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N-glycosylation of blood coagulation factor XIII subunit B and its functional consequence

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Abstract

Background: The protective/inhibitory B subunits of coagulation factor XIII (FXIII-B) is a ~80 kDa glycoprotein containing two N-glycosylation sites. Neither the structure nor the functional role of the glycans on FXIII-B has been explored.

Objective: To reveal the glycan structures linked to FXIII-B, to design a method for deglycosylating the native protein, to find out if deglycosylation influences the dimeric structure of FXIII-B and its clearance from the circulation.

Methods: Asparagine-linked carbohydrates were released from human FXIIII-B by PNGase F digestion. The released N-linked oligosaccharides were fluorophore labeled and analyzed by capillary electrophoresis. Structural identification utilized glycan database search and exoglycosidase digestion based sequencing. The structure of deglycosylated FXIII-B was investigated by gel filtration. The clearance of deglycosylated and native FXIII-B from plasma was compared in FXIII-B knock out mice.

Results: PNGase F completely removed N-glycans from the denatured protein. Deglycosylation of the native protein was achieved by repeated digestion at elevated PNGase F concentration. The total N-glycan profile of FXIII-B featured nine individual structures; three were fucosylated and each structure contained at least one sialic acid. Deglycosylation did not change the native dimeric structure of FXIII-B, but accelerated its clearance from the circulation of FXIII-B knock out mice.

Conclusion: Characterization of the glycan moieties attached to FXIII-B is reported for the first time. Complete deglycosylation of the native protein was achieved by a deglycosylation workflow. The associated glycan structure is not required for FXIII-B dimer formation, but it very likely prolongs the half-life of FXIII-B in the plasma.

KEYWORDS

capillary electrophoresis, deglycosylation, factor XIII, factor XIII subunit B, glycan

Boglárka Hurják and Zsuzsanna Kovács contributed equally to the study; Both senior authors László Muszbek and András Guttman contributed equally to the manuscript.

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1 | INTRODUCTION

Coagulation factor XIII (FXIII) is a transglutaminase proenzyme essential for maintaining hemostasis by stabilizing the fibrin clot and protecting it against fibrinolytic degradation.[1-3] FXIII circulates in blood as a heterotetramer ($FXIII\text{-}A_2B_2$) consisting of two potentially active FXIII-A and two inhibitory/protective FXIII-B subunits.[4] In plasma, FXIII-B is in excess over FXIII-A; about 50% of $FXIII\text{-}B_2$ exists in free non-complexed form.[1] The cleavage of FXIII-A by thrombin and the Ca^{2+} induced dissociation of the two subunits transform it into an active transglutaminase that cross-links fibrin chains and $\alpha_2$plasmin inhibitor to fibrin. FXIII-A deficiency causes serious bleeding diathesis and deficient patients require regular substitution therapy.[5-7] The non-enzymatic FXIII-B subunit prolongs the lifespan of FXIII-A in the circulation and prevents its spontaneous activation.[8-10] FXIII-B is a ~80 kDa glycoprotein that contains approximately 8.5% carbohydrate on two N-glycosylation sites (Asn142 and Asn525) in the third and ninth sushi domains.[11,12] Neither the structure nor the functional role of these glycans has been studied in detail as yet, in spite of the fact that the importance of such modifications has been confirmed by numerous studies in immunology, oncology, hematology, etc.[13-15] Several members of the blood coagulation cascade are glycoproteins, eg, factors II, VII, VIII, and IX (FII, FVII, FVIII, and FIX) suggesting the importance of attached carbohydrates. It has been demonstrated that the stability and macromolecular interactions of FVIII changed by deglycosylation.[16] It is also important to note that glycosylation can aid in promoting protein solubility.[17] Furthermore, in vivo efficacy of several therapeutically relevant recombinant clotting factors increased by their natural glycosylation.[18]

The aim of our study was to comprehensively characterize the N-glycan structure of FXIII-B and achieve N-glycan removal under non-denaturing conditions for downstream experiments to understand the biological relevance associated with its glycosylation. In this study the effect of deglycosylation on the dimeric structure of FXIII-B and on its plasma clearance was investigated.

