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UDP-*D*-Xylose : Proteoglycan Core Protein β -*D*-Xylosyltransferase: A New Marker of Cartilage Destruction in Chronic Joint Diseases

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Summary: We investigated the diagnostic significance of UDP-*D*-xylose : proteoglycan core protein β -*D*-xylosyltransferase (EC 2.4.2.26) in different chronic joint diseases. This enzyme is located almost exclusively within chondrocytes, where it initiates the formation of chondroitin sulphate during the biosynthesis of proteoglycans and from which it is easily released after damage of articular cartilage.

Xylosyltransferase activity was determined in synovial fluid and serum by a radiochemical method, based on the incorporation of [14 C]xylose from UDP-[14 C]xylose into an exogenous acceptor protein.

Serum has been shown to be the appropriate material for the determination of xylosyltransferase activity in blood, since in plasma fibrinogen causes an inhibition of enzyme activity of about 50%.

The catalytic concentrations of xylosyltransferase in synovial fluids and sera of patients with chronic joint diseases ($n = 131$) ranged from 0.5 to 22.0 mU/l and from 0.8 to 5.6 mU/l, respectively. On most cases we found higher xylosyltransferase activities in synovial fluids than in the corresponding sera. The highest catalytic concentrations of the enzyme were observed in the synovial fluids of patients suffering from rheumatoid arthritis (median value: 5.56 mU/l, 90%-range: 3.2–22.0 mU/l). Synovial fluids of patients with arthritis urica, however, showing a comparable high degree of inflammation, contained lower enzyme catalytic concentrations (median value: 2.38 mU/l, 90%-range: 0.7–5.2 mU/l), which were in the range of those in osteoarthritis (median value: 2.50 mU/l, 90%-range: 0.8–4.8 mU/l). The higher xylosyltransferase activities in rheumatoid synovial fluids seem to be attributed to an increased cartilage destruction during the course of this disease.

Our results demonstrate that xylosyltransferase activity in the synovial fluid represents an appropriate clinical-chemical parameter, indicating the degree of cartilage destruction in chronic joint diseases. The serum xylosyltransferase activities of patients with various chronic joint diseases, however, showed no significant differences.

Introduction

Chondroitin sulphate is present in the articular cartilage as a component of a multichain proteoglycan in which the carbohydrate moiety is covalently bound to a core protein. In the biosynthesis of chondroitin sulphate proteoglycan several glycosyltransferases and sulphotransferases are involved, including UDP-*D*-xylose : proteoglycan core protein β -*D*-xylosyltransferase (EC 2.4.2.26) (1, 2). The initial, apparently

rate-limiting step, which involves the transfer of xylose from UDP-xylose to serine hydroxyl groups in the core protein, is catalysed by this enzyme (3–5).

We examined the diagnostic significance of xylosyltransferase as a clinical-chemical parameter in chronic joint diseases, since the enzyme is highly specific to cells, which are able to synthesize proteoglycans, especially to chondrocytes in articular cartilage. In these cells the xylosyltransferase seems to be

only weakly bound to the membranes of the rough endoplasmic reticulum (6), from which it is released easily after disruption of the cells. Since destruction of articular cartilage is the common pathogenic process of all chronic joint diseases, the activity of xylosyltransferase in synovial fluids of patients with chronic joint diseases will indicate the extent of cartilage destruction.

Some of the results presented in this paper have been published previously as a short communication (7).

Materials and Methods

Chemicals

UDP-*D*-xylose, hyaluronate-4-glycanohydrolase (from sheep testes; EC 3.2.1.35), heparin (170 USP units/mg, according to the United States Pharmacopeial Convention), benzamidine hydrochloride, iodoacetic acid sodium salt, and ficoll were obtained from Sigma (München, FRG), UDP-*D*-[¹⁴C]xylose (specific activity: 9.88 TBq/mol = 267 nCi/nmol) was purchased from New England Nuclear Corporation (Dreieich, FRG). Guanidine hydrochloride, phenylmethanesulphonyl fluoride, tris(hydroxymethyl)aminomethane (tris), and ethylene diamine tetraacetic acid sodium salt (EDTA) were obtained from Serva (Heidelberg, FRG) and Q-sepharose Fast Flow from Pharmacia (Uppsala, Sweden). Human granulocyte elastase (EC 3.4.21.37) was a research product of the Biochemical Research Department of E. Merck (Darmstadt, FRG). Hydrogen fluoride in pyridine (70%) and caesium chloride were purchased from E. Merck. Lithium thiocyanate and trifluoromethane sulphonic acid were obtained from Ventron GmbH (Karlsruhe, FRG). Silk from the silkworm *Bombyx mori* was a gift of Dr. Z. Nevo, Tel Aviv University, Israel. The defined chondroitin sulphate peptidoglycans were a gift of Professor H. Stuhlsatz, Aachen University of Technology, FRG.

