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Determination of Chondroitin-6-Sulphate by a Competitive Enzyme Immunoassay Using a Biotinylated Antigen

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Dedicated to Prof. Dr. Dr. Helmut Greiling on the occasion of his 65th birthday

Summary: A competitive enzyme immunoassay was developed to determine chondroitin-6-sulphate in body fluids and cell cultures. The assay uses a monoclonal anti-chondroitin-6-sulphate antibody, immobilised to microtitre plates, and it involves a competitive binding reaction between chondroitin-6-sulphate in the samples and the biotinylated antigen.

This assay enables the quantification of chondroitin-6-sulphate in the low concentration range of 16–120 μg/l. The intra-assay and inter-assay coefficients of variation are below 6.5% and 9.0%, respectively. More than 90% of chondroitin-6-sulphate was recovered when added to 0.1 mol/l phosphate-buffered saline, an albumin solution (40 g/l in phosphate-buffered saline) and cell culture medium (containing 100 ml/l foetal calf serum).

Chondroitin-6-sulphate was also determined in sera of healthy male (n = 90) and female (n = 90) blood donors. The normal range was 55–169 μg/l. In men the mean value was estimated at 102.2 ±37.1 μg/l and in women at 98.7 ± 26.4 μg/l. No age or sex dependence was observed.

The urine excretion of chondroitin-6-sulphate in men (n = 16) was 44.5 ± 21.1 mg/kg creatinine (mean ± standard deviation) and in females (n = 10) 53.5 ± 21.3 mg/kg creatinine. The clearance rate in men was 0.41 ± 0.22 ml × min^{-1} and in women 0.38 ± 0.15 ml × min^{-1}. No sex dependence was found.

Furthermore, the enzyme immunoassay was modified to measure the specific incorporation of a radioactively labelled precursor ([^{14}C]galactosamine) into chondroitin-6-sulphate. This modification rapidly gives information on the cellular glycosaminoglycan synthesis in cell culture. Using this method our experiments with cultivated human chondrocytes showed that the synthesis of chondroitin-6-sulphate decreased in the presence of interleukin-1α (60.0% less), tumour necrosis factor α (64.4%), γ-interferon (21.6%) and lipopolysaccharide (53.4%).

Introduction

Chondroitin sulphate consists of repeated disaccharide units containing N-acetyl-*D*-galactosamine and *D*-glucuronic acid. This glycosaminoglycan is sulphated either on the C4 or C6 position of the amino sugar (1) and attached to a core protein to form proteoglycans. The macromolecule is synthesised in the *Golgi* apparatus, transported to the cell surface and secreted into the extracellular space (2). Chondroitin sulphate is a major and ubiquitous component of the extracellular matrix of connective tissues (1). Several methods for the determi-

nation of chondroitin sulphate have been described. These techniques use enzymatic digestion, precipitation and chromatographic methods, especially high performance liquid chromatography (3, 4).

Methods have been reported recently, which use specific antibodies against different components of proteoglycans (e. g. keratan sulphate, dermatan sulphate) (5–8). So far, an enzyme immunoassay of chondroitin sulphate has not been described. The assay described here uses a monoclonal anti-chondroitin-6-sulphate antibody (9) and involves a competitive binding reaction between chondroitin-6-sulphate in the sample and biotinylated chondroitin-4-sulphate as labelled antigen. This enzyme immunoassay enables the determination of chondroitin-6-sulphate in serum, urine and cell culture medium.

Materials and Methods

Materials

Bovine serum albumin was obtained from Merck, Darmstadt. Tween 20, *o*-phenylenediamine, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, monoclonal anti-chondroitin-6-sulphate antibody (CS 56), adipic acid dihydrazide and N-hydroxysuccinimidobiotin were purchased from Sigma, Deisendorf. Streptavidin-coupled horse radish peroxidase[1]) was from Boehringer, Mannheim, and chondroitinase ABC[1]) from Seikagaku, Tokyo. Maxisorb immunoplates were obtained from Nunc, Wiesbaden. Chondroitin-6-sulphate and chondroitin-4-sulphate were obtained from Medac, Hamburg and from Sigma, Deisendorf. All chemicals used were of analytical grade or the highest commercially available grade.

