Biochemical and Biophysical Research Communications 383 (2009) 4–10

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Analysis of xylosyltransferase II binding to the anticoagulant heparin

Javier Carrera Casanova, Michael Ambrosius, Joachim Kuhn, Knut Kleesiek, Christian Götting *

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Georgstraße 11, 32545 Bad Oeynhausen, Germany

ARTICLE INFO

Article history:
Received 26 February 2009
Available online 14 March 2009

Keywords:
Xylosyltransferase
Proteoglycan
Glycosaminoglycan
Heparin
Glutathion-*S*-transferase
Maltose binding protein
Cardin–Weintraub motif

ABSTRACT

The key enzymes in the biosynthetic pathway of glycosaminoglycan production are represented by the human xylosyltransferase I and its isoform II (XylT-I and XylT-II). The glycosaminoglycan heparin interacts with a variety of proteins, thereby regulating their activities, also those of xylosyltransferases. The identification of unknown amino acids responsible for heparin-binding of XylT-II was addressed in this study. Thus, six XylT-II fragments were designed as fusion proteins with MBP and we received soluble and purified MBP/XylT-II from *Escherichia coli*. Heparin-binding studies showed that all fragments bound with low affinity to heparin. Prolonging of XylT-II fragments did not account for a cooperative effect of multiple heparin-binding motifs and in turn for a stronger heparin-binding. Sequence alignment and surface polarity plot led to the identification of two highly positively charged Cardin–Weintraub motifs with surface accessibility, resulting in combination with short clusters of basic amino acids for strong heparin-binding of native xylosyltransferases.

Introduction

Proteoglycans are complex macromolecules which are built up of a core protein to which glycosaminoglycan chains (GAG), such as chondroitin sulphate, dermatan sulphate, heparin, and heparan sulphate [1], are attached. One biological function of glycosaminoglycans is their interaction with ligands. Besides the existence of glycosaminoglycan/glycosaminoglycan-interactions [2], the majority of all interactions are based on glycosaminoglycans with proteins. It is known, for example, that heparin and heparan sulphate bind to diverse proteins and by this means regulate their activity, including that from enzymes, growth factors, chemokines, coagulation clots factors, extracellular matrix proteins, DNA-binding proteins and cell surface proteins from bacterial and viral organisms.

Heparin interacts with proteins in two ways. First, it resembles the polyanionic structure of nucleic acid, which is why DNA-binding proteins can be purified using glycosaminoglycans. Second, heparin acts through its electrostatic interaction as an affinity ligand. In both cases, an increased amount of salt leads to the dissociation of the heparin/protein-complex.

The key enzymes for the biosynthesis of GAGs are the xylosyltransferases I and II (EC 2.4.2.26, XylT-I and XylT-II). Both enzymes transfer xylose from UDP-xylose to specific serine residues of a core protein [3,4]. The xylosyltransferases possess a high affinity to the GAG heparin, thereby showing the ability of heparin severely to inhibit their activities and its use as an efficient affinity chromatographic purification step [5–7]. Moreover, heparin-binding of xylosyltransferases seems to be independent of correctly folded protein [8].

Analyses of the primary structure of known heparin-binding proteins have revealed the existence of conserved motifs, though binding to glycosaminoglycans may occur. Cardin and Weintraub [9] identified two basic amino acid clusters with the sequence [-X-B-B-X-B-X-] or [-X-B-B-B-X-X-B-X-], where B represents a basic amino acid, often arginine or lysine, and X symbolizes uncharged or hydrophobic amino acids. Analyses with synthetic peptides which were homologous to the consensus sequence have shown that single heparin-binding sequences often have a low or no affinity to heparin, whereas multiple copies in the form of concatemers or dimerized sequences possess a high affinity [10]. Because not all heparin-binding proteins contain either consensus sequence, Margalit et al. [11] proposed another mechanism of heparin/protein-interaction. There, a distance of 20 Å between two basic amino acids is crucial, located at opposite positions on an alpha-helix. The xylosyltransferases consist of many short clusters of basic amino acids that are scattered throughout the sequences. As a third possibility of interaction, it is believed that strong binding of these enzymes to heparin depends on a multiplicity of basic clusters in the sequence, and not just on one single binding site [12].

Abbreviations: GAG, glycosaminoglycan; XylT, xylosyltransferase; GST-AC, glutathion-*S*-transferase affinity chromatography; MBP-AC, maltose binding protein affinity chromatography; Hep-AC, heparin affinity chromatography; IB, inclusion body; aa, amino acid.

