Xylosyltransferase II is the predominant isoenzyme which is responsible for the steady-state level of xylosyltransferase activity in human serum

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ABSTRACT

In mammals, two active xylosyltransferase isoenzymes (EC 2.4.2.16) exist. Both xylosyltransferases I and II (XT-I and XT-II) catalyze the transfer of xylose from UDP-xylose to select serine residues in the proteoglycan core protein. Altered XT activity in human serum was found to correlate directly with various diseases such as osteoarthritis, systemic sclerosis, liver fibrosis, and pseudoxanthoma elasticum. To interpret the significance of the enzyme activity alteration observed in disease states it is important to know which isoenzyme is responsible for the XT activity in serum. Until now it was impossible for a specific measurement of XT-I or XT-II activity, respectively, because of the absence of a suitable enzyme substrate. This issue has now been solved and the following experimental study demonstrates for the first time, via the enzyme activity that XT-II is the predominant isoenzyme responsible for XT activity in human serum. The proof was performed using natural UDP-xylose as the xylose donor, as well as the artificial compound UDP-4-azido-4-deoxyxylose, which is a selective xylose donor for XT-I.

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1. Introduction

Xylosyltransferases I and II (XT-I, XT-II, EC 2.4.2.26) catalyze the initial step in the biosynthesis of glycosaminoglycans by the transfer of xylose from UDP-xylose to specific serine residues of the core protein in proteoglycans (PGs), the major components of connective tissue [1–5]. It is somewhat surprising that all higher organisms have two xylosyltransferase enzymes, whereas only a single isoform exists for the other glycosyltransferases involved in the biosynthesis of the common GlcAβ1,3-Galβ1,3-Galβ1,4-Xyl-O-Ser glycosaminoglycan tetrasaccharide linker in PGs [6–8].

To date, the enzymatic function of both xylosyltransferase isoforms has seemed to be identical although the xylosyltransferase genes have been shown to be differentially expressed in mammalian tissues and cell lines [4,5,9]. XT-I and XT-II shared approximately 55% overall sequence homology, with regions showing more than 80% identity [4]. In almost all cell types and tissues XT-II expression is detectable, while XT-I mRNA expression was only marginal in multiple cell lines and undetectable in seven cell lines [10]. Moreover, we hypothesized that the physiological role of XT-I and XT-II is largely dependent upon the acceptor affinity because of the different K_m values observed [11]. A systematic evaluation of acceptor peptides carrying a G-S-G or G-S-x-G motif demonstrated that XT-I acceptor xylosylation was less stringent but had a higher kinetic turnover since K_m and V_{max} values were slightly higher than those for XT-II. In contrast, XT-II has stronger affinity for acceptor peptides while showing low enzymatic efficiency [10]. Although both isoforms were profoundly biochemically characterized and were proven to bear many similarities regarding substrate and acceptor specificity as well as enzymatic kinetics in vitro, the precise physiological function of each XT isoform is still unknown. It is

likely that acceptor peptides exist, which are specific for either XT-I- or XT-II-mediated xylosylation.

The presence of two xylosyltransferase isoforms might be crucial for higher organisms to regulate PG biosynthesis and to adjust the PG pool. XT-I plays a key role in chondrocyte maturation, bone development, and matrix mineralization [12–15]. We further demonstrated increased XT-I expression as a marker for myofibroblast differentiation, a key event in wound healing and fibrogenesis [16]. Loss of XT-II has been associated with reduced PG synthesis in the liver and cystogenesis [17].

Numerous studies have reported increased serum XT activity to be associated with disorders characterized by active fibrogenesis, e.g. systemic sclerosis, osteoarthritis and liver fibrosis [18–21]. Therefore, XT activity was suggested to be a promising biomarker in blood reflecting the actual PG synthesis rate. However, the source of serum XT activity is still a matter of discussion. To date, it is not clear if serum XT activity results from both XT isoforms. Data from several in vitro studies proposed that both XT isoforms are actively secreted by tissues to the extracellular space, therefore occurring as active enzyme(s) in the circulation [22]. It was observed that a loss of XT-II activity might be compensated through increased XT-I expression [23]. In this context, the lack of XT serum activity in *Xylt2* knock-out mice [17] lead to the question of the source of XT activity in serum. It was hypothesized that XT-II might be released by the liver or by platelet activation as both exclusively express XT-II [24]. Proteomic data demonstrated XT-II to be the only XT isoform identified in platelets [25]. A specific assay to distinguish XT-I from XT-II activity in blood and other biological fluids was not available until now. In this study, we refined our already published mass spectrometric method in which the original substrate, UDP-xylose, was replaced with UDP-4-azido-4-deoxyxylose (UDP-XylAz) [26]. The use of UDP-XylAz enables us to measure solely and selective XT-I activity.