Methods

Preparation of biotinylated antigen

Chondroitin sulphate (500 mg) was dissolved in 3 ml adipic acid dihydrazide (40 g/l, pH 4.75) and shaken for 30 min at room temperature. The solution was incubated for 1 hour at 4 °C with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in a ratio of 1 : 100 by vol. After dialysis, 2 g/l N-hydroxysuccinimidobiotin were added and the solution was incubated for 2 hours. After further dialysis the biotinylated antigen was stored at −20 °C.

Enzyme immunoassay

The wells of a plastic microtitre plate were coated overnight at 4 °C with the anti-chondroitin-6-sulphate antibody (50 µl/well, 1 : 2000 dilution of the ascitic fluid in 0.1 mol/l phosphate-buffered saline (8.0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 1.1 g/l K_2HPO_4, pH 7.4)) overnight at 4 °C. The coating solution was removed and the plates were washed three times with phosphate-buffered saline and blocked with bovine serum albumin (2 g/l phosphate-buffered saline, 100 µl/well) at room temperature for 1 hour. Samples (50 µl/well) or chondroitin-6-sulphate standard diluted in phosphate-buffered saline-Tween were added. After incubation for two hours at 4 °C, 50 µl of the biotinylated antigen were added to each well for 1 hour at 4 °C. The wells were washed three times with phosphate-buffered saline-Tween, then 50 µl streptavidin horse-radish peroxidase (60 U/l) were added and the solution was incubated for one hour at 4 °C. The wells were washed with phosphate-buffered saline and incubated with 50 µl of freshly prepared *o*-phenylenediamine (60 g/l) at room temperature for 45 minutes. The enzymatic colour development was stopped by adding 50 µl 0.5 mol/l sulphuric acid and the absorbance was measured at 492 nm with a Behring microtitre enzyme immunoassay reader.

Analysis of chondroitin sulphates by high performance liquid chromatography

The chondroitin-6-sulphate used as standard and the chondroitin-4-sulphate used as biotinylated antigen were analysed by high performance liquid chromatography to determine the composition of unsaturated chondroitin-sulphate disaccharide. The HPLC analysis of the disaccharide was carried out according to a modified method of *E. Gurr* et al. (4).

Modification of the enzyme immunoassay to determine the chondroitin-6-sulphate synthesis of cultured cells by monitoring [^{14}C]galactosamine incorporation

The concentration of ^{14}C-labelled chondroitin-6-sulphate in the medium of cultured human chondrocytes was measured by the enzyme immunoassay as described above. In a parallel experiment, after the competitive binding reaction, the microtitre wells were incubated with trypsin (3 g/l) to detach the ^{14}C-labelled antigen/antibody complex from the microtitre plates. This enzymatic digestion enabled quantification of radioactivity in a scintillation well. This method will be published elsewhere (10).

Recovery, precision and detection limit of the enzyme immunoassay

The accuracy of the enzyme immunoassay was investigated by adding a constant amount of chondroitin-6-sulphate (60 mg) to 0.1 mol/l phosphate-buffered saline, to an albumin solution (40 g/l in phosphate-buffered saline), and to a cell culture medium (containing 100 ml/l foetal calf serum). The concentration of chondroitin-6-sulphate was determined before and after addition, and the recovery was calculated.

Different concentrations of chondroitin-6-sulphate (20, 80 and 120 µg/l) were used to determine the inter-assay (n = 70) and intra-assay (n = 15) variation coefficients.

To determine the detection limit of the enzyme immunoassay, chondroitin-6-sulphate was added in concentrations of 0, 4, 8 and 16 µg/l to 0.1 mol/l phosphate-buffered saline and to an albumin solution (40 g/l in phosphate-buffered saline). The low 3s-limit ($\bar{x}$-3s) of the chondroitin-6-sulphate-free samples was calculated after a series of 48 determinations. The lowest chondroitin-6-sulphate concentration (mean of 48 determinations), which was significantly different from this calculated value, was defined as the detection limit.