* Corresponding author. Fax: +49 (0) 5731 97 2013.
E-mail address: cgoetting@hdz-nrw.de (C. Götting).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.03.050

Materials and methods

Construction of expression plasmids and production in Escherichia coli. Synthetic oligonucleotide primers that included at their 5′-end the restriction site BamHI were designed based on the XylT-II cDNA sequence of pCMV-XL6-XYLT2 (Genbank Accession No. NM_022167, Origene, Rockville, MD). Various fragments from *XylT-II* were amplified, purified through gel extraction and subjected to BamHI digestion. Then the PCR products were purified and ligated into the multiple cloning site of the BamHI-digested and SAP-dephosphorylated (Shrimp Alkaline Phosphatase, USB) pMAL-c4E expression vector (New England Biolabs), in the frame with the N-terminal located maltose binding protein (MBP). All constructs were verified for right orientation and base sequence by DNA sequencing. Faultless constructs were transformed into the *E. coli* strain BL21(DE3), which was used as a general expression strain for the production of recombinant MBP fusion proteins. Thereby, MBP/XylT-II expression is under the control of the IPTG-inducible "tac" promoter. Expression was induced with 1 mM IPTG at room temperature for 2 h in order to minimize the production of potentially insoluble material.

Cell lyses. After harvesting the cells, the cell pellet was dissolved in 1/30th of the original culture volume with lysis buffer that contained 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 Unit/ml Benzonase (Novagen) und 0.2 mg/ml lysozym. Cell lyses proceeded at room temperature for 20 min. Insoluble material was removed by centrifugation at 10,000*g* at 4 °C for 20 min and the soluble protein supernatant was utilized for purification.

Maltose binding protein affinity chromatography. In order to purify fusion proteins with N-terminal located maltose-binding-protein (MBP), their affinity to an amylose resin (New England Biolabs) was utilized. The purification was done in batch modus. For that, the cell-free protein supernatant was added to an amylose matrix which had previously been equilibrated with LowSalt-buffer (20 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, pH 6.0). Binding was allowed to occur at 4 °C for 15 min. The amylose matrix was washed once with LowSalt-buffer, twice with HighSalt-buffer (20 mM Tris–HCl, 500 mM NaCl, 1 mM EDTA, pH 6.0) and reequilibrated twice with NoSalt-buffer (20 mM Tris–HCl, 1 mM EDTA, pH 6.0). The fusion proteins were eluted competitively with 10 mM Maltose in NoSalt-buffer. MBP fusion proteins were detected with a monoclonal anti-MBP antibody derived from mice (New England Biolabs).

Heparin affinity chromatography. For heparin analyses of xylosyltransferase II, MBP/XylT-II fusion proteins were used that were purified using MBP affinity chromatography. For this, MBP/XylT-II fragments were incubated with heparin immobilized on agarose beads. The beads were equilibrated with phosphate-buffered saline (PBS), pH 7.4, MBP/XylT-II fragments were added and incubated at 4 °C for 15 min for efficient binding. Unbound protein was removed by washing the heparin beads with PBS, pH 7.4, several times. Fusion proteins were eluted by extraction with SDS containing sample buffer and analyzed with SDS–PAGE. MBP/XylT-II fragments were detected using the monoclonal anti-MBP antibody (New England Biolabs).

Results

Cloning, expression and purification of XylT-II fragments as GST fusion proteins

The human xylosyltransferase comprises the proteins XylT-I and its isoform XylT-II. Both proteins can be efficiently purified using heparin affinity chromatography and their activity is also blocked by heparin. Information about the primary structure of the interaction site between the heparin and xylosyltransferase is needed, because besides the use of heparin as an anticoagulant, GAGs are targets for therapeutic drugs.

In order to identify the binding region that forms part of this interaction, we have expressed and purified to homogeneity full-length XylT-II as inclusion bodies (IB), thus overcoming a potential disruption of a heparin-binding sequence and simultaneously increasing the likelihood of finding a specific amino acid sequence (data not shown). Since IBs are insoluble, the purification via metal affinity chromatography was performed under denaturing conditions using 8 M urea as a solubilizing agent. We encountered difficulties during the dialyses against heparin-binding compatible buffer systems because the removing of urea immediately led to precipitation of XylT-II (data not shown). For that reason, we evolved a second strategy. We separately amplified six fragments, approximately 500 bp long, which comprised the whole cDNA of XylT-II and then we cloned them as N-terminal-located GST fusion proteins (Table 1). Thereby, every fragment was designed in such a way as to possess ~40 aa as overlapping sequences to the previous and next fragment, hence avoiding a potential disruption of a heparin-binding sequence (Fig. 1A). The fusion proteins were produced in the strain BL21(DE3) and purified over a GST matrix. In all cases a predominant band of the expected size was visible after IPTG induction. Additionally, they were verified as GST fusion proteins since they were recognized through a monoclonal anti-GST antibody. Nevertheless, the recombinant proteins could not be purified, as shown as an example with GST/XylT-II fragment 5 (Fig. 1B). Besides the main band, the immunological detection revealed few faint bands, the molecular weight of which was between the main band and the size of GST alone (MW ~26 kDa), indicating either the presence of a proteolytic activity or interme-

Table 1
Primers used in this study.