Essentials

- Factor XIII B subunit (FXIII-B) is a glycoprotein with two glycosylation sites.
- The asparagine linked carbohydrates of FXIII-B were fully characterized and sequenced.
- Nine glycan structures were identified, all variously sialylated and three core fucosylated.
- Deglycosylation accelerated the clearance of FXIII-B from the circulation.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Acetonitrile was purchased from Molar Chemicals (Halásztelek, Hungary). Sodium hydrogen carbonate, DL-dithiothreitol solution (1 mol/L in H_2O), sodium dodecyl sulfate, D-(+)-maltose monohydrate and 1 mol/L sodium cyanoborohydride in tetrahydrofolic acid (THF) were from Sigma-Aldrich (St. Louis, MO). Peptide-N4-(N-acetyl-beta-glucosaminyl) asparagine amidase (PNGase F; 500 mU) was the product of Asparia Glycomics (San Sebastian, Spain). The exoglycosidases: sialidase A, α(1-2,3,4,6) fucosidase, β(1-4,6)-galactosidase and β-N-acetylhexosaminidase were from ProZyme (Hayward, CA). 8-aminopyrene-1,3,6-trisulfonate (APTS), the maltooligosaccharide ladder, the internal standard, and the N-CHO Carbohydrate Labeling and Analysis kit were from SCIEX (Brea, CA). FXIII-B was purified from human plasma in our laboratory.[19,20]

2.2 | PNGase F digestion under native conditions

Eighty micrograms of FXIII-B was dissolved in 50 µL of 20 mmol/L $NaHCO_3$ (pH 7.0) followed by the addition of 8.1 mU PNGase F enzyme in 10 µL, ie, 10 times higher than that regularly used for denatured proteins. This reaction mixture was transferred to a 10 kDa spin-filter (VWR, Radnor, PA) and incubated overnight at 37°C. Then, 100 µL HPLC grade water (Millipore, Darmstadt, Germany) was added to the reaction mixture and the released N-glycans were centrifuged through spin-filters at 11 270 × *g* for 10 minutes, followed by drying in a SpeedVac system (Thermo Scientific) prior to fluorophore labeling. The remaining pellet was dissolved in 50 µL 20 mmol/L pH 7.0 $NaHCO_3$ buffer and re-digested with 1 µL (0.81 mU) PNGase F enzyme by incubating overnight at 37°C. Then, 100 µL HPLC grade water was added to the reaction mixture and the released N-glycans were centrifuged through the spin-filter at 11 270 × *g* for 10 minutes, followed by drying in a SpeedVac system prior to fluorophore labeling.

2.3 | PNGase F digestion under denaturing conditions

After digestions of the native protein, as described in Section 2.2, the remaining pellet was denatured and digested again with PNGase F. The pellet was dissolved in 10 µL of high-performance liquid chromatography (HPLC) grade water and 1 µL denaturing buffer (400 mmol/L DTT, 5% SDS) was added to the mixture on the 10 kDa filter. After incubation at 65°C for 10 minutes 100 µL HPLC grade water was added and the filter was centrifuged at 11 270 × *g* for 10 minutes to remove any remaining denaturing buffer. FXIII-B was digested in situ on the filter by the addition of 49 µL 20 mmol/L $NaHCO_3$ buffer (pH 7.0) and 1 µL (0.81 mU) PNGase F. The reaction mixture was incubated at 37°C overnight. Then, 100 µL HPLC grade water was added to the reaction mixture and the released N-glycans

were centrifuged through the spin-filters at 11 270 × *g* for 10 minutes. The samples were dried in SpeedVac. The liberated N-glycans were then APTS labeled as described below. Results with FXIII-B sample treated with PNGase F under the denaturing protocol were used for comparison to establish the effectiveness of the digestion of non-denatured FXIII-B.

2.4 | Fluorophore labeling

Six microliter 20 mmol/L APTS in 15% acetic acid and 2 µL 1 mol/L $NaCNBH_3$ (in THF) was added to the dried glycan samples and incubated at 37°C overnight. The labeled samples were magnetic bead purified (SCIEX Fast Glycan Sample Preparation and Analysis kit) following the instruction manual of the kit and immediately analyzed by capillary electrophoresis with laser induced fluorescence detection (CE-LIF) analysis or stored at −20°C for later work.