Sample preparation

1. Venous blood was obtained from healthy male (n = 90) and female (n = 90) donors aged 18–65. After clotting, followed by centrifugation for 10 minutes at 4000 min^{-1}, the serum was frozen and stored until use.

2. The urine collection started in the morning by rejecting the previous night urine and finished after 24 hours by including the subsequent night urine. This urine collection avoids any influence of circadian rhythm effects on the chondroitin-6-sulphate determination. Urine from 16 healthy male and 10 healthy female volunteers were tested.

[1]) Enzymes:
Chondroitinase ABC EC 4.2.2.4
Horse-radish peroxidase EC 1.11.1.7

3. Human chondrocytes were precultivated as a monolayer for 24 hours in *Dulbecco's* modified *Eagles* medium supplemented with heat-inactivated foetal calf serum (50 ml/l), antibiotics and mediators. After 24 hours, fresh medium containing the mediators and [^{14}C]glucosamine hydrochloride 56 MBq/l was added. The culture media were collected and the chondroitin-6-sulphate concentration and specific incorporation rates were determined. The following mediator concentrations were used: interleukin-1α (10^4 U/l), tumour necrosis factor α (100 µg/l), γ-interferon ($2 \cdot 10^5$ U/l) and lipopolysaccharide (10 µg/l). The mediator concentrations were chosen according to dose-response studies in which the efficacy of these concentrations was demonstrated (10).

Statistical analysis

The significance of the mean value difference and the age and sex dependence of chondroitin-6-sulphate were calculated using *Student's* t test and linear correlation analysis, respectively.

Results

Analysis of chondroitin-6-sulphate and chondroitin-4-sulphate by high performance liquid chromatography

The chondroitin-6-sulphate used as a standard contained 78.3% chondroitin-6-sulphate and 21.7% chondroitin-4-sulphate. The dose response curve of the enzyme immunoassay was established on the basis of the amount of chondroitin-6-sulphate in the standard. The composition of chondroitin-4-sulphate was 89.5% chondroitin-4-sulphate, 4.5% chondroitin-6-sulphate and 6% non-sulphated chondroitin (chondroitin-0-sulphate).

Biotinylation of antigens

The binding affinity of the biotinylated chondroitin-6-sulphate and biotinylated chondroitin-4-sulphate to the monoclonal antibody was investigated. The antigenicity of chondroitin-6-sulphate was considerably reduced after biotinylation (fig. 1). Despite several modifications of the biotinylating reaction it was not possible to increase the antigenicity of biotinylated chondroitin-6-sulphate. However, the antigenicity of chondroitin-4-sulphate was not influenced by biotinylation, so this was used as the competitive antigen in an optimal concentration of 100 mg/l.

Dose response curve of the enzyme immunoassay

The dose response curve of the enzyme immunoassay was obtained by using the biotinylated chondroitin-4-sulphate as competitive antigen (fig. 2). Corresponding standard curves were obtained using 0.1 mol/l phosphate-buffered saline and albumin (40 g/l in 0.1 mol/l phosphate-buffered saline). In addition, the standard curve of chondroitin-6-sulphate in foetal calf serum (100 ml/l) showed a similar course. The linearity of the dose response curve was found at a concentration of 16–120 µg/l. Matrix effects were observed only at a chondroitin-6-sulphate concentration of 80 µg/l. The difference between the phosphate-buffered saline standard curve and