2. Materials and methods

2.1. Materials

JAR choriocarcinoma cells were purchased from ATCC (Rockville, MD) and Wild-type CHO cells (CHO-K1) derived from DSMZ (Braunschweig, Germany). UltraDOMA-PF cell culture medium was from BioWhittaker (Vervier, Belgium) and aqua ad injecta from Braun (Melsungen, Germany). Fetal calf serum was obtained from Biowest (Nuaille, France). UDP-xylose was purchased from Carbo-Source Services (Athens, Georgia). The synthetic peptide Bio-BIK-F (Xylosyltransferase acceptor peptide: Biotin-NH-QEEEGSGGGQKK(5-fluorescein)-CONH₂) was obtained from Thermo Electron GmbH (Ulm, Germany). HPLC-grade water and methanol were purchased from Fisher Scientific GmbH (Schwerte, Germany). All other reagents were of research grade or better and were purchased from commercial sources.

2.2. Cell culture

JAR cells were cultured according standard procedures [27]. When confluency was reached in the T-flasks, the adherent cells were removed, washed twice with DPBS, suspended in protein-free Ultradoma-PF medium, and cultured in the same medium supplemented with 1x antibiotic/antimycotic solution without fetal calf serum. After confluence was reached, the cell culture supernatant was harvested for XT activity measurement.

Cloning of human XT-I cDNA and XT-II cDNA, respectively, were performed as described previously [5,28,29]. The XT-I, as well as the XT-II containing culture medium was collected separately and clarified by centrifugation at 1500 *g* for 5 min.

2.3. Serum samples

Venous blood samples from blood donors were collected for routine analysis in serum monovettes from KABE Labortechnik GmbH (Nümbrecht-Elsenroth, Germany). Anonymized samples were left over from routine analysis; hence, no medical-ethical approval was necessary for this study.

2.4. Synthesis of UDP-XylAz

UDP-XylAz was prepared as described previously [30]. Briefly, the 1-, 2- and 3-hydroxyl groups of ʟ-arabinose, the C-4 epimer of xylose, were selectively benzolyated. The remaining free hydroxyl group at the C-4 position of 1,2,3-tri-O-benzoyl-ʟ-arabinose was then converted to an azide. Following exchange of the benzoyl groups with acetyl groups, selective deprotection of the anomeric position was performed with hydrazine. Reaction of the resulting free hydroxyl group with diallyl-N,N-diisopropylphosphoramidite gave the globally protected azido xylose-1-phosphate product. After deprotection of the phosphate group and immediate reaction with UMP-N-methylimidazolide, UDP-XylAz was obtained.

2.5. Sample preparation for XT activity measurement

The XT activity measurement is based on the xylosyltransferase mediated incorporation of xylose into the synthetic acceptor peptide Bio-BIK-F containing the XT recognition sequence. UDP-xylose, as well as UDP-XylAz was used as xylose donor. The reaction mixture for the assay contains, in a total volume of 100 μL: 50 μL sample, 20 μl acceptor solution (33 μmol/L Bio-BIK-F), 25 mmol/L MES (pH 6.5), 25 mmol/L KF, 5 mmol/L MnCl₂, 5 mmol/L MgCl₂, and 150 μmol/L UDP-xylose or UDP-XylAz. After incubation at 37 °C for 12 h, the reaction was stopped by heating the reaction mixtures at 99 °C for 10 min. The samples were centrifuged at 10,000 g, and the clear supernatant was transferred to an autosampler vial, and used for further analysis.

2.6. Mass spectrometric XT activity measurement

The xylosylated peptides Bio-BIK-F-Xyl and Bio-BIK-F-XylAz, respectively, were quantified according to the mass spectrometric assay described previously [26], with various modifications. Separation of the enzyme product Bio-BIK-F-Xyl, as well as Bio-BIK-F-XylAz was performed on a 2.1×50-mm reverse phase column (UPLC BEH C18, 1.7 mm) maintained at 60 °C using a UPLC (ultra-performance liquid chromatography) system directly coupled to a tandem mass spectrometer (Waters, XEVO TQ-S System). A 1.0 μl sample was injected at a flow rate of 0.5 ml/min. The gradient program was 90%/10% water/methanol containing 0.1% formic acid for 1.0 min, followed by a linear gradient over 0.8 min of 5%/95% water/methanol. After additional 0.5 min, the mobile phase was revered to the initial state, and the run was terminated at 3.0 min. The mass spectrometer was operated in electrospray positive ionization mode. Nitrogen was used as the nebulizing gas and argon was used as the collision gas. Instrument settings were as follows: capillary voltage, 2.0 kV; source temperature, 150 °C; desolvation temperature, 600 °C; collision gas pressure, 3.0×10^{-3} mbar. Sample analysis was performed in the multiple reaction monitoring mode (MRM) of the instrument, with a dwell time of 0.16 s for all compounds. Mass transition, sample cone energy, and collision energy for Bio-BIK-F-Xyl were 975.2 → 909.0, 10 eV, and 29 eV and for Bio-BIK-F-XylAz 987.6 → 909.0, 10 eV, and 29 eV, respectively. The enzyme activity was 1 mU = 1 nmol of incorporated xylose per min, which is equal to the synthesis of

175.5 μg/L Bio-BIK-F-Xyl or 177.8 μg/L Bio-BIK-F-XylAz, respectively, under the assay conditions (incubation time = 90 min).