Chromatography paper No. 2043 b mgl for the xylosyltransferase assay was obtained from Schleicher & Schüll (Düren, FRG). The synthetic peptides *L*-prolyl-*L*-seryl-*L*-glycine and *L*-leucyl-*L*-prolyl-*L*-seryl-*L*-glycine were kindly provided by Dr. Monika Casaretto of the Deutsches Wollforschungsinstitut, Aachen University, FRG.

Patients and reference group

We investigated 131 synovial fluids and 79 sera from patients with inflammatory and non-inflammatory joint diseases: rheumatoid arthritis (*n* = 24; 18 females and 6 males, age: 32–78 years, mean: 57.2 years); osteoarthritis (*n* = 32; 20 females and 12 males, age: 47–83 years, mean: 61.8 years); ankylosing spondylitis (*n* = 10); Reiter's syndrome (*n* = 4); septic arthritis (*n* = 6); arthritis urica (monosodium urate crystals detected in synovia cell pellet) (*n* = 30; 1 female and 29 males, age: 30–80 years, mean: 52.3 years); calcium pyrophosphate deposition disease (pseudogout) (*n* = 18; 7 females and 11 males, age: 29–85 years, mean: 61.1 years); psoriatic arthritis (*n* = 7). In 79 cases synovial fluid and serum were taken simultaneously from the same patients.

The diagnosis of rheumatoid arthritis was based on the criteria of the American Rheumatism Association. The patients suffering from ankylosing spondylitis met the New York and Rome criteria. Microorganisms were shown to be present in all synovial fluids of patients with septic arthritis. Xylosyltransferase activities were also determined in sera of randomly selected blood donors (reference group) (*n* = 20; 6 females and 14 males, age: 19–53 years, mean: 33.4 years).

Sample preparation

Synovial fluid

Samples of synovial fluid were centrifuged immediately after aspiration of the synovial fluid from the knee joint at 1000 *g* for 10 min at 4 °C to remove the synovial cells. The supernate was centrifuged again at 5000 *g* for 10 min at 4 °C to remove any remaining cells and cell fragments. Total absence of cells was checked by light microscopy and the supernate was stored at –70 °C until assayed. In order to diminish the viscosity of the synovial fluids, 1 ml of synovial fluid was depolymerized by preincubation with 10 µl (33 units) of hyaluronate-4-glycanohydrolase for 20 min at 37 °C before further investigation. From control experiments a possible interference of hyaluronate-4-glycanohydrolase with xylosyltransferase activity could be excluded.

Serum

Venous blood samples were collected in plastic tubes containing polystyrol beads (serum monovettes, Sarstedt, Nümbrecht, FRG). Serum was separated from blood cells by centrifugation (5000 *g*; 4 °C; 10 min) within 1 h. The samples were stored in several aliquots at –70 °C until analysed.

Plasma

Venous blood was drawn into EDTA-coated tubes and centrifuged at 5000 *g* for 10 min at 4 °C within 1 h after blood sampling. The plasma was frozen in plastic tubes at –70 °C.

Tissue extract from human costal cartilage

Human costal cartilage was extracted with tris buffer (0.1 mol/l, pH 7.5), containing 0.5 mol/l KCl, 10 mmol/l benzamidine hydrochloride, 5 mmol/l EDTA, 1 mmol/l iodoacetate and 0.1 mmol/l phenylmethanesulphonyl fluoride for 20 h at 4 °C. After centrifugation at 5000 *g* for 15 min the extract was treated with hyaluronate-4-glycanohydrolase (33 kU/l; 4 h; 37 °C) and dialysed exhaustively against 0.1 mol/l tris buffer, 0.1 mol/l NaCl (pH 8.5). After centrifugation of the solution (50 000 *g*; 30 min; 4 °C) xylosyltransferase was enriched by ion exchange chromatography on Q-sepharose Fast Flow. Xylosyltransferase-containing fractions were concentrated by ultrafiltration (Amicon, PM-10) and stored at –70 °C.

