

Posttranslational modifications of tissue factor

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Posttranslational modifications and activity of tissue factor
 - 3.1. Glycosylation
 - 3.1.1. Carbohydrate composition
 - 3.1.2. Activity
 - 3.2. Phosphorylation
 - 3.3. Palmitoylation
 - 3.4. Disulfide bond(s) and “encryption-decryption” of TF
4. Perspective
5. Acknowledgement
6. References

1. ABSTRACT

Tissue factor (TF), a membrane protein, is an initiator of blood coagulation *in vivo*. In this review we discuss how posttranslational modifications affect activity and other properties of TF. Glycosylation of the extracellular domain and the composition of carbohydrates at three glycosylation sites have an influence on TF activity in the extrinsic FXase by increasing the rate of FX proteolysis. No influence of TF glycosylation on the activity of the FVIIa/TF complex towards small synthetic substrates was observed, suggesting that glycosylation has no effect on TF interaction with FVIIa. There are no published data suggesting a direct influence of phosphorylation or palmitoylation in the cytoplasmic domain on TF procoagulant activity. There has been a debate in the recent literature related to the role and formation of the Cys¹⁸⁶-Cys²⁰⁹ disulfide bond. Published opinions from various laboratories range from this bond being essential for the expression of cell TF activity to having no role in it. Overall, it is clear that some modifications of TF have an effect on TF procoagulant activity, signaling functions and trafficking. The influences of other modifications are debatable.

2. INTRODUCTION

Tissue factor (TF) is a trans-membrane protein that is an essential component of the factor VIIa-TF enzymatic complex (extrinsic factor Xase). TF is expressed in a variety of cells and is found in the central nervous system, lungs, and placenta at relatively high concentrations(1-3). Some blood cells, such as monocytes and macrophages, can express detectable amounts of TF when they are stimulated *in vitro* by various, primarily by inflammation-related, agents(4-6). Additionally, TF has been identified in atherosclerotic plaques, which has suggested a role for TF in the progression of cardiovascular diseases(7, 8). In healthy individuals, however, cells in contact with blood do not contain physiologically active TF. Upon a damage of the vascular wall, subendothelial TF is expressed/exposed to the blood flow and binds factor VIIa, an enzymatic component of the extrinsic factor Xase, which circulates at a concentration of approximately 0.1 nM (9). This enzymatic complex activates the zymogens factor IX and factor X to their respective serine proteases, factor IXa and factor Xa, leading to thrombin generation and consequential initiation of the blood coagulation process.

Natural full-length TF is expressed as a 263 amino acid protein and is composed of three distinct domains: an NH₂-terminal extracellular domain (residues 1-219), which is composed of two fibronectin type III domains and is responsible for factor VIIa binding and extrinsic factor Xase formation, the transmembrane domain (residues 220-242), which spans the membrane and anchors TF to it and a cytoplasmic carboxyterminal domain (residues 243-263), which is involved in signal transduction(10). Thus, two of the three domains of TF (extracellular and transmembrane) play distinct roles in the blood coagulation process and it has been generally accepted that TF lacking the cytoplasmic domain is functionally identical to the full-length protein in the initiation of blood coagulation.

The amino acid sequence data of TF indicate that there are four potential N-glycosylation sites; three of them are in the extracellular domain at Asn11, Asn124 and Asn137 and one in the cytoplasmic domain at Asn261. Although a partial identification of carbohydrates attached to the glycosylation sites of the extracellular domain was accomplished(11), a more detailed analysis of carbohydrate moieties and their role on TF affinity for factor VIIa and that of the complex enzyme (factor VIIa-TF) for its natural substrates factor IX and X are missing. Similarly, there are no convincing data published describing the influence of glycosylation on the TF-related activity in processes leading to thrombin generation and clot formation. TF also contains two potential disulfide bonds (Cys49-Cys57 and Cys186-Cys209) located in the extracellular domain(12). The carboxy-terminal cytoplasmic domain contains a single Cys245 residue and two Ser residues (Ser253 and Ser258). The Cys245 residue is potentially linked to a palmitate or stearate fatty acyl chain(12) while one or both Ser residues can be phosphorylated by a protein kinase C-dependent mechanism(13).