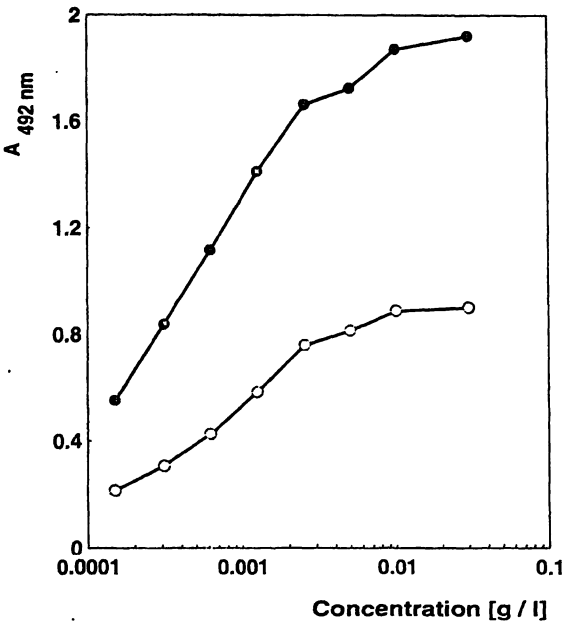

Fig. 1 Binding of biotinylated chondroitin-6-sulphate (○—○) and biotinylated chondroitin-4-sulphate (●—●) to the monoclonal anti-chondroitin-6-sulphate antibody. The labelled chondroitin sulphates were added in different concentrations to the anti-chondroitin-6-sulphate antibody. The antigen-antibody complex was incubated with streptavidin horse-radish peroxidase and the absorbance at 492 nm was measured after substrate addition.

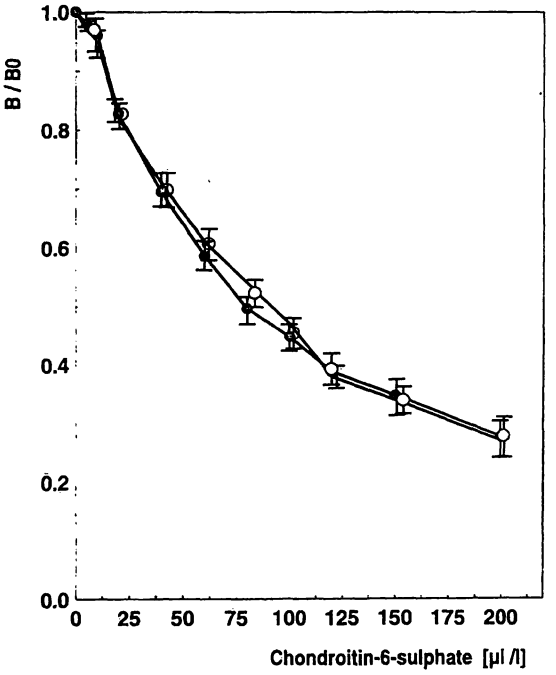

Fig. 2 Dose-response curve of the chondroitin-6-sulphate enzyme immunoassay in different matrices: 0.1 mol/l phosphate-buffered saline (○—○) and albumin (40 g/l) (●—●). Mean value and standard deviation of 48 determinations are given.

the albumin standard curve was 8%. However, the difference was within the standard deviation. At all other concentrations the differences were less than 5%.

Cross-reactivity of the enzyme immunoassay with other glycosaminoglycans

Cross-reactivity is defined as 50% inhibition of binding of glycosaminoglycans to the monoclonal antibody. The 50% inhibition point of chondroitin-6-sulphate was found at 27 µg/l. The concentration of chondroitin-4-sulphate required for 50% inhibition was 112 g/l, i.e. about 4000 times greater than that required for the equivalent inhibition by chondroitin-6-sulphate. The cross-reaction of other glycosaminoglycans was considerably outside the linear range of chondroitin-6-sulphate (fig. 3). The concentrations of these glycosaminoglycans causing 50% inhibition were: dermatan sulphate 1 g/l, keratan sulphate > 1 g/l, hyaluronan > 5 g/l, and heparin > 10 g/l.

Precision, recovery and detection limit of the chondroitin-6-sulphate immunoassay

The inter-assay and intra-assay variation coefficients are less than 9.0% and 6.5%, respectively (fig. 4). The recovery of chondroitin-6-sulphate was in the range of 87.5%–109.6% (tab. 1). The detection limit was 16 µg/l (tab. 2).