Preparation of substrates

Preparation of core protein from bovine nasal septum cartilage

Chondroitin sulphate proteoglycan was extracted from bovine nasal septum cartilage with 4 mol/l guanidine hydrochloride and purified by density gradient centrifugation under dissociative conditions in 4 mol/l guanidine hydrochloride and caesium chloride (initial density 1.5 kg/l) using the method of Sajdera & Hascall (8). After digestion with testicular hyaluronate-4-glycanohydrolase (9), the freeze-dried chondroitin sulphate proteoglycan preparation, which must be completely free from water, was deglycosylated either with 70% hydrogen fluoride in pyridine (10, 11) or with trifluoromethane sulphonic acid (12). The resulting core protein preparations were investigated for serine content by amino acid analysis.

Preparation of silk for assay

Silk from *Bombyx mori* (1 g) was dissolved in 10 ml of 600 g/l lithium thiocyanate (13, 14) with stirring and gentle heating. After dilution with deionized water to about 80 ml, the solution was filtered, dialysed for 48 h against deionized water until free

of thiocyanate, and equilibrated with tris buffer (0.1 mol/l, pH 7.0). Subsequently, the volume was adjusted to 100 ml and 0.5 ml fractions of the silk solution were stored at -70°C .

Assay of xylosyltransferase activity

Xylosyltransferase activity was assayed by a procedure which has been described previously (15, 16), using silk as exogenous acceptor (13). The method is based on radiochemical quantification of xylosyltransferase-catalysed binding of [^{14}C]xylose to serine residues in the acceptor:

Incubation mixtures contained the following components in a final volume of 110 μl : 50 μl of the specimen, 0.3 mg of silk in tris buffer (0.1 mol/l, pH 7.0; 30 μl), 10 μl of MnCl_2 (0.02 mol/l), and 20 μl of coenzyme solution, containing 0.561 nmol of unlabelled UDP-xylose, 0.187 nmol UDP- ^{14}C xylose (specific activity: 9.88 TBq/mol = 267 nCi/nmol) and 0.5 μmol of KF in tris buffer (0.1 mol/l, pH 7.0). After incubation of the reaction mixture for 45 min at 37°C , the reaction was terminated by adding 10 μl of EDTA (0.125 mol/l) and cooling the mixture in an ice-bath. Control incubation mixtures without exogenous acceptor (specimen blank) were kept at 0°C for 45 min. In order to separate non-incorporated UDP-xylose from xylosylated silk, each reaction mixture was applied to Schleicher & Schüll No. 2043 b mg/l paper over an area of approximately 2 cm^2 for descending paper chromatography. Non-bound UDP-xylose was eluted with ethanol/ammonium acetate (1 mol/l, pH 5.5; 7 + 3 by vol.) for 48 h. After drying, 9 cm^2 pieces were transferred to a scintillation vial. Subsequently, 1 ml of water and 10 ml of scintillation fluid were added and the radioactivity was measured using a liquid scintillation counter (LKB Rack Beta II, Bromma, Sweden).

In comparative studies the following acceptor proteins were also used: core protein from HF-degraded and trifluoromethane sulphonic acid-degraded proteoglycan of bovine nasal septum cartilage, and the synthetic peptides Leu-Pro-Ser-Gly and Pro-Ser-Gly. All experiments were carried out in the presence of an excess of the substrates (13.8–23-fold K_m) in order to reach V_{max} . The results were expressed as enzyme activity (xylose, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$) or acceptor activity (xylose, $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mmol}^{-1}$ serine).

In diagnostic studies the determination of xylosyltransferase activity in synovial fluids and sera of patients with chronic joint diseases and the reference group was carried out only with silk as substrate.

Radioactivity of acceptor-bound [^{14}C]xylose and the specific activity of UDP- D - ^{14}C xylose were used to calculate xylosyltransferase activity ($\text{Bq} \times 5.574 = \text{mU/l}$).

Evaluation of imprecision, detection limit, and working range of the xylosyltransferase assay

The imprecision of the xylosyltransferase determination was investigated with synovial fluids at 3 different activity levels (2.71, 4.89, and 6.85 mU/l). Within-run imprecision was obtained by analysis of 8 replicate samples of each pool. Between-day imprecision was calculated from the results obtained from the same synovial fluid pools, which were stored in small aliquots at -70°C , on 6 different days spread over a 4-week period.

For the investigation of the detection limit of the assay, synovial fluid was heated for 30 min at 56°C , in order to completely abolish the enzyme activity. Thereafter the xylosyltransferase

activity was assayed in 10 parallel trials and the detection limit (mean value + 3 SD) was calculated from the intra-assay variation.