A variety of human recombinant TF species have been produced, from those containing only the extracellular domain to the full-length protein. These recombinant proteins have been extensively used in research and clinical laboratories worldwide due to the limited availability of natural tissue factor. Alternatively, many laboratories have been using natural "thromboplastin" reagents as a source of TF in their experiments. These reagents are made by homogenizing natural tissues (most often non-human) that contain a relative abundance of TF such as brain, placenta and lung. Experimental results acquired using recombinant TF and thromboplastins *in vitro* are frequently used for understanding coagulation processes occurring *in vivo*. Unfortunately, the non-availability of isolated natural TF does not allow the confirmation (or rejection) of results obtained with recombinant TF or that present in homogenates of natural tissues. The goal of this review is to compare posttranslational modifications of natural TF purified from placenta with those of recombinant protein and to discuss the influence of these modifications on the functional activity of TF proteins.

3. POSTTRANSLATIONAL MODIFICATIONS AND ACTIVITY OF TISSUE FACTOR

3.1. Glycosylation

Posttranslational modifications are a common feature of proteins and usually have an effect on protein properties including their function, stability and localization. According to some estimates, between 140 and more than 200 types of posttranslational modifications of proteins have been described(14), ranging from quite common and widespread such as glycosylation, phosphorylation, acylation, methylation and ubiquitination to more rare such as sulfation, hydroxylation, etc.

Glycosylation, the attachment of carbohydrates to proteins and lipids, is the most common and complex form of posttranslational modifications and requires between 1-2% of human genes, which encode proteins responsible for this modification(15). The complexity of carbohydrates attached to the proteins makes their characterization difficult and challenging. Only advancements in methodology of the last decade (mass spectrometry; MS, high-pressure liquid chromatography; HPLC and nuclear magnetic resonance; NMR) allowed characterization of complex carbohydrate moieties of proteins more precisely and with lower labor intensity(16).

TF protein was purified and partially characterized for the first time 40 years ago(17). Shortly after, an evidence for the presence of carbohydrates on bovine TF was obtained by Pitlick(18). It was based upon the observation that concanavalin A, a plant lectin which binds preferentially to glucosyl and mannosyl residues(19), efficiently but reversibly inhibits TF procoagulant activity. Moreover, based on concanavalin binding, it was concluded later that glycosylation of TF from various species is different and there is certain heterogeneity in glycosylation of human TF extracted from various tissues(20). In 1985 Shands observed that tunicamycin, a mixture of homologous nucleoside antibiotics which prohibits posttranslational glycosylation(21), interfered with TF activity(22). Based on this observation, it was hypothesized that glycosylation of TF could be important for its function, although no direct evidence was provided. Two years later, the primary structure of the TF protein was established and potential glycosylation sites were suggested(10).

Although it has been known for almost 40 years that TF contains carbohydrate moieties, no attempts to precisely characterize their structure and abundance were taken. Moreover, the influence of glycosylation on functions of TF proteins, both natural and recombinant, is clearly underrepresented in the existing literature. In a side-by-side activity comparison for glycosylated and non-glycosylated full-length recombinant TF, it has been stated that glycosylation is not required for TF procoagulant activity(11). Since no quantitative data were provided, it is impossible to check the validity of that suggestion. Similarly, a statement suggesting that the activity of recombinant full-length TF is identical to that of natural protein from brain was not supported by any data(23). One

Table 1. Molecular masses (Da) of TF proteins

TF species ^a	Source	Calculated ^b	MALDI-TOF	Difference ^c	SDS-PAGE
rTF ₁₋₂₄₃	E.Coli	27,423	27,800	377	31,000
rTF ₁₋₂₄₃ D ^r	E.Coli	27,423	27,800	377	31,000
rTF _{1-263G}	Sf9	29,592	33,196	3,604	37,000
rTF _{1-263D}	Sf9	29,592	30,202	610	33,000
pTF _G	Human placenta	29,592	36,179	6,605	45,000
pTF _D	Human placenta	29,592	ND	ND	34,000