Designation	Sequence (5′→3′)	Description
BamHI_XylT-II_(1for)	AATTGGATCC**ATGGTGGCGAGCGCGCGAG**	Amplification of XylT-II fragment 1 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(558rev)	AATTGGATCC*CTA***GGCGATCTCCTGCTGGCAC**	
BamHI_XylT-II_(460for)	AATTGGATCC**ACGGACAATGGCTTCACC**	Amplification of XylT-II fragment 2 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(960rev)	AATTGGATCC*CTA***CCAGCCAGGCACCTCTAGC**	
BamHI_XylT-II_(820for)	AATTGGATCC**CACCGGGAGGTGGTGGAGC**	Amplification of XylT-II fragment 3 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(1350rev)	AATTGGATCC*CTA***GGTCTCACAGGCCAGGCT**	
BamHI_ XylT-II_(1198for)	AATTGGATCC**GACTGGTTCGTGCTGACA**	Amplification of XylT-II fragment 4 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(1722rev)	AATTGGATCC*CTA***GGGTGCAGCAGTGGCGGCAT**	
BamHI_ XylT-II_(1591for)	AATTGGATCC**CCAGCCCTCAAGGCCTAC**	Amplification of XylT-II fragment 5 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(2091rev)	AATTGGATCC*CTA***CACCACATAGGTTGGGTCG**	
BamHI_ XylT-II_(1939for)	AATTGGATCC**GAGGTTGGCACTGATTGG**	Amplification of XylT-II fragment 6 for cloning into pGEX-6P-1 and pMAL-c4E
XylT-II_Stop_BamHI _(2598rev)	AATTGGATCC***CTA*CCTGAGTCGCCCGTCT**	

Homologous sequences to the target sequence are bold. The restriction site BamHI is underlined. Stop-codons, which were introduced into the sequences, are highlighted in italic style. Expression vectors containing prolonged fragments were constructed by combining together different forward and reverse primers.