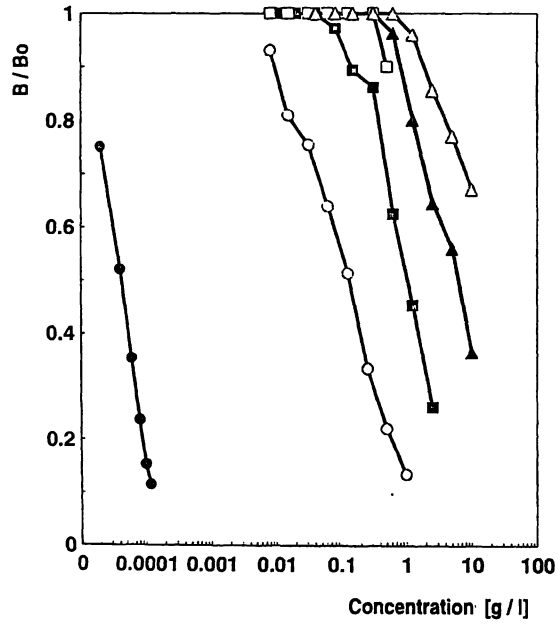

Fig. 3 Dose-response curves of the monoclonal anti-chondroitin-6-sulphate antibody with different glycosaminoglycans. The cross-reactivities defined as the glycosaminoglycan concentration at 50% inhibition were calculated: chondroitin-6-sulphate (●–●): 27 µg/l; chondroitin-4-sulphate (○–○): 112 mg/l; dermatan sulphate (◘–◘): 1 g/l; keratan sulphate (□–□): > 1 g/l; hyaluronan (▲–▲): > 5 g/l; heparin (△–△): > 10 g/l.

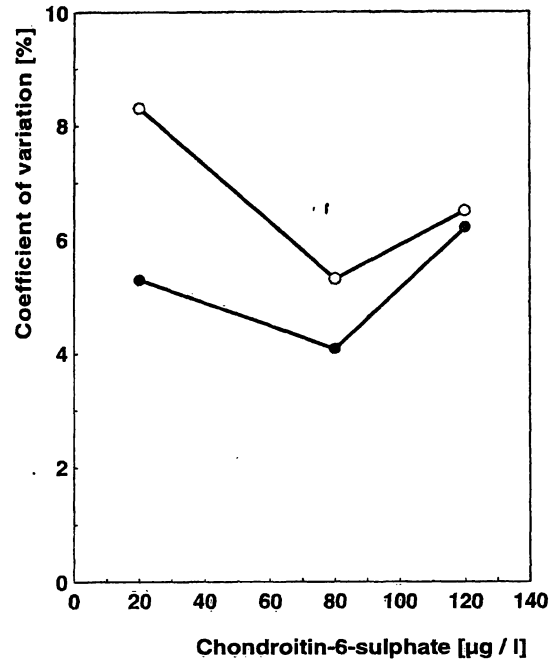

Fig. 4 Imprecision of the enzyme immunoassay. The intra-assay (n = 15) (●–●) and inter-assay (n = 70) (○–○) variation coefficients of different chondroitin-6-sulphate concentrations are given.

Tab. 1 Determination of chondroitin-6-sulphate in the presence of different matrices and recovery of exogenously added chondroitin-6-sulphate. The experiments were carried out in quadruplicate. Mean values ($\bar{x}$) and standard deviations (s) are given.

Matrix	No addition of chondroitin-6-sulphate [µg/l]	Addition of 60 µg/l chondroitin-6-sulphate [µg/l]	Recovery [%]
Phosphate-buffered saline [0.1 mol/l]	not detectable	55.8 ± 2.2	96.3
Foetal calf serum [100 ml/l]	68 ± 2.1	121.3 ± 2.9	94.7
Albumin [40 g/l]	not detectable	65.7 ± 2.2	109.6
Culture medium of human fibroblast	600 ± 19.3	577.5 ± 22	87.5

Concentration of chondroitin-6-sulphate in blood serum

The normal range of chondroitin-6-sulphate in blood serum was 55–169 µg/l. In the serum of healthy males the mean chondroitin-6-sulphate concentration was 101 µg/l (95% range: 56–169 µg/l) and in females 99 µg/l (95% range: 61–150 µg/l). No sex or age dependence was found (fig. 5).

Tab. 2 Evaluation of the detection limit of the enzyme immunoassay. The absorbance of the enzyme immunoassay was measured using samples with and without small amounts of chondroitin-6-sulphate in 0.1 mol/l phosphate-buffered saline and in albumin (40 g/l). Mean values ($\bar{x}$) and standard deviations (s) are given. The low 3s-limit ($\bar{x}$-3s) of a chondroitin-6-sulphate-free sample was calculated after a series of 48 determinations. The lowest chondroitin-6-sulphate concentration (mean of 48 determinations), which was significantly different from this calculated value, was defined as the detection limit (16 µg/l).