^ap-placental, r-recombinant, G-glycosylated, D-deglycosylated, ^bcalculated from amino acid composition, ^cdifference between calculated mass by amino acid composition and that obtained by MALDI-TOF. Adapted with permission from (26).

of the rare studies addressing the issue of TF glycosylation and its influence on procoagulant activity of the protein was published by Stone and co-workers(24). The results of that study suggested that activity of a soluble form of TF, which is in fact the extracellular domain of the protein, is independent of the presence or absence of carbohydrate moieties on the protein. However, soluble forms of TF used by the authors of the study are physiologically irrelevant with respect to the initiation of blood coagulation due to their inability to bind to the membrane and in a complex with factor VIIa, efficiently activate factor X and factor IX(25).

Recently, the activity comparison of two forms of recombinant TF proteins (recombinant TF 1-243 produced in *E. coli* and recombinant TF 1-263 produced in insect Sf9 cells) and that of the natural human placental TF was accomplished in our laboratory(26). Deglycosylation, tryptic digestion, HPLC and MS techniques were used to accomplish this task. It was established that three of the four potential glycosylation sites have asparagine-linked (N-linked) oligosaccharides in their structure. All three of them were located in the extracellular domain of TF at Asn¹¹, Asn¹²⁴ and Asn¹³⁷, i.e. at the sites predicted by the amino acid sequence of the protein(27). Similar to previous publications(10, 11), no carbohydrates were detected at Asn²⁶¹. The N-linked glycosylation is typical for the majority of proteins that enter the secretory pathway in eukaryotic cells and occurs at the sites which contain Asn-X-Thr/Ser amino acid sequence(28, 29). It is catalyzed by the hetero-oligomeric oligosaccharyltransferase(30).

3.1.1. Carbohydrate composition

Based on the MS data, it was determined that, consistent with the expression system (bacteria), recombinant TF 1-243 had no carbohydrates attached to the backbone of the protein and that placental TF was modified more heavily than recombinant full-length protein rTF 1-263 (Table 1; 26). The extent of glycosylation and carbohydrate composition was different between the two proteins as well as between each glycosylated site within the protein. Additionally, a quite high heterogeneity of carbohydrates was observed at each glycosylation site. Only 20% of Asn¹¹ in recombinant tissue factor 1-263 is glycosylated, predominantly with high mannose carbohydrates. In contrast, more than 75% of Asn¹¹ is glycosylated in placental tissue factor. This site in placental tissue factor is heavily fucosylated and no mannose-containing carbohydrates are detected. Asn¹²⁴ in both tissue factor proteins (natural and recombinant) is almost completely glycosylated, however the composition of carbohydrates is distinctly different. In recombinant tissue

factor 1-263, more than 80% of this site is modified with high-mannose carbohydrates, whereas in placental tissue factor Asn¹²⁴ is glycosylated with hybrid and sialylated carbohydrates. Similarly, pronounced differences in carbohydrate structure are observed for Asn¹³⁷, although in both proteins this site is almost completely glycosylated. In recombinant tissue factor 1-263, the abundance of high-mannose carbohydrates is almost matched by that of fucosylated sugars, whereas in placental tissue factor Asn¹³⁷ is modified exceptionally with fucosylated-(sialylated) carbohydrates. Differences observed in carbohydrate composition between human placental tissue factor and the recombinant protein produced in Sf9 cells are consistent with general patterns of protein glycosylation in mammalian and insect cells(31-33).