3. Results

3.1. General approaches of the UPLC-electrospray ionization tandem mass spectrometry investigations

By UPLC, both Bio-BIK-F-Xyl and Bio-BIK-F-XylAz were successfully separated from most impurities in the samples, an operation that facilitates the tuning of the mass spectrometer during method development (Fig. 1). After separation by UPLC, the xylosylated peptides were measured by scanning positively charged ions. As shown in the inserts of Fig. 1 we detected only double charged ions of Bio-BIK-F-Xyl (m/z 975.2) and Bio-BIK-F-XylAz (m/z 987.6), respectively. Therefore, we performed daughter-ion scanning of the positively double-charged xylosylated peptides indicating that double-charged dexylosylated peptide Bio-BIK-F (m/z 909.0) was the major daughter ion of both xylosylated peptides. Optimal sensitivity for the detection of Bio-BIK-F-Xyl, as well as Bio-BIK-F-XylAz by mass spectrometry in the MRM mode was achieved at collision energy of 29 eV.

3.2. UPLC-MS/MS-based approach for selective measurement of XT-I

As no xylose acceptor peptide has yet been identified that is specific for one of the two XT isoforms, we focused on the xylosyl donor as a means of differentiating between the activities of each enzyme. At first we produced a cell culture supernatant, which should contain both XT isoforms, using JAR choriocarcinoma cells expressing both XT-I and XT-II mRNA [10]. The ability of the XT(s) found in JAR choriocarcinoma cells to transfer XylAz to a known acceptor peptide, Bio-BIK-F, using UDP-XylAz as a donor was then assessed, and revealed that UDP-XylAz is a substrate for the XT(s). XT activity measurement of the supernatant was 23.0 mU/L for UDP-xylose and 0.35 mU/L for UDP-XylAz. The limit of detection of the XylAz transfer was determined to be 0.04 mU/L using Bio-BIK-F as xylose acceptor and UDP-XylAz as xylose donor under the measurement conditions.

Furthermore, in two separate procedures we produced recombinant human XT-I and XT-II, respectively. Using Bio-BIK-F as the xylose acceptor, the XT activity of the cell culture supernatant of High Five/pCG255-1 insect cells, which expressed the recombinant

XT-I enzyme, was determined to be 38.5 mU/L using UDP-xylose as xylose donor and 2.87 mU/L using UDP-XylAz (see also Fig. 2A and E, respectively). The XT activity of the XT-II preparation in which pgsA-745 CHO cells expressed the full-length human XT-II in serum- and protein-free cell culture medium was 40.5 mU/L if UDP-xylose was used as xylose donor; however no XT activity was detected when UDP-XylAz was used as the xylose donor instead of UDP-xylose (see also Fig. 2B and F, respectively). These investigations were verified two times with the same results, indicating that only the XT-I isoform transfers XylAz from UDP-XylAz to the Bio-BIK-F peptide. A schematic view of the results of these investigations is shown in Fig. 3.

Furthermore, we mixed XT-I and XT-II samples and measured the XT activity of the mixtures using UDP-XylAz as sugar donor. The results show that only mixtures which contain XT-I transfer XylAz from UDP-XylAz to the Bio-BIK-F peptide, and the transfer rate increased with increasing proportion of XT-I in the mixture (0% XT-I and 100% XT-II = not detectable; 25% XT-I and 75% XT-II = 315 μg/L Bio-BIK-F-XylAz; 50% XT-I and 50% XT-II = 736 μg/L Bio-BIK-F-XylAz; 75% XT-I and 25% XT-II = 1195 μg/L Bio-BIK-F-XylAz; 100% XT-I and 0% XT-II = 1819 μg/L Bio-BIK-F-XylAz). These results confirm once again that UDP-XylAz is a selective sugar donor only for the XT-I isoform. The XT-II isoform was unable to transfer XylAz from UDP-XylAz to Bio-BIK-F probably because of steric inhibition of donor sugar binding in the catalytic site of XT-II (illustrated in Fig. 3).

### 3.3. Measurement of XT activity in human serum

Having shown that UDP-XylAz is only a substrate for XT-I, we next used it to investigate the origin of the XT activity observed in human serum. The XT activity of pooled serum from blood donors was measured using Bio-BIK-F as sugar acceptor and UDP-xylose, or UDP-XylAz as the sugar donor. The XT activity of the serum pool was measured to be 26.9 mU/L, which was higher than the XT activity of the JAR cell culture supernatant. However, as shown in Fig. 2C and G, sugar transfer was only performed when UDP-xylose was used as the donor, indicating that no XT-I but only XT-II contributes to XT activity in human serum.