Chondroitin-6-sulphate [µg/l]	Absorbance at 492 nm $\bar{x}$	s	Absorbance at 492 nm $\bar{x}$-3s
	Matrix: Phosphate-buffered saline		
0	1.35		
4	1.33	0.031	1.26
8	1.29		
16	1.16		
	Matrix: Albumin		
0	1.41		
4	1.39	0.032	1.29
8	1.37		
16	1.18		

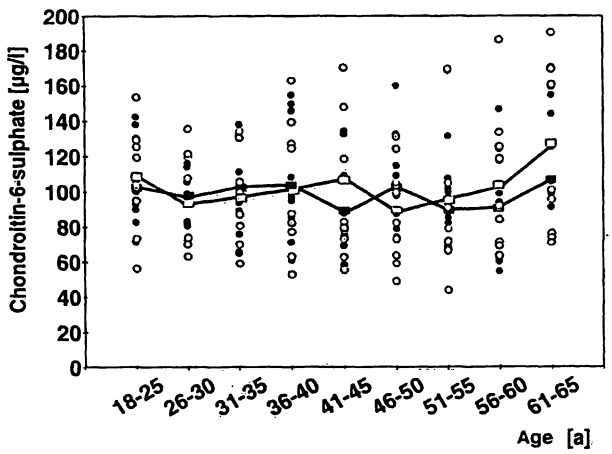

Fig. 5 Chondroitin-6-sulphate concentration in the serum of male (○) and female (●) blood donors of different ages. The means of each age group (n = 10) are given for male (□—□) and female (■—■).

Concentration of chondroitin-6-sulphate in urine

The urine concentration of chondroitin-6-sulphate in males was 44.5 ± 21.1 mg/kg creatinine (mean ± standard deviation) and in females 53.5 ± 21.3 mg/kg creatinine. The chondroitin-6-sulphate clearance in men was determined as 0.41 ± 0.22 ml × min^{-1} and in women as 0.38 ± 0.15 ml × min^{-1}. No sex dependence was observed.

Determination of chondroitin-6-sulphate synthesis rate in chondrocyte culture by modification of the enzyme immunoassay

The synthesis of chondroitin-6-sulphate was investigated in the medium of human chondrocytes cultivated with and without agents. The incorporation of ^{14}C-labelled galactosamine into chondroitin-6-sulphate was used as a specific index of the synthesis rate. In comparison with chondrocytes incubated without agents, the chondroitin-6-sulphate synthesis decreased in the presence of the following agents: tumour necrosis factor α (TNF α): 64.4%, interleukin-1α (IL-1α): 60.0%, bacterial lipopolysaccharide (LPS): 53.4%, and γ-interferon (γ-INF): 21.6% (tab. 3).

Discussion

Development of an enzyme immunoassay for chondroitin-6-sulphate was made difficult by the availability of only one monoclonal IgM antibody against chondroitin-6-sulphate. We therefore used a competitive method. In our assay, a biotinylated chondroitin-4-sulphate antigen competes with the antigen in the sample for the binding sites of the antibody which was immobilised on the plastic surface of the microtitre plate.

Tab. 3 Measurement of [^{14}C]galactosamine incorporation in chondroitin-6-sulphate by modification of the enzyme immunoassay. After the determination of the concentration of chondroitin-6-sulphate in the culture medium, the chondroitin-6-sulphate antibody complex was enzymatically detached from the microtitre plates and the radioactivity of the [^{14}C]chondroitin-6-sulphate was measured. The calculation of the [^{14}C]chondroitin-6-sulphate provides a specific value related to the synthesis rate of this glycosaminoglycan. This is shown in studies with human chondrocyte cultures incubated with different agents for 48 hours. Agent concentrations: interleukin-1α: 10^4 U/l; tumour necrosis factor α: 100 µg/l; γ-interferon: $2 \cdot 10^5$ U/l; lipopolysaccharide: 10 µg/l; [^{14}C]galactosamine: 56 MBq/l. Mean and standard deviation of four experiments are given.

