However, analysis of separate gel-filtration pools indicated that the two $PG_{(IdoA)}$s were not being separated under our chromatographic conditions. It was thought that the major iduronic acid-containing PG was decorin [$PG_{(IdoA)}$-II], while a minor component might be biglycan [$PG_{(IdoA)}$-I]. These differ in their functional properties. Both of these PGs have glycosaminoglycan (GAG) chains in their *N*-terminal region. The difference between these two macromolecules is that biglycan has two GAG chains attached at two different sites [13].

The criterion for purity of the small $PG_{(IdoA)}$s was that the first two amino acids at the *N*-terminus were Asp-Glu, identical with those found in all small, iduronic acid-containing PGs examined to date {with the exception of fibromodulin, which has pyroglutamic acid at the *N*-terminus and will not give an *N*-terminal sequence by direct Edman degradation [14], and lumican, which is also blocked (Neame, Lorenzo and Heinegård, unpublished)}. In all cases except pool A, $PG_{(IdoA)}$-II was the major component present (70–90%). The majority of the $PG_{(IdoA)}$ was found in pools B and C, the major uronic acid containing fractions. In contrast to previous work [1,2], we were able to differentiate two *N*-terminal sequences after Edman degradation had progressed beyond five cycles. Human biglycan [$PG_{(IdoA)}$-I] and decorin [$PG_{(IdoA)}$-II] differ at residues 6 and 7. The *N*-terminal sequence of human biglycan is Asp-Glu-Glu-Ala-Ser-Gly-Ala-Asp-Thr..., whereas human decorin is Asp-Glu-Ala-Ser-Gly-Ile-Gly-Pro-Glu.... In all cases, we detected Ile at position 6, consistent with human decorin [15]. In the majority of cases, we were also able to detect some Ala at position 7, which is found in human biglycan [16]. Using the molar yields of these amino acids, we were able to analyze the relative amounts of biglycan and decorin in human skin samples.

The results of the sequence analysis of the crude pools of $PG_{(IdoA)}$s are given in Table 1. There is some risk associated with using *N*-terminal sequencing as a mode of quantitation, in that *N*-terminals can be partially blocked, and the repetitive yield is sequence-dependent. However, as the *N*-terminals of these two $PG_{(IdoA)}$s are similar, their *N*-terminal yields will be a good indication of the relative amounts of the two proteoglycans. Attempts were made to separate the two species of iduronic acid-containing PGs on Sepharose CL-6B columns under associative conditions [8]; the elution profiles of epidermis, dermis, and normal and hypertrophic scars are shown in Fig. 1a–d, respectively. The pooled fractions are represented by bars. Based on *N*-terminal analysis of the GAG-containing pools, there was no separation of the two iduronic acid-containing proteoglycans.

These data indicate that the quantities of two different types of proteoglycans,

Table 1
Edman degradation of skin and scar $PG_{(IdoA)}s$ [a]

| Pool | Cycle | Amino acid | (pmol) | Yield pmol (estimated) | |
|---|---|---|---|---|---|
| | | | | Biglycan | Decorin |
| *Epidermis* | | | | | |
| B | 6 | Ile | 62 | | |
| | 7 | Ala | 6 | | |
| C | 6 | Ile | 162 | | |
| | 7 | Ala | 6 | 12 | 224 |
| *Dermis* | | | | | |
| B | 6 | Ile | 81 | | |
| | 7 | Ala | 3 | | |
| C | 6 | Ile | 250 | | |
| | 7 | Ala | 22 | 25 | 331 |
| *Normal scar* | | | | | |
| B | 6 | Ile | 121 | | |
| | 7 | Ala | 0 | | |
| C | 6 | Ile | 93 | | |
| | 7 | Ala | 8 | 8 | 214 |
| *Hypertrophic scar* | | | | | |
| B | 6 | Ile | 2 | | |
| | 7 | Ala | 79 | | |
| C | 6 | Ile | 217 | | |
| | 7 | Ala | 23 | 102 | 219 |

[a] Yields of Ile (cycle 6) and Ala (Cycle 7) obtained during sequence analysis of pools B and C from Fig. 1. The nmol yield was corrected for background, which represented between 5 and 15% of the total signal. Pool A did not contain significant levels of leucine-rich PG sequence.

biglycan and decorin, in scar tissues differ substantially in comparison to normal skin and normal scar. It is known that decorin binds to type I collagen and that biglycan[$PG_{(IdoA)}$-I] does not. Decorin [$PG_{(IdoA)}$-II] also binds TGF-ß [17], type VI collagen [18] and inhibits fibroblast adhesion to fibronectin substrates [19]. The exact role of biglycan is unknown. Biglycan is found in the pericellular region of the extracellular matrix and may modulate the interaction of cells with their environment [20]. TGF-ß has been shown to up-regulate biglycan and versican gene expression while at the same time it reduces the expression of decorin [21]. It is possible, therefore, that the high levels of biglycan found in hypertrophic scar are a result of TGF-ß activity. A further possibility, comparing small PGs in skin with those in cartilage, is that the biglycan is normally not substituted with glycosaminoglycan, but in hypertrophic scar it is substituted with glycosaminoglycan [22]. If it was not substituted with glycosaminoglycan, then we would not isolate it using conventional methods for purifying proteoglycans.

## 4. Conclusions

This method, utilizing *N*-terminal sequence of characterization of $PG_{(IdoA)}$-I and II can determine the amounts of two types of $PG_{(IdoA)}$s present in inseparable iduronic acid-containing PGs. This information is very useful as far as human skin and post-burn scar PGs are concerned, in order to understand the mechanisms uniquely associated with post-burn wound healing. Hopefully, this knowledge will be beneficial to plan strategies to modulate scar formation after burn injury, which is our ultimate goal.

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