To investigate the linearity of the assay, serial dilutions from a tissue extract of human costal cartilage with high xylosyltransferase activity (70.3 mU/l) were examined. Each solution was analysed in duplicate.

In order to evaluate the recovery of xylosyltransferase activity, inactivated aliquots (56°C ; 30 min) of inflammatory and non-inflammatory synovial fluids were spiked each with 2 different xylosyltransferase activities from human costal cartilage (1.1 and 10.9 mU/l) and the recovery rates were expressed as percent of the added activities.

Effect of agents on xylosyltransferase activity

Fibrinogen

The effect of human fibrinogen on xylosyltransferase activity was investigated in synovial fluid, serum and tissue extract from human costal cartilage, supplemented with bovine serum albumin (18 g/l).

Fibrinogen concentrations between 0 and 21.5 g/l in the incubation mixture were used. Xylosyltransferase activity was also determined in fibrinogen-containing plasmas and corresponding fibrinogen-depleted sera of blood donors ($n = 20$).

Chondroitin sulphate peptidoglycans and heparin

Tissue extracts of human costal cartilage were incubated with chondroitin sulphate peptidoglycans of different degree of sulphation (SO_4^{2-} , 0.31–0.96 mol/mol disaccharide) and with a persulphated peptidoglycosaminoglycan (SO_4^{2-} , 4 mol/mol disaccharide) in a concentration range between 0 and 1000 $\mu\text{mol/l}$ galactosamine per assay.

The effect of heparin on xylosyltransferase activity was examined in a concentration range of 0 to 566 USP units per ml of incubation mixture, also using serum of blood donors.

Native elastase from granulocytes

A tissue extract of human costal cartilage was incubated with defined quantities of human granulocyte elastase (1.86, 18.6, and 186 mg/l) during 140 min. After an incubation time of 0, 45 and 140 min, aliquots were taken and immediately mixed with the elastase inhibitor phenylmethanesulphonyl fluoride to reach a final concentration of 0.1 mmol/l. The complete abolition of elastase activity in the aliquots was checked by a method described previously (17). Xylosyltransferase activity was measured by the method described above.

Xylosyltransferase activity in cells of the synovial system

Xylosyltransferase activities were investigated in human hip articular cartilage, cultured synovial fibroblasts, pellet of synovial cells from synovial fluids with different degree of inflammation, and in blood granulocytes.

The hip articular cartilage derived from morphologically unchanged biopsy material and was homogenized before extraction. Synovial cells were taken from tissue pieces of the knee-joint capsule obtained during meniscectomy and maintained in monolayer cultures. The synovial cell pellet was obtained after centrifugation (750 g, 5 min) of fresh synovial fluids. Granulocytes were isolated from the buffy coat of centrifuged stored blood by density gradient centrifugation in the presence of ficoll.

Lysis of the different cells in tris buffer (0.1 mol/l, pH 7.4) was achieved by repeated deep-freezing and thawing and subsequent treatment by ultrasound. After centrifugation (10 000 g; 30 min) xylosyltransferase activities were measured in the supernates.

Other methods

Elastase in complex with α_1 -proteinase inhibitor was determined by an enzyme immunoassay described previously (18).

Determination of protein content was done by the method of Lowry (19), using bovine serum albumin as standard.

Quantitation of serine residues of the different acceptors was carried out with a Biotronik LC 6000 E amino acid analyser after hydrolysis in 6 mol/l HCl at 110 °C for 24 h. Galactosamine was determined by the same method after hydrolysis of the sample with 3 mol/l HCl.

Estimation of the cell count in the native synovial fluids was performed with a *Neubauer* haemocytometer. In synovial fluids with high cell counts predilution with 9 g/l NaCl was necessary. Synovial cells were differentiated after centrifugation of the native synovial fluids for 15 min at 1000 g and staining of the cell pellet on a slide, according to the method of *Pappenheim* (20). In order to facilitate the spreading of the cells, the viscous cell pellet was depolymerized by short-term preincubation with 5 μ l (16 units) of hyaluronate-4-glycanohydrolase. The granulocyte number was calculated from its percentage of differential synovial cell count in the total cell count measured.

Statistical analysis

The linear regression function was evaluated according to the method of *Pearson*. The *Spearman* rank correlation coefficients were calculated to prove significant correlations between xylosyltransferase activities in synovial fluids and sera of patients with different chronic joint diseases. Differences in mean values were examined by the *Kruskal-Wallis* H-test and the *Wilcoxon* signed ranks test (21); P values < 0.02 were considered as significant.