4. Discussion

Xylosylation of the PG core protein is the initial step in biosynthesis of the GlcAβ1,3-Galβ1,3-Galβ1,4-Xyl tetrasaccharide
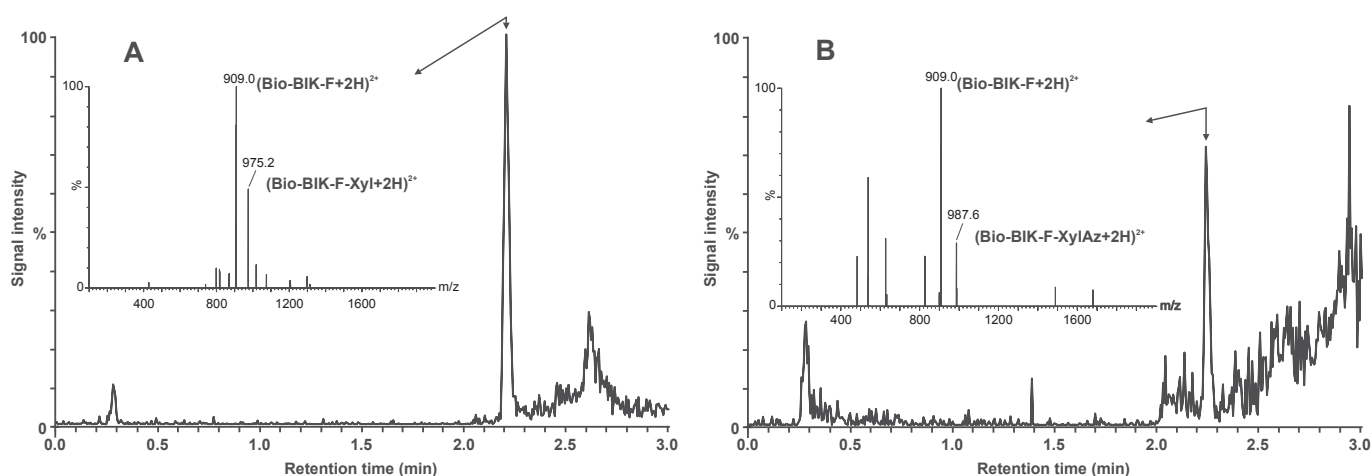
Fig. 1. RP-UPLC-electrospray ionization mass spectrometry chromatogram from A, Bio-BIK-F-Xyl and B, Bio-BIK-F-XylAz crude preparation. Daughter ion scan (scanning from m/z 100 to m/z 2000) of the crude preparations. A, the *insert* shows the doubly charged Bio-BIK-F-Xyl (m/z 975.2) and its main daughter ion (m/z 909.0) and B, the *insert* shows the doubly charged Bio-BIK-F-XylAz (m/z 987.6) and its main daughter ion (m/z 909.0).
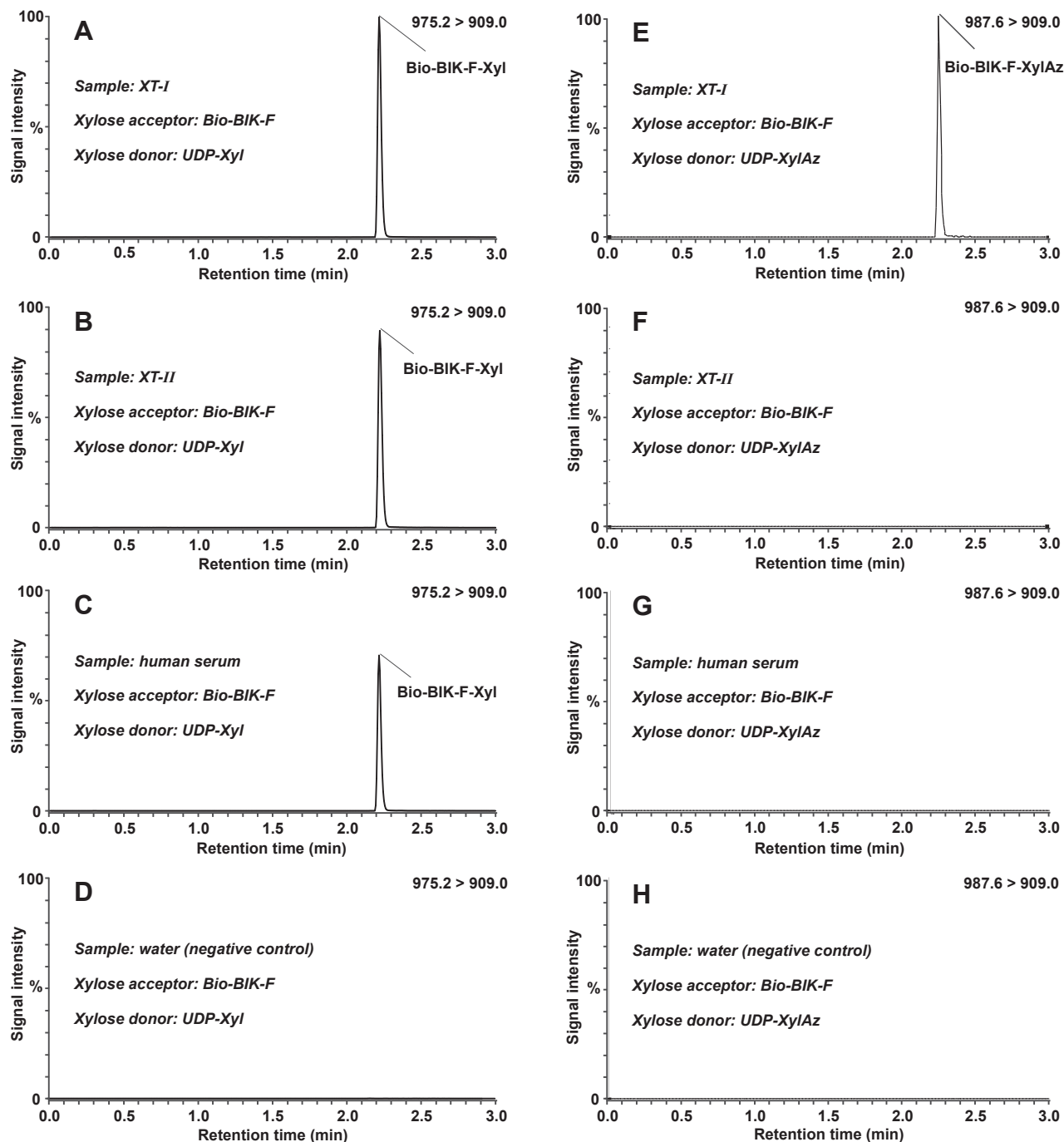
Fig. 2. MRM chromatograms of different sample preparations detecting the xylosyltransferase products Bio-BIK-F-Xyl, as well as Bio-BIK-F-XylAz by measurement of the mass transition m/z 975.2 > 909.0 and m/z 987.6 > 909.0, respectively. A, XT-I was used as the enzyme, UDP-xylose as the xylose donor, and Bio-BIF-F as the xylose acceptor; B, XT-II was used as the enzyme, UDP-xylose as the xylose donor, Bio-BIK-F as the xylose acceptor; C, human serum which contains xylosyltransferase was used as the enzyme sample, UDP-xylose as the xylose donor, and Bio-BIK-F as the xylose acceptor; D, water was used as the negative control sample, UDP-xylose as the xylose donor, and Bio-BIK-F as the xylose acceptor; E, XT-I was used as the enzyme, UDP-XylAz as the xylose donor, and Bio-BIF-F as the xylose acceptor; F, XT-II was used as the enzyme, UDP-XylAz as the xylose donor, Bio-BIK-F as the xylose acceptor; G, human serum which contains xylosyltransferase was used as the enzyme sample, UDP-XylAz as the xylose donor, and Bio-BIK-F as the xylose acceptor; H, water was used as the negative control sample, UDP-XylAz as the xylose donor, and Bio-BIK-F as the xylose acceptor.