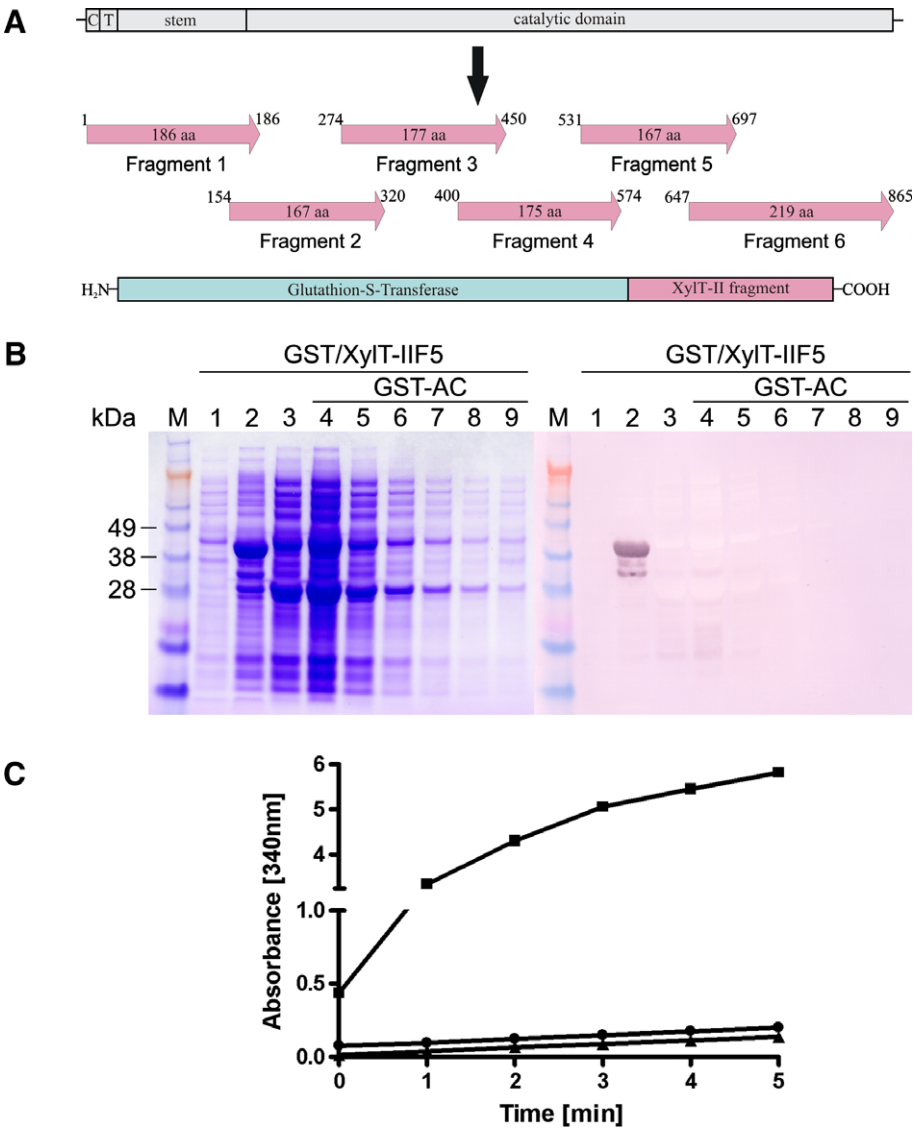

Fig. 1. Production of GST/XylT-II fusion proteins for heparin binding studies. (A) Schematic representation of the cloning strategies. Six fragments of approx. 500 bp in length were constructed as fusion proteins with N-terminal located glutathion-*S*-transferase. The fragments comprise the whole *XylT-II* cDNA and every fragment was designed to have ~40 aa of overlapping sequence to the previous and next fragment, respectively. The first and last amino acid of the fragments and their lengths are indicated by numbers. C, cytoplasamatic loop; T, transmembrane domain (B) GST affinity purification of GST/XylT-II fragment 5 (GST/XylT-IIF5). GST/XylT-IIF5 was purified over glutathion beads in batch modus and detected with a monoclonal anti-GST antibody derived from mice. M, Marker; 1, pre-induction; 2, IPTG post-induction; 3, crude sample (total protein); 4, unbound protein; 5, 1. Wash (PBS, pH 7.4); 6, 2. Wash (PBS, pH 7.4); 7, 1. Elution (50 mM Tris–HCl, 10 mM red. glutathion, pH 8.0); 8, 2. Elution (50 mM Tris–HCl, 10 mM red. glutathion, pH 8.0); 9, 3. Elution (0.5 % SDS). (C) Enzymatic detection of functional GST/XylT-II fusion proteins through CDNB activity assay. Aliquots of crude soluble supernatant of GST (■) and GST/XylT-IIF5 (▲) were incubated with the GST substrates 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione to produce a GST-dependent conjugate (CDNB–GSH) that can be detected through its absorbance at λ 340 nm. Insoluble material from GST/XylT-IIF5 (●) was solubilized with 0.5% Tween 20, 1 mM EDTA and assayed for activity, too. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

diate translation products that start after shifting from the *GST*-mRNA to the *XylT-II*-mRNA. Intermediate translation products originate from the different codon-usage of the organisms. Because the binding of GST depends on the right structure, we assumed that the fusion proteins are highly produced in *E. coli*, but present as insoluble aggregates. Fractionated solubility studies and immunological studies have already indicated the localization of the GST/XylT-II fusion proteins in the pellet if crude protein extract is centrifuged at low speed (data not shown). Since an antigen/antibody reaction detects simultaneous functional and non-functional protein, we performed the GST-activity assay [13,14], which detects only functional and therefore right-folded GST. For this, the crude soluble protein extracts of GST and GST/XylT-IIF5 were used for the activity assay. Moreover, the protein pellet of GST/XylT-IIF5 was solubilized under mild conditions (0.5% Tween 20, 1 mM EDTA) (Fig. 1C). After one minute an absorbance of 3.0 was reached for GST, whereas no transfer could be observed for the soluble and solubilized samples of GST/XylT-IIF5. These results confirm that GST/XylT-II fusion proteins are produced in sufficient amounts, but are present as misfolded proteins since they are neither active nor isolatable.

Cloning, expression and purification of XylT-II fragments as MBP fusion proteins. The expression of XylT-II fragments as fusion proteins with glutathion-*S*-transferase resulted in the formation of IBs. Thus, we chose the maltose binding protein (MBP) as a second high-molecular weight affinity tag [15,16] in order to enhance solubility [17]. For MBP/XylT-II fusion protein construction, the same primers could be utilized again, resulting in the same fragment length and biochemical properties. The expression vectors were transformed into BL21(DE3) and the proteins purified over an amy-

lose resin. In all cases, a predominant band of the expected size was visible after IPTG induction, as shown in Fig. 2A for MBP/XylT-II fragment 3 (MBP/XylT-IIF3). MBP/XylT-IIF3 could be purified to near homogeneity and detected utilizing a monoclonal anti-MBP antibody. Together with the main product, several faint bands were also immunologically visualized in the elution fraction. According to their molecular weights, which were positioned between 38 and 62 kDa, this indicated either proteolysis or interme-