Results

Precision, accuracy, working range, and detection limit of the xylosyltransferase assay

The intra-assay coefficients of variation, calculated from measurements of samples with high (mean value = 6.85 mU/l), intermediate (mean value = 4.89 mU/l), and low (mean value = 2.71 mU/l) xylosyltransferase activities, were 7.5%, 8.2% and 9.5%, respectively. Using aliquots of these samples for evaluation of the inter-assay imprecision, coefficients of variation of 10.2%, 11.1%, and 11.8% were found.

Data on accuracy are not available, since standard reference material is not established yet. Nevertheless, synovial fluids of patients with inflammatory and non-inflammatory joint diseases, mixed with tissue extracts of human costal cartilage gave recovery rates close to 100% (range 97.9–103.6%). The detection limit of xylosyltransferase activity was found to be 0.15 mU/l. The working range of the assay is between 0.5 and 40 mU/l (fig. 1). Thus, it was not necessary

Fig. 1. Linearity of the xylosyltransferase assay. The enzyme activity was determined in dilutions of a xylosyltransferase-enriched tissue extract of human costal cartilage.

to predilute samples of synovial fluid and serum, since all xylosyltransferase activities determined in these body fluids were within the working range.

Xylosyltransferase activities in synovial fluid and serum remained unchanged for at least 1 week at 4 °C, and at –70 °C for longer than 6 months. The enzyme loses about 10–20% activity in 1 week when kept at room temperature.

Studies with different substrates

Silk has been shown to be the substrate with the highest incorporation rate in the xylosyltransferase assay, compared with bovine HF- and CF₃SO₃H-degraded proteoglycans, and the synthetic peptides Leu-Pro-Ser-Gly and Pro-Ser-Gly (tab. 1). No significant incorporation of [¹⁴C]xylose was observed when the peptides were used. While the *K_m* values of silk and HF-degraded proteoglycan (core protein I in tab. 1) are in the same range, the corresponding enzyme and acceptor activities showed clear differences. The enzyme activity (incorporation of xylose, nmol · min⁻¹ · l⁻¹) of xylosyltransferase in the presence of silk was 3.7 and 1.8 times higher than that in the presence of the HF- and CF₃SO₃H-degraded proteoglycans, respectively. The acceptor activity of silk (incorporation of xylose, nmol · min⁻¹ · mmol⁻¹ serine) exceeds that of HF-degraded proteoglycans by a factor 2.

No influence of an endogenous acceptor was observed in any case, since the incubation without exogenous acceptor did not result in a significant incorporation of [¹⁴C]xylose.

Tab. 1. [14 C]Xylose transfer to various exogenous acceptors in the xylosyltransferase assay. Enzyme and acceptor activities were calculated in the presence of the following acceptors: core protein I (from HF-degraded proteoglycan), core protein II (from trifluoromethane sulphonic acid-degraded proteoglycan), silk from *Bombyx mori*, and 2 synthetic peptides.

Acceptor protein	Enzyme activity	Acceptor activity
	[14 C]xylose incorporated (nmol $\cdot$ min $^{-1}$ $\cdot$ l $^{-1}$)	(nmol $\cdot$ min $^{-1}$ $\cdot$ mmol $^{-1}$ serine)
Core protein I	19.5	1.27
Core protein II	39.7	2.57
Silk	72.6	2.63
Leu-Pro-Ser-Gly	<0.2	
Pro-Ser-Gly	<0.2	

Xylosyltransferase activity in human cells

Examination of defined cells of the synovial system demonstrates the presence of xylosyltransferase activities predominantly in articular cartilage chondrocytes (41 – 196 μ U/g wet weight). A minor enzyme activity (< 8 μ U/ 10^6 cells) was also detectable in cultured synovial fibroblasts, which are able to synthesize small amounts of chondroitin sulphate (< 10% of the total glycosaminoglycans). Xylosyltransferase activity was found neither in the total synovia cell pellet from inflammatory joint effusions nor in granulocytes.

Effect of fibrinogen on xylosyltransferase activity

When we determined xylosyltransferase activities in sera and plasmas of blood donors ($n = 20$), significantly lower enzyme activities were found in all fibrinogen-containing plasma samples. The mean value in plasma was diminished to 46.3% (SD = 2.8%) of that in serum.

Moreover, the addition of human fibrinogen to serum caused a marked inhibition of xylosyltransferase activity (fig. 2). At physiological concentrations the degree of inhibition was about 50%. In synovial fluids and tissue extracts of human costal cartilage, however, the fibrinogen-induced inhibition was less pronounced (22% and 12% of inhibition in the presence of physiological concentrations of fibrinogen, respectively) (fig. 2).