linker in PGs and has previously been demonstrated to be the rate-limiting step in glycosaminoglycan biosynthesis [31]. Xylosyl-transferase activity was one of the first glycosaminoglycan glyco-syltransferase activities detected but decades passed before discovering the presence of two xylosyltransferases in higher organisms, and cloning their corresponding genes [2–4].

However, the distinct role of each of the two xylosyltransferases in PG biology still remains to be elucidated. Both xylosyl-transferases share a high degree of homology, especially in the central and carboxy-terminal region of the protein where the pu-tative catalytic domain is located [9]. Even more, phylogenetic an-alyses demonstrate that two xylosyltransferases are found in nearly
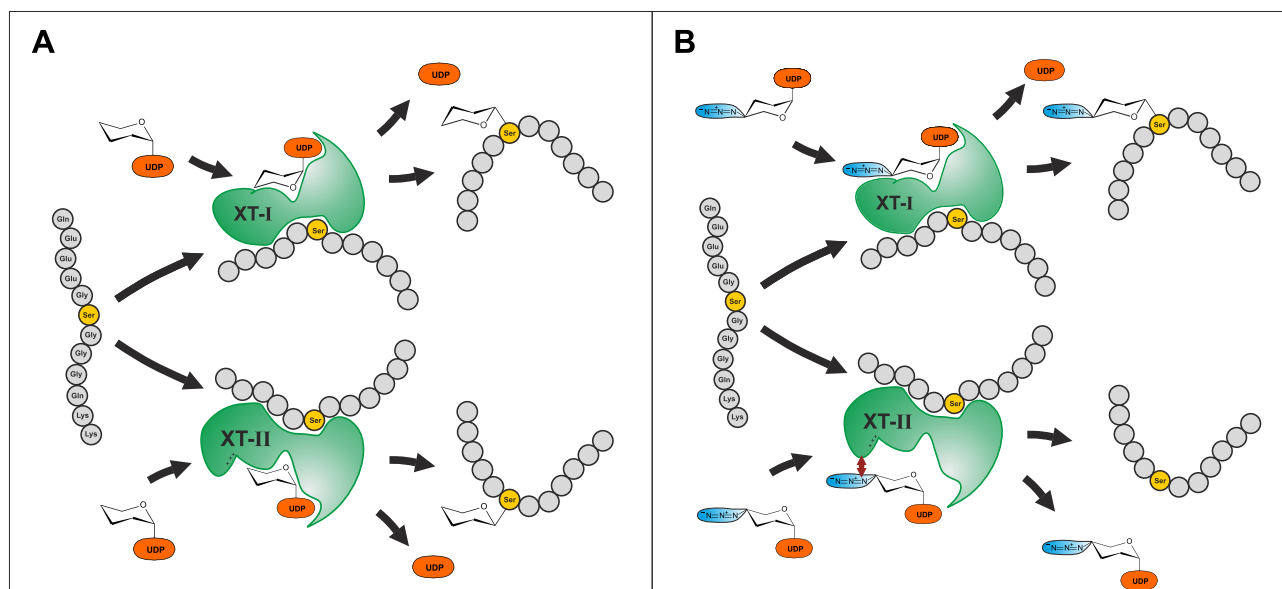
Fig. 3. Schematic view of the enzyme reaction of xylosyltransferase I and II, respectively. A, the upper section illustrates the xylosyltransferase I-catalyzed incorporation of xylose from UDP-xylose into the Bio-BIK-F peptide containing the xylosyltransferase recognition sequence. The lower section illustrates the same reaction, however, catalyzed by xylosyltransferase II. B, the upper section illustrates the xylosyltransferase I-catalyzed incorporation of XylAz from UDP-XylAz into the Bio-BIK-F peptide containing the xylosyltransferase recognition sequence. The lower section illustrates the same reaction catalyzed by xylosyltransferase II; however, the enzyme reaction does not occur, likely because of the steric inhibition of donor sugar binding in the catalytic site of XT-II.

all higher organisms and that the amino acid sequences are highly conserved [4]. These findings indicate that both enzymes have relevant but different functions in development and life for higher organisms. Besides their similarity regarding enzymatic function, the xylosyltransferase genes are differentially expressed in mammalian tissues and cell lines [32]. In most tissues, both xylosyltransferase genes are expressed and in numerous cell lines XT-II is the major xylosyltransferase. In liver tissue XT-II has been found to be exclusively expressed [32]. However, differences in the tissue-expression patterns are probably not the only difference as both enzymes are expressed in most tissues. Therefore, it has been suggested that donor or acceptor specificities exist for either XT-I- or XT-II-mediated xylosylation. However, all acceptor proteins and peptides used as substrate for xylosylation revealed distinct acceptor affinities but did not show an exclusive xylosylation by one of the xylosyltransferases.

In the present study we show different donor properties of XT-I and XT-II using the artificial UDP sugar, UDP-XylAz (Fig. 2). UDP-XylAz, an azide modified UDP-xylose analog, could only be used as a donor substrate by XT-I for the site-specific xylosylation of the bikunin-derived peptide QEEEGSGGGQKK, while no XT-II mediated xylosylation of the central serine residue was observed by mass spectrometric analysis (Fig. 2). Conversely, both XTs efficiently transfer xylose to the peptide serine when UDP-xylose was used as donor (Fig. 2). No xylosylation was detected when XT-II and UDP-XylAz were used in the enzyme assay probably due to steric inhibition of donor sugar binding in the catalytic site of XT-II (Fig. 3).

In addition to recombinant XT-I and XT-II enzyme sources, the capability of using UDP-XylAz as the donor sugar was verified using cell culture supernatants and body fluids. We have demonstrated before that the mass spectrometric assay used allows a precise and sensitive determination of xylosyltransferase activity [26]. Using UDP-XylAz we are now able for the first time to differentiate between XT-I and XT-II activities in blood and other body fluids.

Xylosyltransferase activities in body fluids have been shown to reflect the PG biosynthesis rate. Although xylosylation of the core protein occurs in the Golgi apparatus, the majority of the xylosyltransferase activity in cultured cells is found in the culture supernatant, where it is accumulated in a time-dependent manner [18,33]. Shedding of the xylosyltransferases from the Golgi surface and simultaneous secretion of xylosyltransferase and large PGs suggested that quantification of xylosyltransferase activity in body fluids could be a valuable tool for determining the actual PG synthesis rate, which is of importance in tissue remodeling processes [9,18,19].