3.1.2. Activity

To evaluate the influence of glycosylation on TF activity, we compared glycosylated and fully deglycosylated forms of these three TF proteins. As expected, “deglycosylation” did not affect recombinant TF 1-243 activity and affinity for FVIIa and FX in the fluorogenic (membrane-independent) and the extrinsic factor Xase (membrane-dependent) assays (Table 2 and 3, respectively). Somewhat surprisingly, deglycosylation had no effect on the activity of recombinant and placental TF in the fluorogenic assay (Figure 1), suggesting that differences in activity in this assay observed for different forms of TF could be related to other posttranslational modifications. These results were consistent with observation of Stone *et al.* suggesting that the soluble form of TF, which does not interact with the membrane, is not influenced by deglycosylation(24). In contrast to the fluorogenic assay, deglycosylation of the full-length recombinant TF 1-263 slightly decreased the efficiency of this protein in the extrinsic factor Xase complex. However, the most pronounced decrease in the activity of this enzymatic complex was observed when natural placental TF was deglycosylated. It caused a decrease in affinity of factor X for the factor VIIa/TF complex, a decrease in the catalytic constant and, as a consequence of these changes, an almost 7-fold decrease in the second order rate constant (Table 3, Figure 2). Upon deglycosylation, all the parameters of the Michaelis-Menten kinetics of the extrinsic factor Xase were almost identical for both recombinant TF 1-263 and placental TF, suggesting that the differences in the activity observed for the glycosylated forms of these two TF proteins are related to the structure of carbohydrates attached to the peptide chain and to the extent of glycosylation. A similar decrease in activity of the extrinsic factor Xase was observed by the Edgington’s laboratory when, upon mutation of two cysteines in the extracellular

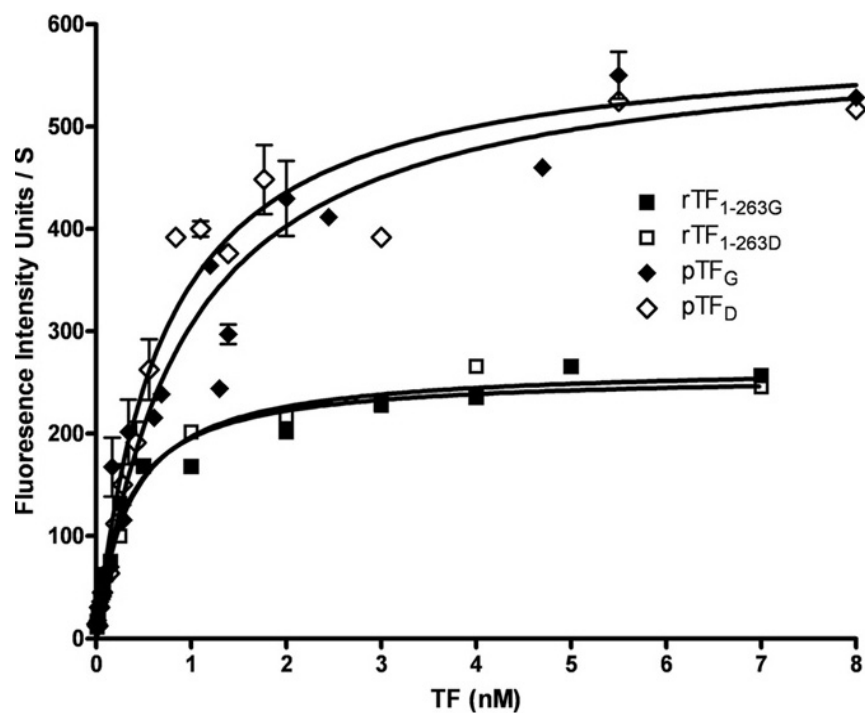


Figure 1. Amidolytic activity of FVIIa in complex with placental TF (pTF; diamonds) and recombinant full-length TF (rTF₁₋₂₆₃; squares) both glycosylated (G; filled symbols) and deglycosylated (D; open symbols). Increasing concentrations of TF were incubated with 0.5 nM FVIIa followed by the addition of 50 μ M fluorogenic substrate. The rate of substrate hydrolysis was recorded. Reproduced with permission from (26).