Inhibition studies with chondroitin sulphate peptidoglycans and heparin

Chondroitin sulphate peptidoglycans and glycosaminoglycan polysulphates show dose-dependent inhibition of xylosyltransferase activities (fig. 3). The degree

Fig. 2. Inhibitory effect of fibrinogen on xylosyltransferase activity in tissue extracts of human costal cartilage (A), synovial fluid (B), and serum (C). The hatched area indicates the physiological concentration range of fibrinogen.

Fig. 3. Effect of peptidochondroitin sulphates of different degrees of sulphation on xylosyltransferase activity in tissue extracts of human costal cartilage (PCS = peptidochondroitin sulphate; GAGPS = persulphated peptidoglycosaminoglycan).

The content of sulphate (mol/mol disaccharide) was as follows: PCS-17 = 0.31; PCS-23 = 0.52; PCS-29 = 0.71; PCS-35 = 0.96; GAGPS = 4.0.

of sulphation of the glycosaminoglycans also exerts an important influence on the extent of inhibition. A complete suppression of xylosyltransferase activity was observed in the presence of a persulphated peptidoglycosaminoglycan at concentrations of 10 μ mol/l GalN. Chondroitin sulphate peptidoglycans with sulphate contents of 0.52–0.96 mol per mol disaccharide caused a 50% inhibition in the concentration range between 200 and 600 μ mol/l GalN, whereas lower sulphated chondroitin sulphate peptidoglycans

(SO_4^{2-} , 0.31 mol/mol disaccharide) did not show a significant inhibitory effect on xylosyltransferase activity.

Also the polyanion heparin exhibits a dose-dependent inhibition of xylosyltransferase activity in serum and tissue extracts of human costal cartilage (fig. 4). Heparin added to the assay mixture at concentrations usually found in plasma during therapy (0.2–1 USP units/ml), caused only a slight inhibition (2–5%) of serum xylosyltransferase activity. However, in the presence of heparin concentrations, which are used in blood sampling to prevent clotting (10–15 USP units/ml plasma), the serum xylosyltransferase activity was inhibited by about 40%. The inhibitory effect of heparin is more pronounced on xylosyltransferase activities from tissue extracts of human costal cartilage (fig. 4).

Fig. 4. Effect of heparin on xylosyltransferase activity in serum (A) and tissue extracts of human costal cartilage (B). The hatched areas represent heparin concentrations in plasma during therapy (I), and in blood sampling with heparin as anticoagulant (II).

Fig. 5. In vitro effect of human granulocyte elastase on xylosyltransferase activity in tissue extracts of human costal cartilage. Elastase was added to give the following final concentrations: 1.86 mg/l (○), 18.6 mg/l (x), 186 mg/l (▲), 0 mg/l (●).

Effect of native granulocyte elastase on xylosyltransferase activity

Xylosyltransferase activity was almost completely inhibited after incubation of tissue extracts of human costal cartilage with granulocyte elastase (186 mg/l). At a lower elastase concentration of 18.6 mg/l the xylosyltransferase activity was diminished to 61.4% and 9.4% of the initial activity after incubation for 45 and 140 min, respectively (fig. 5).

Xylosyltransferase activities in synovial fluids and sera of patients with chronic joint diseases

In synovial fluids and sera of patients with chronic joint diseases xylosyltransferase activities ranged from 0.5 to 22.0 mU/l and from 0.8 to 5.6 mU/l, respectively (fig. 6). The parallel determination of xylosyltransferase activity in synovial fluid and serum of the same patients showed that in more than 80% of the synovial fluids enzyme activities were higher than those of the corresponding sera (fig. 7). However, no significant correlation between xylosyltransferase activity in synovial fluids and that in sera was observed.

The serum xylosyltransferase activities of patients with chronic joint diseases were not significantly dif-

Fig. 6. Xylosyltransferase activities in synovial fluid (●) and serum (○) from patients with different chronic joint diseases. The median and single values are given. CPDD = calcium pyrophosphate deposition disease.

Fig. 7. Comparison of xylosyltransferase activities in synovial fluids and sera taken from the same patients, which were suffering from the following joint diseases: osteoarthritis (●; $n = 33$), rheumatoid arthritis (○; $n = 24$), ankylosing spondylitis (+; $n = 10$), Reiter's syndrome (▲; $n = 4$), and septic arthritis (△; $n = 6$).

ferent (fig. 6) and were similar to those of the reference group (median value: 1.42 mU/l, 90%-range: 1.21–1.67 mU/l).