2.5 | Exoglycosidase array based carbohydrate sequencing

Four microliter HPLC grade water and 1 µL 50 mmol/L CH_3COONH_4 buffer (pH 7.5) were added to 5 µL of APTS labeled N-glycans. This mixture was digested at 37°C overnight with an array of α(2-3,6,8,9) sialidase A, α(1-2,3,4,6) fucosidase, β(1-4,6)-galactosidase, and β(1-2,3,4,6)-N-acetylhexosaminidase as specified in Table 1. The digested samples were dried in SpeedVac then analyzed by CE-LIF.

2.6 | Capillary electrophoresis with laser induced fluorescence (CE-LIF) detection

A P/ACE MDQ System (SCIEX) was used to perform all capillary electrophoresis analyses. The separations were monitored by LIF detection using a 488 nm Ar-ion laser with a 520 nm emission filter.

TABLE 1 Linkage specificity of exoglycosidases used for sequencing the PNGase F released and APTS labeled N-glycan pool

Exoglycosidase specificity	A	B	C	D	E
α(2-3,6,8,9) sialic acid	−	+	+	+	+
β(1-4,6) galactose	−	−	+	+	+
α(1-2,3,4,6) fucose	−	−	−	+	+
β(1-2,3,4,6) N-acetylglucosamine	−	−	−	−	+

Note: Capital letters in the first row represent enzymes used in the sequencing array (also described in the legend to Figure 1). (A) PNGase F, (B) sialidase, (C) sialidase + galactosidase, (D) sialidase + galactosidase + fucosidase, (E) sialidase + galactosidase + fucosidase + hexosaminidase.

Fifty cm effective length (60 cm total) 50 µm ID bare fused silica capillaries were employed with the N-CHO separation gel buffer (both from SCIEX) for the analysis. The samples were injected at 1 psi for 5 seconds and the separation was accomplished in reversed polarity mode by applying 30 kV. The 32 Karat software (SCIEX) was used for data acquisition and processing.

2.7 | Molecular weight determination by gel filtration

Native and deglycosylated FXIII-B were analyzed by gel filtration using ÄKTA chomatography system (Amersham Biosciences, Uppsala, Sweden). Size-exclusion chromatography was carried out on a HiPrep™ 16/60 Sephacryl®S-300 HR column (GE-Healthcare, Chicago, IL). Elution was performed at room temperature in Tris-buffered saline (50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4) at a flow rate of 0.5 mL/min. Elution of proteins was monitored at 214 nm. Thyroglobulin, ferritin, aldolase, conalbumin, and ovalbumin were used as standards (GE-Healthcare, Chicago, IL) for the calibration curve.

2.8 | Generation of F13B knock-out mouse line and genotyping strategy

CRISPR/Cas9 technology was used to knock-out the F13B gene by introducing inframe stop codons into its second exon (see also in Figure S1 in supporting information). Translation of the modified gene starts from the translation initiation site in the first exon, but it is halted after incorporating 29 amino acids out of the total 669. Modification is based on a double stranded break by the Cas9 enzyme (Integrated DNA Technologies; IDT, Coralville, IA) directed by cr/tracr RNAs (IDT), and homology directed repair for which the template with the desired mutation was provided on a single stranded oligodeoxyucleotide (ssODN) template (IDT). The target specific sequence for the crRNA was: ATCCTTCCATTTTCCACGGT. Cas9 protein (30 ng/µL), cr/tracrRNAs (1-1 pmol/µL), and ssODN (15 ng/µL) were microinjected into the pronuclei of fertilized eggs of C57Bl/6NTac mice. Sequence of the ssODN template (modified bases are in bold and italics): CCTCTCAGGAGAACTCTATGCAGAAGAGAAACAGTGTGATTTTCCT***TAGTGA***GGAAAATGGAAGGATTGCCCAATATTATTATACGTTTAAAAGCTTTT.

A polymerase chain reaction (PCR) was used for genotyping. First from founder mice, the target region was PCR amplified by F13B specific general forward (TGCAAACTGAAAGATCTGCCG) and reverse (TGTAGCACCTTGGGTTTGGAG) primers, and the sequence was verified. Sequencing was repeated in F1 generation. Wild type and knock out (KO) specific forward primers (AACAGTGTGATTTTCCTACCGTG and AACAGTGTGATTTTCCTTAGTG, respectively) together with the general reverse primer (described above) were used to identify the mice carrying the genetic modification. FXIII-B genotyping strategy is demonstrated in Figure S2 in supporting information.