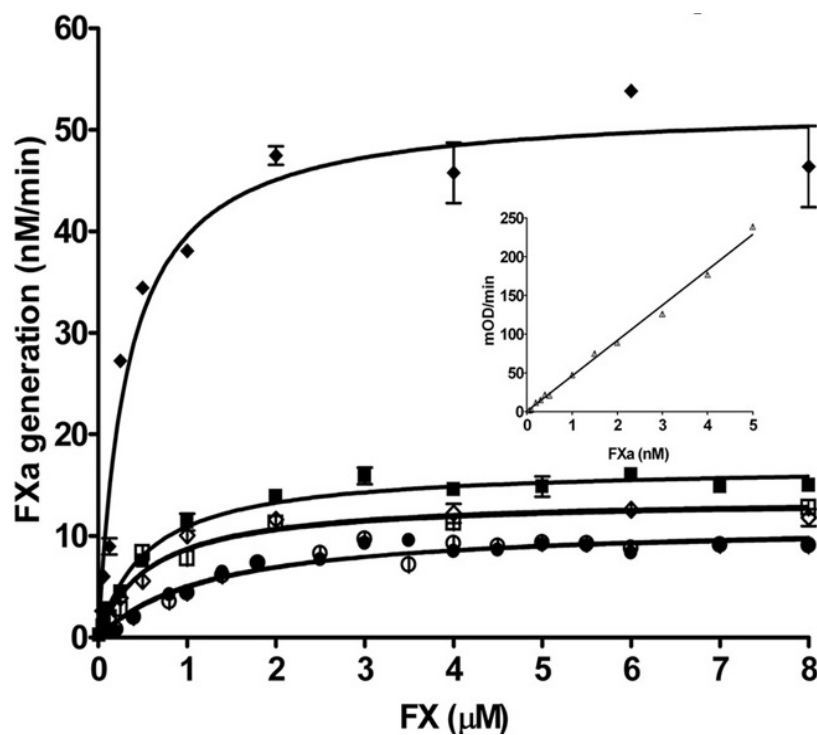


Figure 2. Proteolytic activity of the extrinsic FXase formed by FVIIa and relipidated placental TF (◆ glycosylated and ◇ deglycosylated), full-length recombinant TF₁₋₂₆₃ (■ glycosylated and □ deglycosylated) and truncated recombinant TF₁₋₂₄₃ (● native and ○ “deglycosylated”). FVIIa (5 nM) was incubated with 0.1 nM relipidated TF and increasing concentrations of FX were added. FXa generation was monitored in a chromogenic assay. Reproduced with permission from (26).

Table 2. Affinity and activity of the FVIIa/TF complex in a fluorogenic assay

TF species ^a	K _{d,app} ^b (nM)	V _{max} ^c (pM/s)	Stoichiometry (TF:FVIIa)
rTF ₁₋₂₄₃	0.41 ± 0.40	59.7 ± 1.2	1.1 : 1.0
rTF ₁₋₂₄₃ "D"	0.47 ± 0.03	61.2 ± 1.0	0.9 : 1.0
rTF _{1-263G}	0.31 ± 0.02	75.8 ± 1.6	0.9 : 1.0
rTF _{1-263D}	0.35 ± 0.02	78.3 ± 1.4	1.0 : 1.0
pTF _G	0.92 ± 0.15	173.1 ± 8.4	0.9 : 1.0
pTF _D	0.69 ± 0.09	172.8 ± 10.0	1.0 : 1.0

^a p-placental, r-recombinant, G-glycosylated and D-deglycosylated, ^b apparent dissociation constant, ^c maximum rate of substrate hydrolysis at 0.5 nM FVIIa and saturating TF. Adapted with permission from (26).