The highest enzyme activities were found in synovial fluids of patients suffering from rheumatoid arthritis (median value: 5.56 mU/l, 90%-range: 3.2–22.0 mU/l). Xylosyltransferase activities determined in synovial fluids of patients with osteoarthritis (median value: 2.50 mU/l, 90%-range: 0.8–4.8 mU/l), and calcium pyrophosphate deposition disease (median value: 1.78 mU/l, 90%-range: 0.6–3.2 mU/l) were significantly lower ($P < 0.0001$). That was also true for enzyme activities in synovial fluids of patients with arthritis urica (median value: 2.38 mU/l, 90%-range: 0.7–5.2 mU/l, $P < 0.0001$), although granulocyte counts and elastase concentrations in synovial fluids of patients with rheumatoid arthritis and arthritis urica were in the same range, showing a high degree of inflammation. The median value and 90%-range (in parenthesis) of granulocyte numbers in synovial fluids of patients with rheumatoid arthritis and arthritis urica were 3.25 (0.96–11.0) and 7.76 (2.24–26.9) $\times 10^9/l$, respectively. The corresponding values of granulocyte elastase were 7.65 (2.52–23.2) and 6.89 (1.56–30.2) $\mu g/l$, respectively. In synovial fluids of patients with arthritis urica, the measured xylosyltransferase activities were similar to those in osteoarthritis ($P = 0.741$). Statistical analysis revealed no significant correlation between synovial fluid activity of xylosyltransferase and corresponding granulocyte count or elastase concentration, respectively.

Discussion

Xylosyltransferase activity is present in the rough endoplasmic reticulum of chondrocytes of various species (6). The present information about the involvement of the xylosyltransferase in the biosynthesis of carbohydrate side chains of proteoglycans is mainly based on the investigations of Schwartz & Dorfman (22), which suggest that the xylosyltransferase molecule is provided with binding sites to UDP-xylose, the core protein and galactosyltransferase I. After xylosylation of serine residues in the core protein, the transfer of galactose from UDP-galactose to the xylose residue occurs. However, the course of the proteoglycan synthesis between formation of core protein and addition of carbohydrate side chains has not been elucidated in detail so far (23). In analogy to glycoprotein synthesis (24) it is suggested that the initiation of chondroitin sulphate chains already occurs while the protein core is still nascent (5, 25).

Only a little information about the specificity of xylosyltransferase is available. It is not known whether there is a specific xylosyltransferase for each type of proteoglycan. According to previous findings, the *in vivo* substrate for xylosyltransferase is not a single, well-defined compound but rather a spectrum of molecules, ranging from the shortest possible nascent peptide capable of serving as an acceptor in the first xylosyl transfer reaction to larger peptides species in which almost all of the reactive serine residues have been glycosylated. The only constant feature of the substrate structure discovered so far is the presence of a glycine residue at the C-terminal side of the xylose-accepting serine residue (26). Thus, the search for appropriate substrates of the xylosyltransferase was focused on proteins containing the Ser-Gly pair and it led to silk from *Bombyx mori*, which contains a large number of Ser-Gly sequences in the repeating hexapeptide Ser-Gly-Ala-Gly-Ala-Gly (13). It should be emphasized that the presence of a Ser-Gly pair is not in itself sufficient to convey acceptor activity to a protein, since collagen which contains several such groups is not an acceptor (27).

The K_m values which have been reported for silk (182 mg/l) (13) and protein core of Smith-degraded proteoglycan (143–240 mg/l) (13, 15) are similar, when expressed on a weight basis. Since precise information regarding the relative proportions of reactive and unreactive serine residues is not available for either substrate, the more appropriate comparison based on the molar concentrations of reactive serine residues cannot be made at the present time.

However, our study shows that the acceptor activity, i.e. the incorporation rates of [^{14}C]xylose in our test

system, is about two to three times higher than that of the proteoglycan derivatives. Compared with core protein acceptors, which often show the disadvantage of considerably varying acceptor activities between different preparations, silk is more invariably available. Since the practical and economic advantages of using silk as substrate are evident, in our study this acceptor was employed to measure xylosyltransferase activities in synovial fluids and sera of patients with chronic joint diseases.

