Glycosaminoglycan synthesis in skin fibroblasts from patients with osteogenesis imperfecta

R. Kapoor, S. Bourier and P. Prehm

Department of Cell Biology, Max-Planck-Institute for Biochemistry, D-8033 Martinsried, FRG

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Glycosaminoglycans were analysed from skin fibroblasts with osteogenesis imperfecta (OI) IIA and IIB. The content of sulphated glycosaminoglycans was greatly increased over age-matched controls and to a lesser extent with respect to older age control. Dermatan sulphate in comparison with older control was unaltered in the cells of OI IIA and IIB. The concentration of heparan sulphate was higher in the cells than in the medium, whereas hyaluronic acid, chondroitin sulphate and dermatan sulphate content was higher in the medium. The level of hyaluronic acid was greatly elevated in the medium of OI IIB with respect to both controls.

Osteogenesis imperfecta *Glycosaminoglycan* *Skin* *Fibroblast* *Ageing*

1. INTRODUCTION

Osteogenesis imperfecta (OI) is a genetically inherited disorder of the connective tissues which has been subdivided into at least 5 types according to clinical and biochemical similarities [1]. At present the pathogenesis of this disease is not clearly understood although ultrastructural [2,3] and biochemical [4,5] studies of this disorder in various tissues have been concerned with the analysis of the extracellular matrix components like collagen, glycosaminoglycans (GAGS) and glycoproteins [6,7]. These studies generally reflect an altered metabolism of one or more of the extracellular matrix components, such as an alteration in the ratio of the type I to type III Collagen [8,9]. However, the studies on GAGS produced by cells in culture and tissues with different types of OI have been inconclusive, and both an increase and a decrease in their content has been reported [10–12]. Some of the changes in GAGS have been attributed to an altered metabolism of hyaluronic acid (HA), but very little is known of changes in OI of the different sulphated GAGS such as chondroitin sulphate (CS), heparan sulphate (HS) and dermatan sulphate (DS). This study was undertaken on skin fibroblasts representing a rare but lethal form of OI (OI IIA and IIB) and our purpose was to examine whether the metabolism of some or all of the different GAGS produced by these cells is altered in comparison with normal adult and foetal skin fibroblasts.

2. MATERIALS AND METHODS

2.1. *Cell culture*

Normal human skin fibroblasts from newborn infants and foetuses, skin fibroblasts from foetuses with OI IIA and IIB, were obtained from Dr D.W. Hollister and had been used in a previous study on collagen related to OI [13]. These cells were plated in 25 cm^2 falcon flasks at a density of 1.5×10^3 cells/cm^2. The cells were cultured at 37°C in Eagle's Minimal Essential medium containing 100 units/ml streptomycin–penicillin and 100 mcg/ml kanamycin. After 3 days the culture medium was replaced by Eagle's Minimal Essential medium containing 5 μCi/ml [^{3}H]glucosamine hydrochloride.

2.2. *Preparation of GAGS*

GAGS were isolated separately from the cells and their culture medium. The medium was decanted from the culture flasks and the cells detached

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from the dish by incubation with 0.5 ml trypsin at 37°C for 3–5 min. The cell suspension was then homogenized with a teflon homogenizer followed by digestion with papain [14], in the presence of 0.5–1.0 mg carrier chondroitin-4-sulphate. The digest was dialysed against distilled water and lyophilised.

The medium was dialysed against distilled water and lyophilised. This sample was then digested with papain [14] in the presence of 0.5–1.0 mg chondroitin-4-sulphate followed by dialysis against water and lyophilisation. The lyophilised fractions of cells and medium were applied to columns (0.5 × 5 cm) of DEAE-sephacel in 1 ml water. The columns were eluted with 3 bed volumes each of 0.02 M NaCl and 2.0 M NaCl. Majority of the labelled GAGS were recovered in the 2.0 M NaCl eluate, which was lyophilised for further analysis.