Table 3. Activity of the TF/FVIIa complex in the extrinsic FXase assay

TF species ^a	K _M ^b (μM)	k _{cat} ^c (s ⁻¹)	k _{cat} /K _M (μM ⁻¹ s ⁻¹)	relative activity ^d	Stoichiometry (TF:FVIIa)
rTF ₁₋₂₄₃	1.19 ± 0.22	1.8	1.5	1.0	1.0 : 1.0
rTF ₁₋₂₄₃ "D"	1.31 ± 0.26	1.9	1.4	0.9	1.0 : 1.0
rTF _{1-263G}	0.54 ± 0.05	2.8	5.2	3.5	1.0 : 1.0
rTF _{1-263D}	0.57 ± 0.17	2.2	3.8	2.5	0.9 : 1.0
pTF _G	0.32 ± 0.04	8.7	26.8	17.9	0.9 : 1.0
pTF _D	0.57 ± 0.07	2.3	4.0	2.7	0.9 : 1.0

^a p-placental, r-recombinant, G-glycosylated and D-deglycosylated, ^b Michaelis constant, ^c catalytic constant, ^d in comparison with rTF₁₋₂₄₃. Adapted with permission from (26).

domain of TF, glycosylation of the mutant protein became impaired(34). In addition to the effect of glycosylation on TF procoagulant activity, it plays an important role in the transport of cell TF to the plasma membrane and in the microparticle generation(35).

3.2. Phosphorylation

Among numerous posttranslational modifications of proteins, phosphorylation is the most important because it plays a critical role in the regulation of many protein functions. Protein phosphorylation is carried out by protein kinases, which transfer phosphate to the hydroxyl groups of the side chains of three amino acids – serine, threonine and tyrosine(36). Hydroxyl groups of serine represent the major site of phosphorylation in proteins (90-95% of total phosphorylation sites) and 5-10% of total phosphorylation occurs at the threonine side chain, whereas tyrosine phosphate represents less than 1% of total protein phosphorylation in eukaryotic cells.

In 1992, Zioncheck *et al.* determined that TF protein contains two phosphorylation sites, both of them in the cytoplasmic domain(13). They suggested that phosphorylation is regulated by the protein kinase C-dependent mechanism, because it was induced by the PKC activator phorbol 12-myristate 13-acetate, whereas staurosporine, a potent PKC inhibitor, abolished phosphorylation of TF. From the alignment of cDNA sequences of several species (including human) it was concluded that phosphorylation sites contain a conserved amino acid sequence X-Ser*/Thr*-Pro-X with asterisk indicating the phosphorylation residue. In a later publication, Mody and Carson suggested that the cytoplasmic domain of TF can be phosphorylated *in vitro* at multiple sites, particularly at Ser²⁵³ and Ser²⁵⁸(37). The mutational data presented by Dorfleutner and Ruf suggested that initial phosphorylation at Ser²⁵³ enhances the subsequent phosphorylation at Ser²⁵⁸(38).

There are several publications describing the influence of protein kinase C activators and inhibitors on cell TF activity(39), primarily by altering expression of TF

antigen on the cell surface(40). In addition to the regulation of TF expression, phosphorylation of TF influences cell signaling, migration and angiogenesis(41-43). Ryden and coworkers showed that TF phosphorylation related protease-activated receptor-2 signaling plays an important role in breast cancer recurrence(44).

3.3. Palmitoylation

One of the common posttranslational modifications of eukaryotic proteins is acylation with fatty acids, primarily with myristate and palmitate. Myristoylation usually takes place at the N-terminal glycine by the formation of an amide bond, whereas palmitoylation occurs at a cysteine residue via a thioester bond formation(45). S-palmitoylation corresponds to the binding of a saturated fatty acid containing 16 carbon atoms to an unpaired cysteine. Other fatty acids than palmitic of various length, both saturated and unsaturated can also participate in S-acylation of proteins(46). In addition to cysteine, ε-amino group of lysine can be modified with the palmitate residue(47). S-palmitoylation is almost an exclusive feature of membrane proteins, although there is no well-defined sequence for this modification other than the presence of a free cysteine. S-palmitoylation occurs in the vicinity of cell membranes and directs proteins to the membrane lipid rafts, presumably due to a high affinity of proteins modified with fatty acids for these subdomains of cell membranes(48).