Serum xylosyltransferase activity has been shown to be a marker for fibrosis and sclerosis and to be elevated in tissue remodeling processes like pseudoxanthoma elasticum, fibrosis or systemic sclerosis [9]. Using our new assay design with UDP-xylose or UDP-XylAz as donor sugars we are now able to distinguish between XT-I and XT-II activities. In the present study we show for the first time that XT-II is the predominant contributor to the total xylosyltransferase activity in human serum.

Previous studies suggested already that in mice and humans the predominant XT in serum is XT-II [24]. It was further shown that serum XT activity is about 200% higher than those in plasma. Conclusively, only a negligible amount of serum XT activity seemed to be caused by active XT secretion from tissues. Moreover, analyzing proteomic data of platelets suggested XT-II as exclusive isoform to be present in this blood cell type ([25], http://plateletweb.bioapps.biozentrum.uni-wuerzburg.de/plateletweb.php). Our data support these findings since no XT activity was measured in human serum when using UDP-XylAz as donor sugar (Fig. 2). Serum XT activity was found to be increased in systemic disorders characterized by high extracellular matrix remodeling, whereas serum XT activity remained unchanged in disorders with locally restricted fibrogenesis, e.g. arthrofibrosis. Since XT-II was not actively upregulated in fibrogenesis [23,16] but was found to be the predominant enzyme in serum, the high levels of XT serum activity in disorders characterized by active fibrogenesis remain unclear. Therefore, it is of great interest to investigate if the higher XT serum activities relied on altered platelet count, a higher rate of

XT secretion from specific organs, or cell death of myofibroblasts in fibrotic organs. In this context, our new assay provides a powerful tool to distinguish between XT-I and XT-II activity. It will be of high interest to rerun the former studies using this new method.

In conclusion, we have developed a new enzymatic assay that allows us to differentiate between XT-I and XT-II activity. Consequently, these findings and our new assay design offer a tool to shed light onto the role of the two differentially regulated xylosyltransferases in cell biology and its clinical and diagnostic importance in fibrosis and tissue remodeling processes.

Conflict of interest

None.

Acknowledgments

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.129.

References

[1] U. Lindahl, L. Roden, The role of galactose and xylose in the linkage of heparin to protein, J. Biol. Chem. 240 (1965) 2821–2826.

[2] H.C. Robinson, A. Telser, A. Dorfman, Studies on biosynthesis of the linkage region of chondroitin sulfate complex, Proc. Natl. Acad. Sci. U. S. A. 56 (1966) 1859–1866.

[3] J. Kuhn, C. Götting, M. Schnölzer, et al., First isolation of human UDP-D-xylose: proteoglycan core protein β-D-xylosyltransferase secreted from culture JAR choriocarcinoma cells, J. Biol. Chem. 276 (2001) 4940–4947.

[4] C. Götting, J. Kuhn, R. Zahn, et al., Molecular cloning and expression of human UDP-d-Xylose:proteoglycan core protein beta-d-xylosyltransferase and its isoform XT-II, J. Mol. Biol. 304 (2000) 517–528.

[5] C. Pönighaus, M. Ambrosius, J.C. Casanova, et al., Human xylosyltransferase II is involved in the biosynthesis of the uniform tetrasaccharide linkage region in chondroitin sulfate and heparan sulfate proteoglycans, J. Biol. Chem. 282 (2007) 5201–5206.

[6] H. Kitagawa, Y. Tone, J. Tamura, et al., Molecular cloning and expression of glucuronyltransferase I involved in the biosynthesis of the glycosaminoglycan-protein linkage region of proteoglycans, J. Biol. Chem. 273 (1998) 6615–6618.

[7] R. Almeida, S.B. Levery, U. Mandel, et al., Cloning and expression of a proteoglycan UDP-galactose:beta-xylose beta1,4-galactosyltransferase I. A seventh member of the human beta4-galactosyltransferase gene family, J. Biol. Chem. 274 (1999) 26165–26171.

[8] X. Bai, D. Zhou, J.R. Brown, et al., Biosynthesis of the linkage region of glycosaminoglycans: cloning and activity of galactosyltransferase II, the sixth member of the beta 1,3-galactosyltransferase family (beta 3GalT6), J. Biol. Chem. 276 (2001) 48189–48195.

[9] C. Götting, J. Kuhn, K. Kleesiek, Human xylosyltransferases in health and disease, Cell. Mol. Life Sci. 64 (2007) 1498–1517.

[10] C. Roch, J. Kuhn, K. Kleesiek, C. Götting, Differences in gene expression of human xylosyltransferases and determination of acceptor specificities of various proteoglycans, Biochem. Biophys. Res. Commun. 391 (2010) 685–691.

[11] J.C. Casanova, J. Kuhn, K. Kleesiek, C. Götting, Heterologous expression and biochemical characterization of soluble human xylosyltransferase II, Biochem. Biophys. Res. Commun. 365 (2008) 678–684.