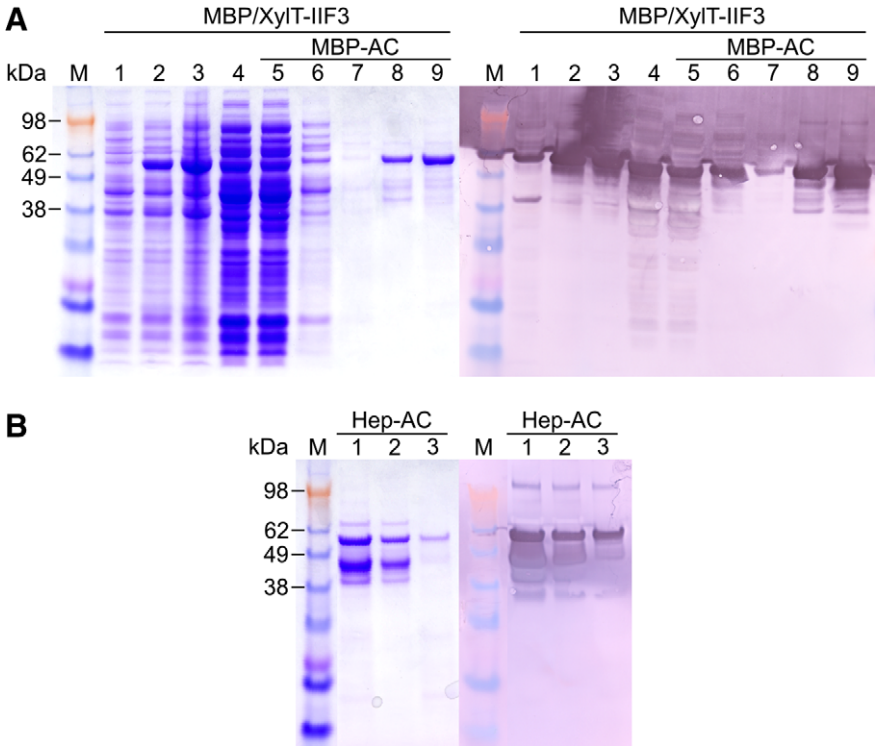

Fig. 2. Expression and purification of MBP/XylT-II fusion proteins for heparin binding studies. (A) MBP/XylT-II fragment 3 (MBP/XylT-IIF3) was expressed in BL21(DE3) and purified in batch modus over an amylose matrix. Fusion proteins were detected with a mouse monoclonal anti-MBP antibody. M, Marker; 1, pre-induction; 2, IPTG post-induction (2 h); 3, IPTG post-induction (3 h); 4, soluble protein; 5, unbound protein; 6, 1. Wash; 7, 2. Wash; 8, 1. Elution; 9, 2. Elution. (B) Heparin affinity purification of MBP/XylT-IIF3. MBP/XylT-IIF3 was purified over heparin-agarose beads and detected with a monoclonal anti-MBP antibody derived from mice. M, Marker; 1, MBP-purified MBP/XylT-IIF3 (before incubation with heparin beads); 2, unbound protein; 3, Elution (SDS-extracted protein).

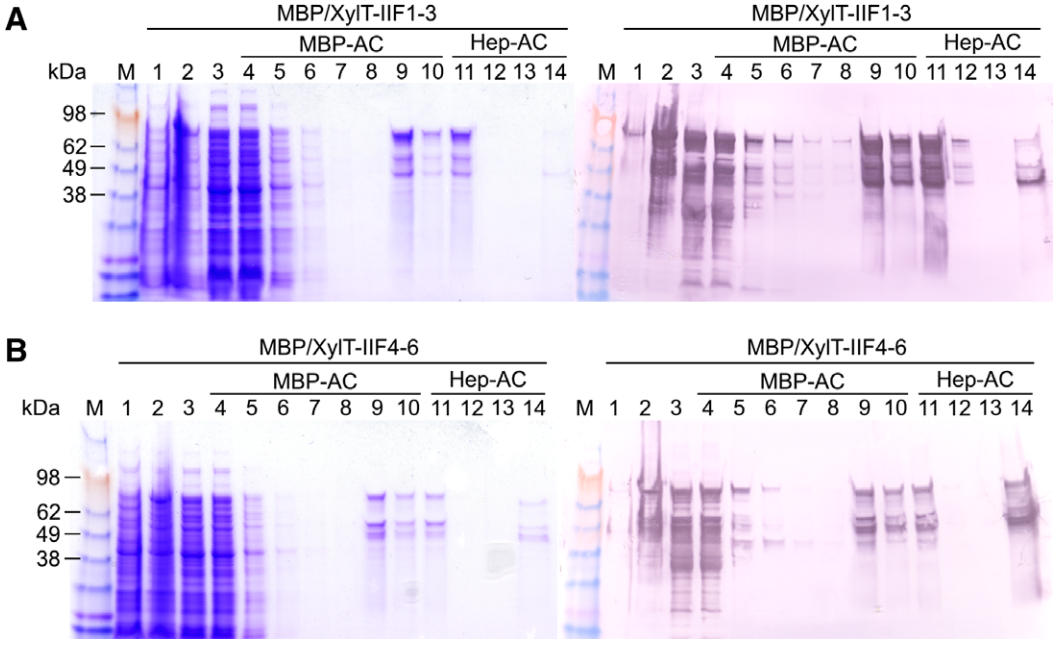

Fig. 3. Production of prolonged MBP/XylT-II fragments for increased heparin binding. Expression vectors containing prolonged MBP/XylT-II fragments MBP/XylT-IIF1-3 (A) and MBP/XylT-IIF4-6 (B) were constructed, the fusion protein expressed and the soluble protein purified over MBP and tested for heparin binding. Thereby, MBP/XylT-II fusion proteins were detected with a monoclonal anti-MBP antibody. M, Marker; 1, pre-induction; 2, IPTG post-induction (2 h); 3, soluble protein; 4, unbound protein; 5, 1. Wash; 6, 2. Wash; 7, 3. Wash; 8, 4. Wash; 9, 1. Elution; 10, 2. Elution; 11, unbound protein; 12, 1. Wash; 13, 2. Wash; 14, 1. Elution.

diate translation products that start after shifting from the *MBP*-mRNA to the *XylT-II*-mRNA. Furthermore, the pre-induced probe shows the endogenous MBP of *E. coli* (~40 kDa) and protein-associated endogenous MBP (~62 kDa) or basally expressed MBP/XylT-IIF3, respectively.