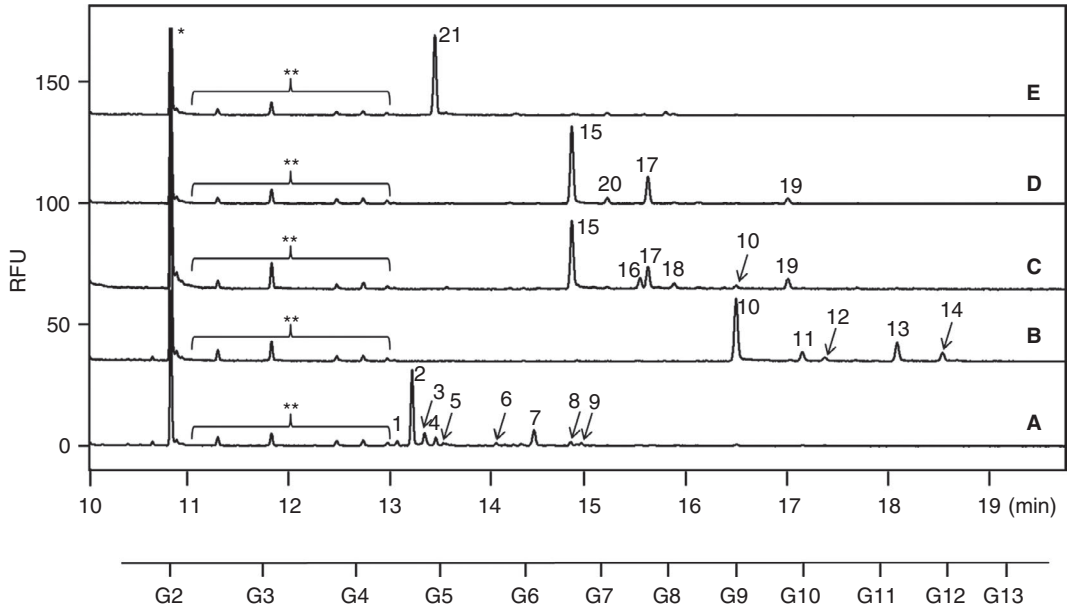


FIGURE 1 Exoglycosidase array based sequencing of denatured human coagulation factor XIII B subunit. Trace (A) PNGase F released and APTS labeled N-glycan pool. This labeled glycan pool was then digested by (B) sialidase, (C) sialidase + galactosidase, (D) sialidase + galactosidase +fucosidase, (E) sialidase + galactosidase +fucosidase + hexosaminidase. RFU: relative fluorescence unit. Separation conditions: bare fused silica capillary with 50 cm effective length (total length 60 cm, 50 μm i.d.), N-CHO separation buffer, temperature: 25°C, voltage: 30 kV (0.17 minutes ramp) reversed polarity, pressure injection: 1.0 psi and 0.5 s. *Internal standard; **peaks which did not respond to the exoglycosidase array treatment

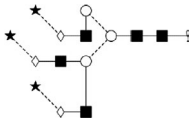
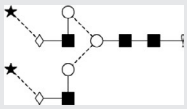
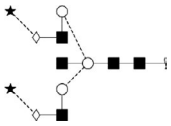
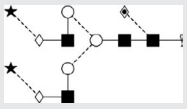
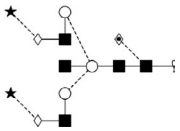
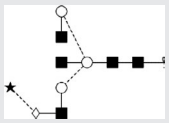
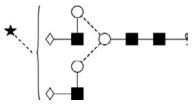
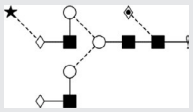
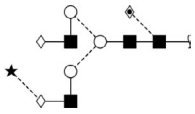
2.9 | Clearance of deglycosylated FXIII-B