It has been shown by Bach and coworkers that TF has one S-palmitoylation site in the intracellular domain at Cys²⁴⁵ (12). In accordance to general features of this modification, Cys²⁴⁵ is at the N-terminus of the intracellular domain and is located close to the membrane surface. The extent of palmitoylation at this site, however, is not clear because it has been shown in several publications that in purified TF proteins, Cys²⁴⁵ can also participate in dimer and higher multimer formation(12, 49-51). Based upon the data presented in those publications, it is hard to conclude whether those multimeric forms of TF are characteristic for the protein in its native environment or they occur during TF purification due to a relatively low stability of S-

palmitoylation. On the other hand, the data suggesting the presence of TF dimers and multimers on the surface of a living cell(52-55) indicate that these forms of TF can exist *in vivo*. Although the existence of TF dimers and multimers has been known for more than a decade, the data related to the activity of these TF forms are scarce and somewhat contradicting. For example, Donate and coworkers showed that the dimerized form of TF has the same factor X-activating efficiency as the monomeric form(55), whereas two other groups of investigators suggested that TF dimers represent an “encrypted” form of protein, which has a relatively low activity(53, 54).

It has been suggested that, in accordance with the properties with S-palmitoylated membrane proteins, this modification should target TF to the cell membrane lipid rafts, which are enriched in sphingolipids and cholesterol(56). During the last decade, increasing experimental data suggest a role for these rafts in modification of TF expression(57, 58) and activity(59-62). It has been shown that one of the components of the lipid rafts, (cholesterol) might play an important role in TF activity(60, 61) although the effect observed by investigators was minimal and did not exceed 2 to 3-fold. Additionally, there was some contradiction related to the role of cholesterol for TF activity. Dietzen and coworkers suggested that cholesterol elimination from the cell membrane lipid rafts increases TF activity(61), whereas two other groups observed a decrease in TF activity caused by cholesterol removal(60) and changes in lipid raft environment(59). It is possible, however, that this controversy could be caused by the use of different cell types by the investigators. Another mechanism, by which S-palmitoylation can regulate TF activity, is its effect on the phosphorylation of the intracellular domain of the protein. It has been shown by Dorfleutner and Ruf that S-palmitoylation at Cys²⁴⁵ inhibits phosphorylation at Ser²⁵⁸ in the intracellular domain of TF(38). The authors suggested several hypotheses explaining this effect, with the leading one implying that palmitoylation favors the association of TF with caveolin-containing lipid rafts. Such association should efficiently inhibit protein kinase C α , involved in TF phosphorylation, by the scaffolding domain of caveolin(63).

Thus S-palmitoylation of the intracellular domain of TF can indirectly alter its procoagulant activity by directing TF towards cell membrane lipid rafts and by influencing phosphorylation of that domain.

3.4. Disulfide bond(s) and “encryption-decryption” of TF

While there is common agreement about the leading role of TF in the initiation of blood coagulation *in vivo*, there are significant controversies related to the expression and regulation of TF activity on the cell surface. It has been suggested that the majority of TF molecules located on the cell surface have low activity (are “encrypted”) and that “decryption” is essential for the expression of TF function(64). Several mechanisms, often contradictory, have been hypothesized in attempts to explain “encryption-decryption” of TF activity.

One of the suggested methods for the “decryption” of TF on the cell surface consists of the treatment of TF-bearing cells with calcium ionophore(65-71). Ionophore increases TF activity by 2 to 10-fold, while some authors assign this increased TF activity to increased expression of TF protein(65), others suggest this arises from changes in the cell membrane environment, particularly in an increased expression of acidic phospholipids(66, 68, 71), sometimes related to cell death(67, 70). Several studies hypothesize a role for cholesterol in cell lipid rafts contributing to the “encryption-decryption” of TF activity(60, 61, 72), although there is little agreement between the suggested mechanisms of this process. An increase in TF activity has been reported when lipopolysaccharide (LPS)-stimulated monocytes are treated with platelets(73-75). This observed increase in activity was quite limited (2 to 3-fold) and could be (in part) assigned to an increase in TF antigen expression by monocytes(75).