Purified MBP/XylT-IIF3 was applied to heparin-agarose beads in order to verify a glycosaminoglycan/protein interaction. After several washes, fusion proteins were eluted by SDS-extraction and detected by Western blot analysis (Fig. 2B). The result shows that MBP/XylT-IIF3 binds to heparin as other fragments do. Neverthe-

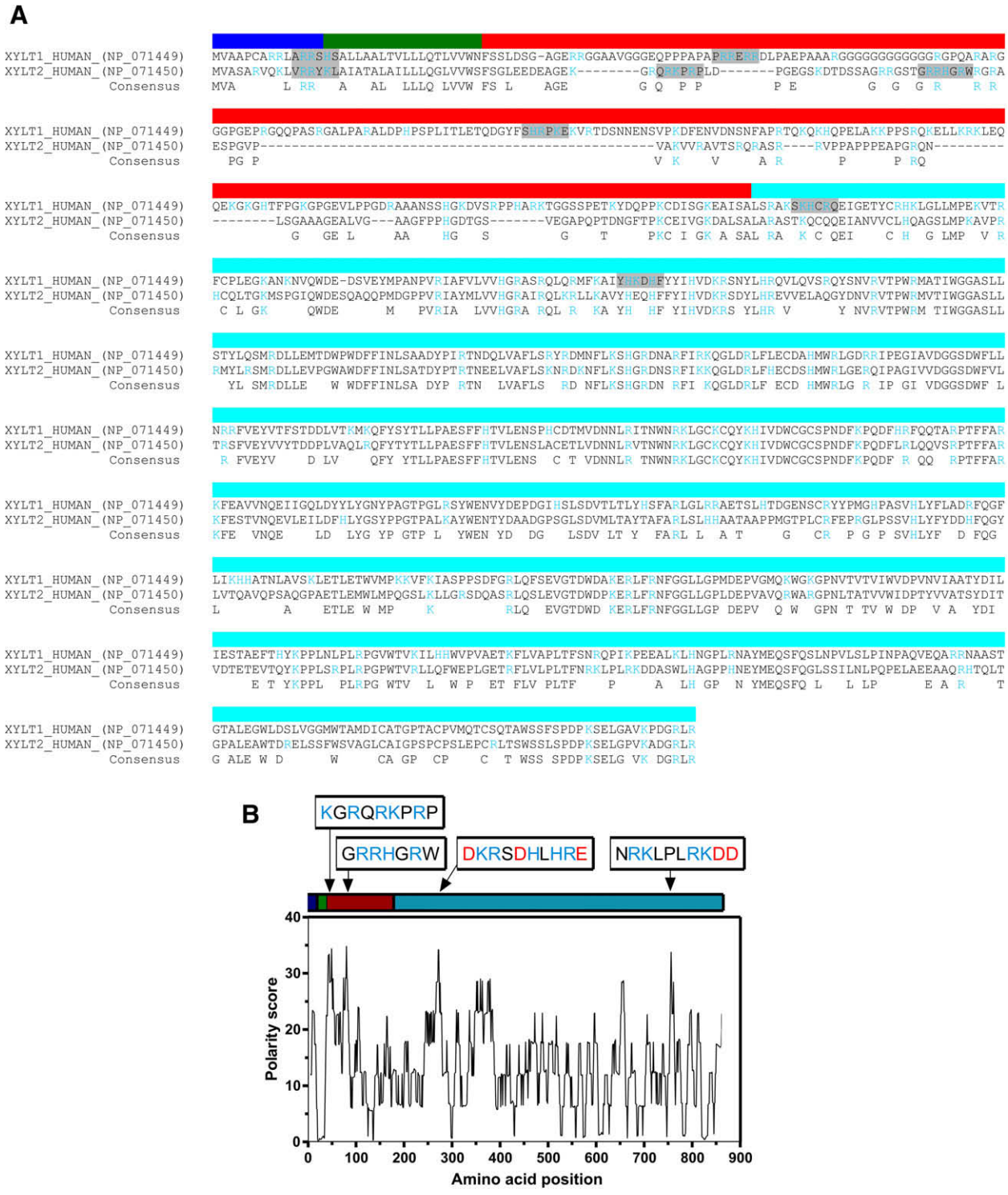

Fig. 4. Sequence homology and topology of the human xylosyltransferases I and II. (A) The protein sequences of human xylosyltransferase I (NP_071449) and II (NP_071450) were aligned using ClusalW. Gaps are indicated by dashed lines. Identical amino acids of XylT-I and XylT-II are shown as consensus sequences. Basic amino acids (H, K and R) which are supposed to interact with negatively charged heparin are highlighted. Grey boxes illustrate the postulated Cardin–Weintraub motifs. The cytoplasmatic region (dark blue), the transmembrane domain (green), the putative stem region (red) and the catalytic domain (light blue) are marked with colored boxes. (B) A Zimmerman–Eliezer–Simha polarity plot of XylT-II shows how polarity is distributed over the whole XylT-II protein. A window size of 9 amino acids was used to generate the plot. Colored bars symbolize the topology of XylT-II, together with specific amino acid clusters that correspond to the high polarity score (<30). Basic amino acids (H, K and R) are marked as blue, and acidic amino acid (D and E) as red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

less, we noticed that the protein amount applied to the heparin beads did not correlate with the protein recovered from the elution step, implicating the fragments to contain low affinity heparin-binding sequences.