The study was approved by the Animal Care Committee of the University of Debrecen. 100 μL 300 μg/mL native non-delycosylated and deglycosylated FXIII-B were injected into the tail vein of seven and six FXIII-B knock out mice, respectively. There were two males in each group. The mice were 8 to 12 weeks old. The body mass of the animals receiving non-deglycosylated and glycosylated FXIII-B was 22.3 ± 2.6 and 22.2 ± 2.3 g, respectively. One hour, 48 hours, and 120 hours later 150 μL blood was drawn from the retro-orbital sinus in heparinized capillary under isofurane anesthesia. Plasma samples were obtained by centrifugation at 1300 g, for 15 minutes. The collected plasma samples were stored at −20°C until the measurement of FXIII-B by enzyme-linked immunosorbent assay (ELISA).[21]

3 | RESULTS AND DISCUSSION

To identify the N-glycan structures on FXIII-B, first comprehensive asparagine linked carbohydrate analysis of the denatured protein was performed. The denatured FXIII-B subunit was treated with PNGase F, the liberated sugars were labeled with APTS, and analyzed by CE-LIF after magnetic bead mediated sample purification.[22] The denaturing step was necessary to completely unfold the glycoprotein, and therefore to ensure full access of the endoglycosidase enzyme to cut off the N-linked sugar structures. Trace A in Figure 1 shows the oligosaccharide profile of the denatured and PNGase F digested FXIII-B N-glycan pool featuring nine peaks. The small peaks migrating prior to peak 1 did not respond to exoglycosidase treatment; therefore, they were not considered as oligosaccharides of interest. GU-unit values of the separated peaks were defined by using GUcal software (GUcal.hu) [22] and the corresponding structures were obtained from the built-in database. In addition, exoglycosidase digestion of FXIII-B was performed to verify the database suggested structures. Traces B-E in Figure 1 depict the electropherograms of APTS-labeled N-glycan pool released from denatured FXIII-B after treating it with an array of exoglycosidase mixtures containing sialidase (B), sialidase + β-galactosidase (C), sialidase + β-galactosidase + fucosidase (D), and sialidase + β-galactosidase + fucosidase +hexosaminidase (E). The sialidase treatment (trace B) released all α(2-3,6,8) linked sialic acids and the fact that all peaks of Trace A (1-9), shifted to the neutral carbohydrate migration region (ie, into peaks 10-14), suggested that all nine glycan structures linked to FXIII-B were sialylated. Treatment of the control glycan pool with the mixture of sialidase and β(1-4,6) galactosidase (Trace C) resulted in the removal of all sialic acid and galactose residues, thus shifting peaks 10-14 to peaks 15-19. This step was followed by digestion of a sialidase, galactosidase, and α(1-2,3,4,6) fucosidase containing reaction mixture. Trace D presents the resulting electropherogram with the shifts into peaks 15, 17, 19, and 20 due to the loss of fucose residues. Finally, Trace E shows the glycan pool treated with reaction mixture E (Table 1), which removed all sialic acid, galactose, fucose, and β(1-2,4,6) linked N-acetylglucosamines. As a result of this treatment, peaks 15, 17, 19, and 20 all consolidated into peak 21 due to the loss of GlcNAc residues. Peak 21 was then identified by its GU value of

TABLE 2 The structure of N-glycans released from factor XIII B subunit

Peak number	Migration timeb (min)	GU_{CE}-unit	Oxford format of glycan structures	Suggested structure
1	11.99	4.407		A3G(4)3S(6,6,6)3
2	12.16	4.584		A2G(4)2S(6,6)2
3	12.3	4.733		A2BG(4)2S(6,6)2
4	12.43	4.867		F(6)A2G(4)2S(6,6)2
5	12.52	4.956		F(6)A2BG(4)2S(6,6)2
6	13.13	5.647		A2[3]BG(4)1S(6)1
7	13.56	6.151		A2G(4)2S(6)1
8	13.99	6.676		F(6)A2[6]G(4)2S(6)1
9	14.11	6.828		F(6)A2[3]G(4)2S(6)1

Note: Numbers correspond to the numbered peaks in Figure 1. GU_{CE}: glucose unit values established by capillary electrophoresis. For abbreviated glycan structural names see reference.[23]