2.3. *Electrophoresis*

Cellulose acetate electrophoresis of samples (10 μl) was carried out in 0.1 M barium acetate (pH 5.0) at 4°C, as in [15] with the following modifications. The electrophoresis was performed at 250 V for 30 min followed by immersion of the strip in 20% alcohol buffer (v/v) for 3 min. Subsequently, the cellulose acetate strip was electrophoresed for a further 20 min at 250 V. The cellulose acetate strip was then cut into 1 mm wide sections, mixed with 5 ml of Rotizant scintillant and counted in a Packard TriCarb 460C scintillation counter.

3. RESULTS

The cellulose acetate electrophoresis separated a

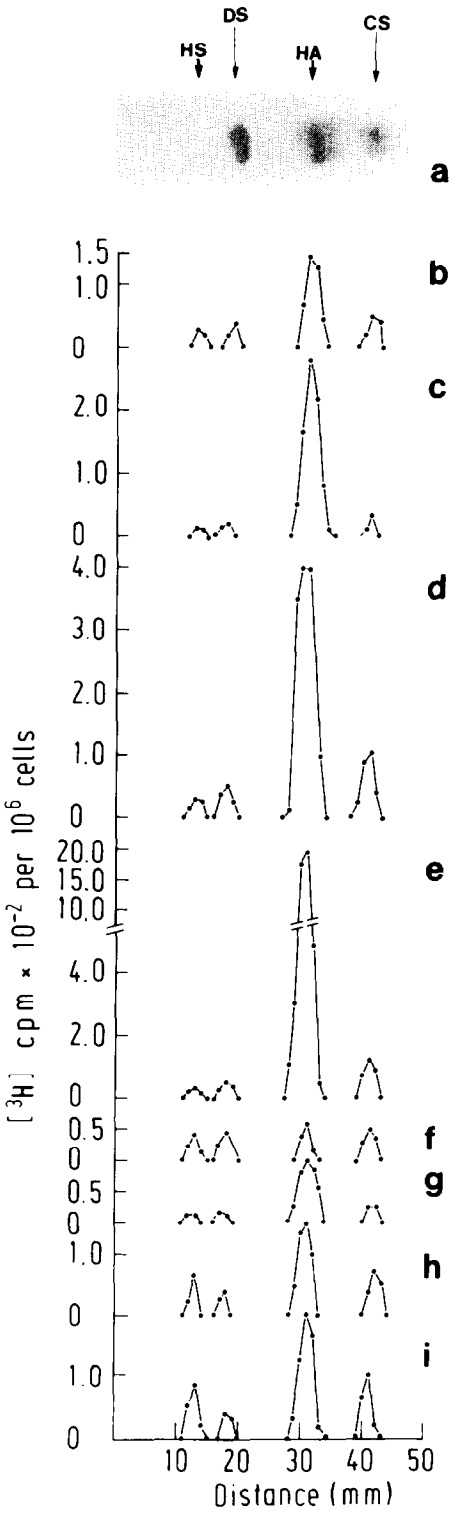

Fig.1. The identification of different GAGS present in skin fibroblasts and their culture medium by cellulose acetate electrophoresis. The background counts have been subtracted from the results shown (see text for details). (a) GAG standards. The position to which HS migrates was determined by using a radiolabelled preparation of HS; (b) culture medium of newborn skin fibroblasts (control); (c) culture medium of foetal skin fibroblasts (control); (d) culture medium of fibroblasts with OI type IIA; (e) culture medium of fibroblasts with OI type IIB; (f) newborn skin fibroblasts (control); (g) foetal skin fibroblasts (control); (h) fibroblasts with OI type IIA; (i) fibroblasts with OI type IIB.

variety of GAGS from cells and their culture medium (fig.1). The total radioactivity incorporated in each GAG was compared directly with radioactivity of the corresponding GAG in the controls.

3.1. *Controls*

The cells and medium of the foetal control contained a large amount of HA and negligible quantities of the sulphated GAGS (table 1). The quantity of HA in the cells and medium of the newborn control was lower and the content of the sulphated GAGS higher in comparison with the foetal controls.

3.2. *OI cells*

The concentration of sulphated GAGS, with the exception of DS, showed small increases over the newborn control, whereas the content of HA was markedly elevated (table 1). In contrast, the level of all sulphated GAGS was greatly increased over the foetal control, particularly HS, which increased in concentration by 5–6-fold. The rise in the content of HA was much smaller than the sulphated GAGS with respect to the foetal control.