Next we constructed and expressed in ER2507 MBP/XylT-II fusion protein that contained prolonged XylT-II fragments (MBP/XylT-IIF1-3 and MBP/XylT-IIF4-6) with the aim of looking for a cooperative effect of several potential available heparin-binding sequences and of generating in turn a stronger binding to heparin. Thereby, increasing the fragment size did not lead to stronger binding and also not to higher yield (Fig. 3A and B). Although heparin-binding was confirmed through western-blot analyses, almost no protein was visible on the corresponding SDS–PAGE.

Computational analysis of potential heparin-binding sites

We performed a sequence alignment of XylT-I and XylT-II to visualize the distribution of basic amino acids which are involved in electrostatic binding to heparin. XylT-I and XylT-II possess many short clusters of basic amino acids, distributed over the whole protein (Fig. 4A). Thereby, the amino acids are highly conserved in the potential catalytic domain of XylTs. While five Cardin–Weintraub motifs are located in the N-terminal region of XylT-I, XylT-II has only three postulated heparin-binding sequences, which are positioned within the first 80 amino acids. The first Cardin–Weintraub motif of XylT-I and XylT-II lies between the cytoplasmatic loop and transmembrane domain. Thus, it is unlikely that this motif will play a role in heparin-binding because XylTs are shedded within the transmembrane domain or stem region from the Golgi-surface and secreted into the extracellular space. In order to measure how surface charge is distributed over the protein sequence, we analyzed XylT-II by using a Zimmerman–Eliezer–Simha polarity plot [18]. The polarity plot shows, among others, two clusters which are located within the catalytic domain and which are not supposed to be involved in heparin-binding because of the latter's high content of acidic amino acids (Fig. 4B). Additionally, two high polarity surface regions were further found in the stem region with the sequence [-K-G-R-Q-R-K-P-R-P-] and [-G-R-R-H-G-R-W-], both also representing Cardin–Weintraub motifs.

Discussion

The interaction of glycosaminoglycans with proteins influences their physiological activity through localization, stabilization, activation or inactivation [19–21]. The xylosyltransferases I and II also bind with a high affinity to heparin, thereby completely blocking their activities. In this study we tried to map the amino acid sequence of XylT-II responsible for heparin-binding because the naturally secreted xylosyltransferases—key enzymes in the GAG pathway—occupy with GAGs the outer space of animal cells, making a physiological interaction probable. Because the presence of other heparin-binding proteins would interfere with identification of the heparin-binding sequence of XylT-II, a prerequisite was the production of purified protein. For this purpose, we used *E. coli* as organisms since correctly folded and functional protein was not necessary for heparin-binding [8] and the recombinant expression in other organisms led to active protein, but also to low-level expression. For this, we developed two strategies for XylT-II production in *E. coli*.

First, we constructed XylT-II as a full-length protein with the aim of targeting the formation of XylT-II as an inclusion body (IB). The advantage in IB production was their stability against proteases, the yield, their "almost" purified status and the possibility of expressing XylT-II as a full-length protein, thereby avoiding a potential disruption of a heparin-binding motif. We succeeded in the expression, solubilization and homogenous purification through metal affinity chromatography of full-length XylT-II, but we encountered difficulties in removing the solubilizing agent. The buffer exchange into heparin compatible buffers resulted immediately in precipitation of XylT-II protein.

The second strategy comprised the cloning of XylT-II fragments, their expression as fusion protein with a high-molecular weight and solubilizing affinity tag (either GST or MBP) and their appropriate purification. Expression analyses with glutathione-*S*-transferase as an affinity tag showed that GST/XylT-II could be produced at a high level in *E. coli*, but they could not be purified over a glutathione matrix. Because glutathione-binding depends on the tertiary structure of GST, we performed solubility tests and GST activity studies and could prove the presence of GST/XylT-II as insoluble protein aggregates.

We have replaced GST with the maltose binding protein (MBP) because this protein resembles GST regarding size and solubility enhancement. We were capable of producing MBP/XylT-II fusion proteins as a soluble protein and we purified them over amylose matrix. However, multiple bands were observed after purification, the molecular weights of which were positioned between MBP alone and full-length MBP/XylT-II. Additionally, these multiple bands were recognized through a monoclonal anti-MBP antibody. Although a homogeneous MBP/XylT-II solution was not attained, it was possible to subject it to heparin-binding analysis. We observed that all fragments bound to heparin independently of each other, as well as of fragments not containing any Cardin–Weintraub motif. To test for a cooperative effect of several available heparin-binding sequences, we prolonged XylT-II fragments more than threefold in size and tested their binding behavior to heparin. Thereby, no stronger binding was generated, implying that multiple copies of heparin-binding sequences do not facilitate interaction with heparin.