★: sialic acid, ◊: galactose, ○: mannose, ■: N-acetylglucosamine, ◆: fucose

4.829 as the N-glycan trimannosyl core structure. As a result of this exoglycosidase enzyme array based carbohydrate sequencing, the following structures were identified: a trisialo (A3G(4)3S(6,6,6)3), a bisialo (A2G(4)2S(6,6)2), a disialo bisecting (A2BG(4)2S(6,6)2), a disialo core fucosylated (F(6)A2G(4)2S(6,6)2), a disialo core fucosylated bisecting (F(6)A2BG(4)2S(6,6)), a monosialylated bisecting (2A2[3]BG(4)1S(6)1), a monosialylated (A2G(4)2S(6)1) and two monosialo core fucosylated structures (F(6)A2[6]G(4)2S(6)1), F(6)A2[3]G(4)2S(6)1, as listed in Table 2. Abbreviated glycan structural names followed the nomenclature proposed by Harvey et al.[23]

Because we were also interested in the deglycosylation efficiency for the native form of FXIII-B, it was treated with PNGase F without denaturation, using a two-step protocol starting with an enzyme concentration 10-fold higher than regularly used for the digestion of denatured proteins. This step was followed by digestion using the regular PNGase F concentration as described in Materials and Methods. Figure 2 demonstrates that PNGase F digestion was also working on the non-denatured protein (Traces A and B). Repeated PNGase F digestion of the native protein using two different enzyme concentrations resulted in successful deglycosylation, which was confirmed by the lack of remaining protein-linked carbohydrate structures. In this experiment the non-denatured protein that remained after this double digestion protocol was denatured and subjected to an additional PNGase F treatment (Trace C). The first

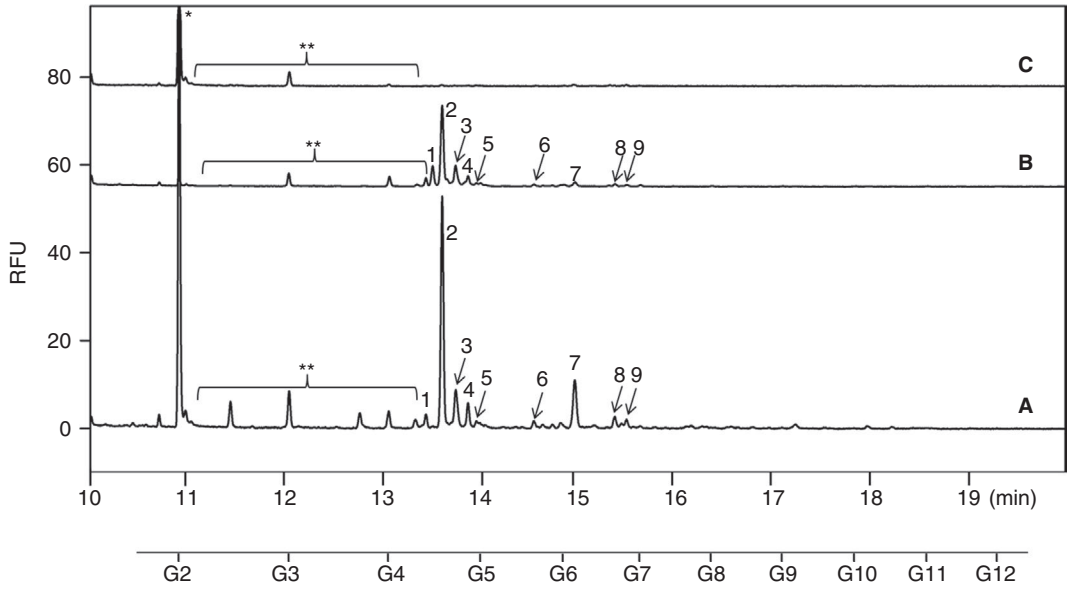


FIGURE 2 Deglycosylation of the native, non-denatured factor XIII B subunit (FXIII-B) by repeated PNGase F digestion. Trace (A) glycans were released from non-denatured FXIII-B by PNGase F using at a concentration 10-fold higher than regularly used for the digestion of denatured proteins. Trace (B) glycans released by regular PNGase F treatment from the protein remaining after step A. Trace (C) the remaining protein after step two was denatured and treated with PNGase F in regular concentration. Separation conditions were the same as described in the legend to Figure 1. *Internal standard; **peaks did not respond to the exoglycosidase array treatment