Incubation with agents	Immunoassays of chondroitin-6-sulphate [µg/l]	Radioactivity of antibody-bound chondroitin-6-sulphate [kBq/l]	Specific incorporation of [^{14}C]galactosamine [mmol/kg chondroitin-6-sulphate]
Without	3100 ± 20	952 ± 16	252
γ-Interferon	2520 ± 160	796 ± 12	197
Lipopolysaccharide	817 ± 22	190 ± 5	118
Interleukin-1α	702 ± 81	136 ± 9	101
Tumour necrosis factor α	605 ± 75	109 ± 6	89

Different procedures were tested to optimise the biotinylation of chondroitin sulphate, since it was observed that the antigenicity of the modified chondroitin-6-sulphate had been altered. The monoclonal anti-chondroitin-6-sulphate antibody recognised the biotinylated chondroitin-6-sulphate to a lesser extent than it did the native form. The decreased antigenicity of biotinylated chondroitin-6-sulphate may be due to steric effects of the sulphate position. In contrast, the antigenicity of biotinylated chondroitin-4-sulphate was unchanged. Consequently, the biotinylated chondroitin-4-sulphate was used as a competitive antigen at a concentration of 100 mg/l and reproducible dose response curves were obtained for the determination of chondroitin-6-sulphate in body fluids and cell culture media. Our method avoids the use of radiolabelled ligands and, by using enzyme immunoassay plates, has proved itself to be a rapid and efficient technique.

The biochemistry of cartilage destruction in inflammatory and degenerative joint disease has been extensively studied, and it has been found that the loss of proteoglycans of the extracellular matrix is one of the earliest events during the pathobiochemical process (11–13). Thus, the measurement of proteoglycans and their catabolic products in synovial fluid and serum may provide useful diagnostic markers for joint diseases. However, the majority of glycosaminoglycans are ubiquitous intercellular matrix components of different connective tissues. It has been suggested that keratan sulphate is a more specific analyte, since it mainly originates from cartilage and, to a lesser extent, from the cornea and aorta (1). Therefore, antibodies against these glycosaminoglycans were developed and used in several immunological assays. In some studies it has been reported that patients with osteoarthritis showed a significantly higher serum concentration of keratan sulphate than healthy volunteers. However, there is still considerable controversy as to whether the serum concentration of keratan sulphate is a specific indication of cartilage destruction (8, 14–15).

Chondroitin-6-sulphate is a glycosaminoglycan, which may also be used as a diagnostic factor in connective tissue diseases, including intracellular degradation of connective tissue and the metabolic activity of connective tissue cells during repair processes. However, the diagnostic relevance of this quantity must be elucidated in further investigations.

The synovial cell culture represents a defined model for obtaining information on the metabolism of chondroitin sulphate in the synovial system and for investigating the influence of different agents on the metabolism of this glycosaminoglycan. In studies on the synthesis of chondroitin sulphate the incorporation of radiolabelled galactosamine into the glycosaminoglycan is usually determined. However, measurement of radiolabelled chondroitin sulphate isolated from cell culture medium may lead to oversimplification of the interpretation, because changes in the measured chondroitin sulphate concentration in the medium can be influenced by partial steps of metabolism, such as intracellular synthesis, secretion into the medium, re-uptake of the macromolecule and intracellular degradation. One unsatisfactory way which has been used to overcome this problem is the simultaneous investigation of the concentration of chondroitin-6-sulphate in the extra-, peri- or intracellular pool (16). The new enzyme immunoassay of chondroitin-6-sulphate, in combination with the assay of the antibody bound to ^{14}C-labelled chondroitin-6-sulphate, now enables measurement of the specific incorporation of [^{14}C]galactosamine into chondroitin-6-sulphate as a means of quantifying the synthesis rate of this glycosaminoglycan. Therefore, amplification of the enzyme immunoassay in this way represents a useful tool for obtaining rapid information on the synthesis of chondroitin-6-sulphate in cell culture (10).