A protein sequence alignment and polarity plot of XylT-I and XylT-II revealed several Cardin–Weintraub motifs and charged surface clusters, which might be involved in electrostatic-mediated heparin-binding. Regarding XylT-II, we could exclude one Cardin–Weintraub motif because of its position within the cytoplasmatic/transmembrane region and two charged clusters because of the presence of acidic amino acids. Only two Cardin–Weintraub motifs in the stem region correlated with their polarity surface accessibility. Because MBP/XylT-II fragments also bound without having any Cardin–Weintraub motif, we conclude that strong binding, which is usually observed with native XylTs, is a combination of the motifs located in the stem region and short clusters of basic amino acids that might come together when the xylosyltransferases fold correctly, accounting for strong heparin binding.

Acknowledgments

The authors thank Sarah L. Kirkby for her linguistic advice. This work was supported by grants from the Forschungsförderung an der Medizinischen Fakultät der Ruhr-Universität Bochum (FORUM, F499-2006).

References

[1] R.V. Iozzo, Matrix proteoglycans: from molecular design to cellular function, Annu. Rev. Biochem. 67 (1998) 609–652.
[2] L.A. Fransson, I. Carlstedt, L. Coster, A. Malmstrom, Proteoheparan sulfate from human skin fibroblasts. Evidence for self-interaction via the heparan sulfate side chains, J. Biol. Chem. 258 (1983) 14342–14345.
[3] C. Götting, J. Kuhn, R. Zahn, T. Brinkmann, K. Kleesiek, Molecular cloning and expression of human UDP-d-Xylose: proteoglycan core protein beta-D-xylosyltransferase and its first isoform XT-II, J. Mol. Biol. 304 (2000) 517–528.
[4] C. Pönighaus, M. Ambrosius, J.C. Casanova, C. Prante, J. Kuhn, J.D. Esko, K. Kleesiek, C. Götting, 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.

[5] K. Kleesiek, R. Reinards, J. Okusi, B. Wolf, H. Greiling, UDP-D-xylose: proteoglycan core protein beta-D-xylosyltransferase: a new marker of cartilage destruction in chronic joint diseases, J. Clin. Chem. Clin. Biochem. 25 (1987) 473–481.
[6] J. Kuhn, C. Götting, M. Schnölzer, T. Kempf, T. Brinkmann, K. Kleesiek, First isolation of human UDP-D-xylose: proteoglycan core protein beta-D-xylosyltransferase secreted from cultured JAR choriocarcinoma cells, J. Biol. Chem. 276 (2001) 4940–4947.
[7] 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.
[8] S. Müller, J. Disse, M. Schöttler, S. Schön, C. Prante, T. Brinkmann, J. Kuhn, K. Kleesiek, C. Götting, Human xylosyltransferase I and N-terminal truncated forms: functional characterization of the core enzyme, Biochem. J. 394 (2006) 163–171.
[9] A.D. Cardin, H.J. Weintraub, Molecular modeling of protein-glycosaminoglycan interactions, Arteriosclerosis 9 (1989) 21–32.
[10] A. Verrecchio, M.W. Germann, B.P. Schick, B. Kung, T. Twardowski, J.D. San Antonio, Design of peptides with high affinities for heparin and endothelial cell proteoglycans, J. Biol. Chem. 275 (2000) 7701–7707.
[11] H. Margalit, N. Fischer, S.A. Ben-Sasson, Comparative analysis of structurally defined heparin binding sequences reveals a distinct spatial distribution of basic residues, J. Biol. Chem. 268 (1993) 19228–19231.
[12] C. Götting, J. Kuhn, K. Kleesiek, Human xylosyltransferases in health and disease, Cell Mol. Life Sci. 64 (2007) 1498–1517.
[13] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974) 7130–7139.
[14] B. Mannervik, U.H. Danielson, Glutathione transferases–structure and catalytic activity, CRC Crit. Rev. Biochem. 23 (1988) 283–337.
[15] C. di Guan, P. Li, P.D. Riggs, H. Inouye, Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein, Gene 67 (1988) 21–30.
[16] C.V. Maina, P.D. Riggs, A.G. Grandea 3rd, B.E. Slatko, L.S. Moran, J.A. Tagliamonte, L.A. McReynolds, C.D. Guan, An *Escherichia coli* vector to express and purify foreign proteins by fusion to and separation from maltose-binding protein, Gene 74 (1988) 365–373.
[17] R.B. Kapust, D.S. Waugh, *Escherichia coli* maltose-binding protein is uncommonly effective at promoting the solubility of polypeptides to which it is fused, Protein Sci. 8 (1999) 1668–1674.
[18] J.M. Zimmerman, N. Eliezer, R. Simha, The characterization of amino acid sequences in proteins by statistical methods, J. Theor. Biol. 21 (1968) 170–201.
[19] S. Faham, R.E. Hileman, J.R. Fromm, R.J. Linhardt, D.C. Rees, Heparin structure and interactions with basic fibroblast growth factor, Science 271 (1996) 1116–1120.
[20] I. Capila, R.J. Linhardt, Heparin–protein interactions, Angew. Chem. Int. Ed. Engl. 41 (2002) 391–412.
[21] M.M. Maimone, D.M. Tollefsen, Structure of a dermatan sulfate hexasaccharide that binds to heparin cofactor II with high affinity, J. Biol. Chem. 266 (1991) 14830.