The xylosyltransferase activities which we have found in the synovial fluid of patients with chronic-inflammatory and degenerative joint diseases ranged between 0.5 and 22.0 mU/l. In the synovial system the articular cartilage represents the main source of these enzyme activities. Our study shows that low xylosyltransferase activity is also detectable in cultured synovial fibroblasts, which synthesize chondroitin sulphate (about 10% of total glycosaminoglycan production) in addition to hyaluronate, which is the main glycosaminoglycan. No xylosyltransferase activity was found in extracts of granulocytes and in synovial cell pellets, not even in highly inflammatory processes.

It is generally accepted that tissue destruction in inflammatory joint diseases is caused by proteinases, which are released from granulocytes (28–31) or other sources, like macrophages, synovial fibroblasts, and chondrocytes (32–34). Particularly elastase, which is secreted in large amounts from granulocytes, plays a crucial role in the destruction of articular cartilage in chronic inflammatory joint diseases, due to its ability to cleave the protein core and the hyaluronate binding region of proteoglycans (35–37).

Since proteoglycan fragments, which may serve as a substrate for xylosyltransferase, are released into the synovial fluid during inflammatory joint processes, we studied the influence of chondroitin sulphate peptidoglycosaminoglycans on the xylosyltransferase activity *in vitro*. Our studies have shown that the xylosyltransferase activity is inhibited by chondroitin sulphate peptidoglycosaminoglycans as a function of concentration and degree of sulphation, and furthermore that granulocytic elastase degrades xylosyltransferase activity. However, an effect of these factors on the enzyme activities measured in synovial fluids from patients with chronic joint diseases seems to be unlikely, since xylosyltransferase activities, added to synovial fluid in defined quantities, showed a high recovery.

When we determined xylosyltransferase activities in synovial fluid and serum of the same patient, no significant correlation between serum activity and

enzyme activity in the synovial fluid was found. While many of the synovial fluids exhibit high enzyme activity, no parallel increase was observed in the corresponding sera. This may be due to the high dilution of the enzyme, which is released from the joint cavity into the blood. Data on the half life of the enzyme in blood are not yet available. Moreover, the organ specificity of this parameter in serum is limited, since different tissues, e.g. liver (38) and skin, must be taken into account as sources of xylosyltransferase activity. Our study rather suggests that the xylosyltransferase activity derived from articular cartilage can be specifically determined in blood only by an immunological method (radioimmunoassay, enzyme immunoassay).

The inhibition studies with fibrinogen also suggest that various xylosyltransferase isoenzymes are present in blood. Addition of human fibrinogen to serum in physiological concentrations caused a marked inhibition (about 50%) of xylosyltransferase activity. A similar degree of inhibition was observed when we compared plasma enzyme activities with those in the corresponding sera. The mechanism of the inhibitory effect of fibrinogen is still unclear. However, the degree of inhibition seems to depend on the origin of the xylosyltransferase isoenzyme, since the blood xylosyltransferase is inhibited more than that derived from human costal cartilage (about 12% of inhibition at physiological concentrations of fibrinogen). The intermediate degree of inhibition of xylosyltransferase activity (about 22%) in synovial fluids from patients with chronic joint diseases may be due to the presence of xylosyltransferase isoenzymes from sources outside the joint, resulting from an enhanced permeability of the blood-synovial-barrier in inflammatory joint processes.

The xylosyltransferase activities in synovial fluids showed considerable differences between clinically defined joint diseases. The highest enzyme activities were found in the synovial fluids of patients with rheumatoid arthritis. It is an interesting finding that the xylosyltransferase activities in synovial fluids of patients with arthritis urica were much lower and in the same range as those in osteoarthritis, although the granulocyte count and elastase concentration, reflecting the degree of inflammation, corresponded with those in synovial fluids of patients with rheumatoid arthritis. Thus, it is obvious that the xylosyltransferase activity represents no parameter of inflammation in the precise sense, although in inflammatory processes the increased destruction of chondrocytes may lead to an enhanced release of xylosyltransferase.

There was no correlation between xylosyltransferase activity in the synovial fluid and granulocyte number or elastase concentration. The higher xylosyltransferase activities in synovial fluids of patients with rheumatoid arthritis, compared with those of patients with arthritis urica and osteoarthritis, is probably attributed to an increased cartilage destruction during the course of this disease.

Our results demonstrate that xylosyltransferase activity in the synovial fluid represents an appropriate clinical chemical parameter, indicating the degree of cartilage destruction in chronic joint diseases. In serum, however, various xylosyltransferase isoenzymes from different tissue sources may be present. The employment of an immunoassay, which is now

under development by our group, seems to be an essential requirement for the determination of cartilage-specific xylosyltransferase in serum.

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