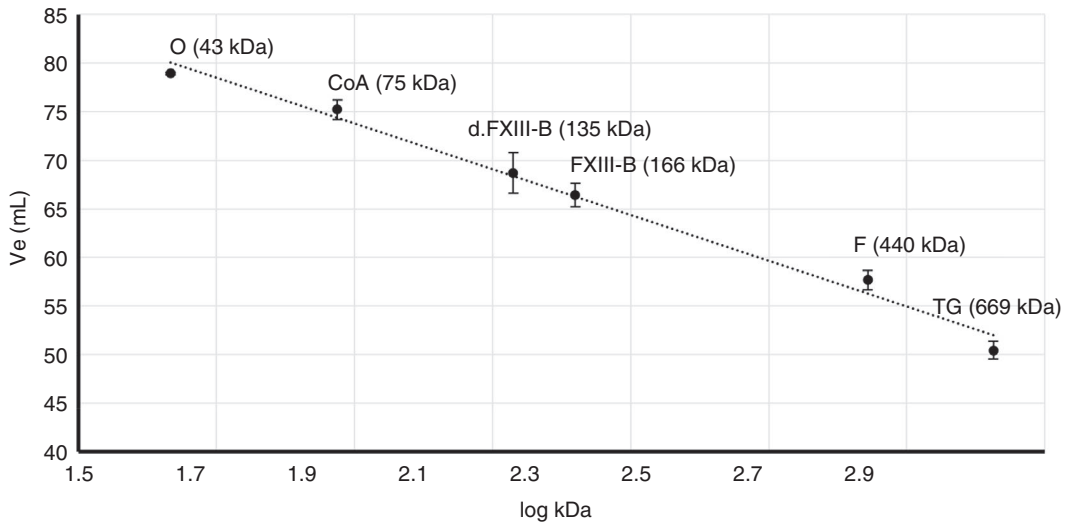


FIGURE 3 Gel filtration analysis of native and deglycosylated factor XIII B subunit (FXIII-B and dFXIII-B). The following molecular weight standards were used for calibration: ovalbumin (O), conalbumin (CoA), ferritin (F), thyroglobulin (TG). The results represent the average ± standard deviation of three independent measurements. Ve: elution volume

endoglycosidase PNGase F digestion in non-denaturing conditions removed about 70% of carbohydrates. The second native condition PNGase F treatment apparently removed the residual sugar structures. Denaturation and another PNGase F digestion of the remaining pellet did not result in any detectable glycans proving complete deglycosylation of the native protein.

To sum up at this point, we comprehensively characterized the N-glycan profile of FXIII-B and developed a protocol for deglycosylation of the protein by PNGase F. Such deglycosylated protein could be used in downstream biochemical experiments aiming at understanding the biological relevance associated with FXIII-B N-glycosylation. The amino acid sequences holding the two glycosylated asparagines in FXIII-B correspond to the consensus sequence of Asn-X-Ser/Thr-Y (X ≠ Pro), ie, at position 142 Asn-Tyr-Ser-Thr and at position 525 Asn-Gly-Ser-Ser. The efficiency of glycosylation also depends on the amino acid in position Y and Ser and Thr are among the most favorable ones.[24,25] Their presence at this position in FXIII-B indicates highly efficient glycosylation.

FXIII-B shows a close structural evolutionary relationship with other proteins encoded by the regulator of complement activation

TABLE 3 The clearance of non-deglycosylated and deglycosylated factor XIII B subunit (FXIII-B) from the plasma of FXIII-B knock-out mice. A, mice injected with native glycosylated (non-deglycosylated) FXIII-B (nD). B, mice injected with deglycosylated FXIII-B (D)