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References

1. Wight, T. N., Heinegard, D. K. & Hascall, V. C. (1991) Proteoglycans – Structure and function. In: *Cell Biology of Extracellular Matrix*, 2nd ed. (Hay, E., ed.) pp. 45–100, Plenum Press, New York.
2. Kimura, J. H., Lohmander, L. S. & Hascall, V. C. (1984) Studies on the biosynthesis of cartilage proteoglycan in a model system of cultured chondrocytes from the swarm rat chondrosarcoma. J. Cell. Biochem. *26*, 261–278.
3. Greiling, H., Stuhlsatz, H. W. & Tillmanns, U. (1984) Chondroitin, chondroitin-4-sulphate, chondroitin-6-sulphate, and dermatan sulphate. In: *Methods of Enzymatic Analysis*, Vol VI (Bergmeyer, ed.), pp. 60–69.
4. Gurr, E., Pallach, G., Tamm, C. & Delbrück, A. (1985) High performance liquid chromatographic assay of disaccharides and oligosaccharides produced by the digestion of glycosaminoglycans with chondroitin sulphate lyases. J. Clin. Chem. Clin. Biochem. *23*, 77–87.
5. Baker, J. R., Caterson, B. & Christner, J. E. (1982) Immunological characterisation of cartilage proteoglycans. Methods of Enzymology *83*, 216–235.
6. Caterson, B., Christner, J. E., Baker, J. R. & Couchman, J. R. (1985) Production and characterisation of monoclonal antibodies directed against connective tissue proteoglycans. Fed. Proc. *44*, 386–393.

7. Kongtawelert, P. & Gosh, P. (1990) A new sandwich-ELISA method for the determination of keratan sulphate peptides in biological fluids emplyoing a monoclonal antibody and labelled avidin biotin technique. Clin. Chim. Acta *195*, 17–26.
8. Alwan, W. H., Carter, S. D., Bennett, D., May, S. A. & Edwards, G. B. (1990) Cartilage breakdown in equine osteoarthritis: Measurement of keratan sulphate by an ELISA system. Research in Veterinary Science *49*, 56–60.
9. Avnur, Z. & Geiger, B. (1984) Immunocytochemical localisation of native chondroitin sulphate in tissues and cultured cells using specific monoclonal antibody. Cell *38*, 811–822.
10. Kähnert, H. & Kleesiek, K. (1993) Influence of the proteoglycan synthesis of cultured human chondrocytes and synovial cells by different cytokines. Eur. J. Clin. Chem. Clin. Biochem. (in preparation).
11. Alexander, C. M. & Werb, Z. (1991) Extracellular matrix degeneration. In: *Cell Biology of Extracellular Matrix*, 2nd ed. (Hay, D. E., ed.), chapter 8, pp. 255–294, Plenum Press, New York.
12. Bayliss, M. T. (1985) Proteoglycan structure in normal and osteoarthrotic human cartilage. In: *Articular Cartilage Biochemistry* (Kuettner, K. E., Schleyerbach, R. & Hascall, V. C., eds.), pp. 295–311, Raven Press, New York.
13. Maroudas, A., Katz, E. P., Wachtel, E. J., Mizrahi, J. & Soundry, M. (1985) Physicochemical properties and functional behavior of normal and osteoarthrotic human cartilage. In: *Articular Cartilage Biochemistry* (Kuettner, K. E., Schleyerbach, R. & Hascall, V. C., eds.), pp. 311–331, Raven Press, New York.
14. Thonar, E. J.-M. A., Lenz, M. E., Klintworth, G. K., Caterson, B., Pachman, L. M., Glickman, P., Katz, R., Huff, J. & Kuettner, K. E. (1985) Quantification of keratan sulphate in blood as a marker of cartilage catabolism. Arthritis Rheum. *28*, 1367–1376.
15. Saxne, T., Heinegard, D. & Wollheim, F. A. (1987) Cartilage proteoglycans in synovial fluid and serum in patients with inflammatory joint disease. Relation to systemic treatment. Arthritis Rheum. *30*, 972–979.
16. Kleesiek, K. & Greiling, H. (1982) Effect of anti-inflammatory agents on the glycosaminoglycan metabolism in cultured human synovial cells. Rheumatol. Int. *2*, 167–174.

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