[12] E.K. Mis, K.F. Liem Jr., Y. Kong, et al., Forward genetics defines Xylt1 as a key, conserved regulator of early chondrocyte maturation and skeletal length, Dev. Biol. 385 (2014) 67–82.

[13] B.F. Eames, Y.L. Yan, M.E. Swartz, et al., Mutations in fam20b and xylt1 reveal that cartilage matrix controls timing of endochondral ossification by inhibiting chondrocyte maturation, PLoS Genet. 7 (2011) e1002246.

[14] J. Yan, S.E. Stringer, A. Hamilton, et al., Decorin GAG synthesis and TGF-β signaling mediate Ox-LDL-induced mineralization of human vascular smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 608–615.

[15] J. Schreml, B. Durmaz, O. Cogulu, et al., The missing "link": an autosomal recessive short stature syndrome caused by a hypofunctional XYLT1 mutation, Hum. Genet. 133 (2014) 29–39.

[16] I. Faust, C. Roch, J. Kuhn, et al., Human xylosyltransferase-I - a new marker for myofibroblast differentiation in skin fibrosis, Biochem.. Biophys. Res. Commun. 436 (2013) 449–454.

[17] E. Condac, R. Silasi-Mansat, S. Kosanke, et al., Polycystic disease caused by deficiency in xylosyltransferase 2, an initiating enzyme of glycosaminoglycan biosynthesis, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 9416–9421.

[18] C. Götting, S. Sollberg, J. Kuhn, et al., Serum xylosyltransferase: a new biochemical marker of the sclerotic process in systemic sclerosis, J. Invest. Dermatol. 112 (1999) 919–924.

[19] S. Schön, G. Huep, C. Prante, et al., Mutational and functional analyses of xylosyltransferases and their implication in osteoarthritis, Osteoarthr. Cartil. 14 (2006) 442–448.

[20] S.Y. McCoy, K.A. Falgowski, P.P. Srinivasan, et al., Serum xylosyltransferase 1 level increases during early posttraumatic osteoarthritis in mice with high bone forming potential, Bone 5 (2012) 224–231.

[21] J. Kuhn, O.A. Gressner, C. Götting, et al., Increased serum xylosyltransferase activity in patients with liver fibrosis, Clin. Chim. Acta 409 (2009) 123–126.

[22] C. Pönighaus, J. Kuhn, K. Kleesiek, C. Götting, Involvement of a cysteine protease in the secretion process of human xylosyltransferase I, Glycoconj. J. 27 (2010) 359–366.

[23] C. Prante, H. Milting, A. Kassner, et al., Transforming growth factor beta1-regulated xylosyltransferase I activity in human cardiac fibroblasts and its impact for myocardial remodeling, J. Biol. Chem. 282 (2007) 26441–26449.

[24] E. Condac, G.L. Dale, D. Bender-Neal, et al., Xylosyltransferase II is a significant contributor of circulating xylosyltransferase levels and platelets constitute an important source of xylosyltransferase in serum, Glycobiology 19 (2009) 829–833.

[25] D. Boyanova, S. Nilla, I. Birschmann, et al., PlateletWeb: a systems biologic analysis of signaling networks in human platelets, Blood 119 (2012) e22–34.

[26] J. Kuhn, C. Prante, S. Schön, et al., Measurement of fibrosis marker xylosyltransferase I activity by HPLC electrospray ionization tandem mass spectrometry, Clin. Chem. 52 (2006) 2243–2249.

[27] M. Butler, M. Dawson, Cell Culture Labfax, Bios Scientific Publishers (Academic Press), Oxford, England, 1992.

[28] J. Kuhn, S. Müller, M. Schnölzer, et al., High-level expression and purification of human xylosyltransferase I in high five insect cells as biochemically active form, Biochem. Biophys. Res. Commun. 312 (2003) 537–544.

[29] J.D. Esko, T.E. Stewart, W.H. Taylor, Animal cell mutants defective in glycosaminoglycan biosynthesis, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 3197–3201.

[30] B.J. Beahm, K.W. Dehnert, N.L. Derr, J. Kuhn, et al., A visualizable chain-terminating inhibitor of glycosaminoglycan biosynthesis in developing zebrafish, Angew. Chem. Int. Ed. Engl. 53 (2014) 3347–3352.

[31] L. Roden, Structure and metabolism of connective tissue proteoglycans, in: W.J. Lennarz (Ed.), The Biochemistry of Glycoproteins and Proteoglycans, Plenum, New York, 1980, pp. 269–314.

[32] K. Cuellar, H. Chuong, S.M. Hubbell, M.E. Hinsdale, Biosynthesis of chondroitin and heparan sulfate in chinese hamster ovary cells depends on xylosyltransferase II, J. Biol. Chem. 282 (2007) 5195–5200.

[33] C. Götting, J. Kuhn, S. Sollberg, et al., Elevated serum xylosyltransferase activity correlates with a high level of hyaluronate in patients with systemic sclerosis, Acta Derm. Venereol. 80 (2000) 60–61.