	Glycosylated FXIII-B (μg/mL)			
	interval after FXIII-B injection			
FXIII-B KO mice	1 hour	48 hours	120 hours	Weight of mice (g)
1nD	10.97	0.570	0.019	20.3
2nD	10.22	0.677	0.045	23.8
3nD	12.48	0.762	0.049	22.0
4nD	12.68	0.609	0.040	24.9
5nD	9.78	0.585	0.032	25.9
6nD	12.17	0.673	0.040	19.8
7nD	11.42	0.635	0.049	19.3
mean	**11.39**	**0.644**	**0.039**	**22.3**
SD	1.13	0.070	0.010	2.6
	Deglycosylated FXIII-B (μg/mL)			
	interval after FXIII-B injection			
FXIII-B KO mice	1 hour	48 hours	120 hours	Weight of mice (g)
1D	5.18	0.0064	<0.001	22.0
2D	4.17	0.0059	<0.001	22.4
3D	3.68	0.0060	<0.001	23.8
4D	4.11	0.0078	<0.001	25.6
5D	4.05	0.0055	<0.001	19.9
6D	4.89	0.0066	<0.001	19.4
mean	**4.35**	**0.0064**	**<0.001**	**22.2**
SD	0.57	0.0010		2.3

Note: FXIII-B was determined from the plasma obtained at various intervals following the injection of native glycosylated (non-deglycosylated) (A) or deglycosylated (B) FXIII-B. The FXIII-B ELISA[21] used for the measurements recognized both deglycosylated and non-deglycosylated FXIII-B to the same extent.

Means are shown in bold.

Abbreviations: D, deglycosylated, nD, non-deglycosylated; KO, knock out.

gene cluster, although it is functionally different from other members encoded by genes of this cluster.[26] The site-specific N-glycan characterization of human complement factor H, a prominent member of this family, has been reported and actual or putative glycosylation sites of factor H related proteins 1-5 have also been identified.[27,28] Factor H, a 155 kD protein, is a soluble regulator of the alternative complement pathway. In factor H there are eight N-glycation sites, all of which, just like in FXIII-B, follow the N-X-S/T rule. Factor H linked N-glycans seem to have structural rather than functional role.[27]

We are aware of only a single attempt to deglycosylate FXIII-B; however, in this case the extent of deglycosylation, the composition of released carbohydrates, and the completeness of deglycosylation were not investigated.[29] Complete deglycosylation of native FXIII-B, as described in this study, provides a tool for exploring the functional/structural role of the N-glycan structure linked to the protein. Here we first studied if deglycosylation influences the dimeric structure of FXIII-B. The sites responsible for forming FXIII-B dimer are on the fourth and ninth sushi domains[29] and considering the closeness of glycan moieties to these sites one may presume that they might influence the dimerization of FXIII-B. The molecular weight of deglycosylated FXIII-B was somewhat less than that of the native one; however, it was expected because the carbohydrate part is removed from the molecule (Figure 3). The closeness of the determined molecular weight of the two species indicates that the dimeric structure of FXIII-B was preserved after deglycosylation.

The effect of deglycosylation on the lifespan of FXIII-B in the circulation was investigated in FXIII-B knock-out mice. The species difference, ie, the injection of human FXIII-B into mice, very likely influenced, and probably accelerated, the rate of elimination. No human data are available on the half-life of free FXIII-B in plasma. In FXIII-A deficient patients the half-life of FXIII-A_2B_2 complex and recombinant FXIII-A_2 that combined with the patients' FXIII-B in the circulation varied within the interval of 6.2 and 16 days.[6,30-33] However, the half-life of FXIII-B_2 in complex might be significantly different from that of the non-complexed protein. Besides, such patients have a significant amount of free FXIII-B_2 in the circulation that might also influence the clearance. The elimination rate of native human FXIII-B_2 in FXIII-B KO mice was faster than expected (Table 3), which might be due to faster elimination of a protein from non-identical species. However, the robust difference in the elimination rate of native and deglycosylated human FXIII-B_2 suggests that the glycan moiety prolongs its lifespan in circulation.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Z. Kovács, B. Hurják, and B. Dönczö performed the deglycosylation experiments and the carbohydrate analysis and they were involved in analyzing the data. B. Hurják performed the gel filtration study. F. Erdélyi was involved in producing FXIII-B knock-out mice. É. Katona and B. Hurják carried out the clearance experiments on FXIII-B

knock-out animals. G. Haramura was involved in protein preparations. A. H. Shemirani, and F. Sadeghi performed genotyping of mice. A. Guttman and L. Muszbek designed the experiments, analyzed the data, and finalized the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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