The level of all GAGS, except DS, was slightly higher in OI IIB than in OI IIA. The content of HS was higher in the cells than in the medium, whereas HA, CS and DS were more concentrated in the medium (table 1).

3.3. *OI medium*

The concentration of HA was increased by about 3.4- and 13-fold in OI IIA and IIB, respectively, when compared with the newborn control. As with the cells, the content of sulphated GAGS was not much increased over this control. However, in comparison with the foetal control their content was greatly elevated, particularly CS, which was increased by about 6.4- and 7.3-fold in OI IIA and IIB, respectively (table 1). The level of HA was 3.8-fold higher in OI IIB than in OI IIA, but the content of the sulphated GAGS was not greatly altered between the two types of OI studied (table 1).

4. DISCUSSION

Changes in the concentration of GAGS in skin fibroblasts cultured from patients with a lethal form of OI were assessed. To eliminate biochemical changes as a result of ageing, two controls of different stages of development were compared. Results showed differences in the content of GAGS between the controls chosen. The level of sulphated GAGS was higher and that of HA lower in the older cells (newborn control) than in the younger cells (foetal control). Such changes in the content of GAGS as a result of ageing are in good agreement with previous studies [16].

The metabolism of GAGS in OI cells and medi-

Table 1

The distribution of various GAGS in skin fibroblasts

Sample	Medium				Cells			
	HA	DS	HS	CS	HA	DS	HS	CS
Newborn control[a]	370	70	50	160	120	70	80	120
Foetal control[a]	800	30	20	40	325	30	20	50
OI, IIA[a]	1260	110	70	255	435	70	95	165
OI, IIB[a]	4740	100	70	290	535	70	115	175
cpm Ratios								
Newborn control/foetal control	0.46	2.33	2.50	4.00	0.37	2.33	4.00	2.40
OI, IIA/newborn control	3.41	1.57	1.40	1.59	3.62	1.00	1.18	1.37
OI, IIB/newborn control	12.81	1.43	1.40	1.81	4.46	1.00	1.44	1.46
OI, IIA/foetal control	1.58	3.67	3.50	6.38	1.34	2.33	4.75	3.30
OI, IIB/foetal control	5.93	3.33	3.50	7.25	1.65	2.33	5.75	3.50

[a] cpm/10^6 cells

The values represented are averages of two determinations

um was also markedly altered in comparison with the controls. The content of all GAGS was greatly elevated with respect to the foetal control, and as the OI cells used in this study were similar in age to these control cells, the differences in GAGS between these cells therefore seem particularly important. In contrast, a large increase in HA and a smaller difference in the content of sulphated GAGS was observed with respect to the newborn control, however, such a comparison is important because it takes into account the differences in age of the cells and emphasizes the altered states of GAGS in skin fibroblasts with a lethal OI.

The increases in the GAGS in cells and medium were variable. The content of HS was higher in the cells than in the medium, which may partly be accounted for by its association with cell membranes as previously demonstrated for a number of different cell types [17,18]. However, HA, CS and DS are not retained at the cell membrane; hence their concentration in the medium is expected to be higher.

In the type of OI studied our results showed an overall increase in the content of GAGS was due to increases in sulphated and non-sulphated GAGS. Earlier studies on mixed populations of GAGS from skin fibroblasts with various forms of OI have revealed both an increase and decrease in their total content [11,19,20]. Such varied observations may partly be due to the biochemical heterogeneity of this disorder which may be reflected by an altered metabolic capacity for these macromolecules at various stages of OI [20]. This view is further enhanced by similar 'mixed' findings on the content of GAGS in many tissues with different types of OI [11,12,21], although comparisons in results between cells in culture and tissues must be made with some caution.

At present there is no correlation between the sulphated GAGS and the severity of the disease. It is also not certain whether the increase in the content of GAGS is a result of their increased synthesis or a reduced breakdown. However, the severity of the disease in lethal and milder forms of OI in skin fibroblasts has been correlated with a large increase in HA concentration, which was paralleled with a rise in the HA synthetase activity [9].

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