It has been also suggested that cell surface TF “decryption” is related to the Cys¹⁸⁶-Cys²⁰⁹ disulfide bond formation. The key data leading to this hypothesis were based on the mutational studies by Edgington’s group(34). They mutated either Cys⁴⁹ and Cys⁵⁷ (which potentially could form a disulfide bridge in the N-terminal part of the extracellular domain of TF) or Cys¹⁸⁶ and Cys²⁰⁹ (which could form a disulfide bond in the vicinity of the membrane) substituting serine residues for cysteines. Mutations of Cys⁴⁹ and Cys⁵⁷ had no effect on TF activity in the extrinsic factor Xase, whereas mutations of Cys¹⁸⁶ and Cys²⁰⁹ decreased the activity of this complex by approximately 3-fold due to an impaired binding of FVIIa to this TF mutant. Based on these data it was concluded that the N-terminal pair of cysteines plays no role in TF activity, whereas the disulfide bridge in the C-terminus of the extracellular domain is essential for the “decryption” of TF activity. However, the Ser¹⁸⁶Ser²⁰⁹ mutant, in contrast to the wild-type protein and Ser⁴⁹Ser⁵⁷ mutant, lacked glycosylation. In a study from our laboratory we showed that deglycosylation of natural TF leads to a similar decrease in activity(26). Thus an impaired activity observed for the Ser¹⁸⁶Ser²⁰⁹ mutant could be caused not by the lack of the disulfide bond but by the lack of glycosylation.

The role (or absence of it) of the Cys¹⁸⁶-Cys²⁰⁹ disulfide in the regulation of TF and the mechanism by which it is formed on the cell surface has been the subject of debates for the last several years. Chen and coworkers observed an increase in cell TF activity upon the treatment of cells with an oxidizing agent mercuric chloride(76). They concluded that this reagent restores Cys¹⁸⁶-Cys²⁰⁹ disulfide bond leading to the observed changes in TF activity. However, no data (experimental or theoretical) are provided supporting the re-formation of the hypothesized reduced disulfide bridge. Existing chemical records conclude that mercuric chloride will oxidize only a single thiol group(77, 78). Moreover, an increase in TF activity on cell surfaces similar to that caused by mercuric chloride can be achieved by treating TF-bearing cells with other metal compounds, such as silver nitrate and phenylmercuric acetate(79).

TF structure and activity

In several publications from Ruf's laboratory, it has been suggested that protein disulfide isomerase (PDI) is responsible for the regulation of cell TF activity via its effect on the status of the Cys¹⁸⁶-Cys²⁰⁹ disulfide bond(80-82). Similarly, studies from Engelmann's laboratory proposed that TF activation by PDI occurs due to the isomerization of a mixed disulfide and an intramolecular Cys¹⁸⁶-Cys²⁰⁹ bond formation(83, 84). However, publications from several other laboratories showed that the TF activity-enhancing effect of PDI is related to the presence of acidic phospholipid phosphatidylserine either present as a contaminant in the PDI preparations(85, 86) or due to its relocation to the TF-bearing cell surface upon treatment with PDI or mercuric chloride(87-90). Moreover, Kothari *et al.* suggested in a recent publication that Cys¹⁸⁶-Cys²⁰⁹ disulfide bond is not essential for the "decryption" of cell TF activity(91).

Thus the question related to the role of this disulfide in the expression of cell TF activity remains open. Moreover, even the presence of this bond in a functional TF protein has to be verified.

4. PERSPECTIVE

Tissue factor has several types of posttranslational modifications, glycosylation being the most complex of them. Although the presence of carbohydrates on tissue factor was identified several decades ago, a thorough characterization of their structure and an evaluation of their influence on tissue factor activity were, with rare exceptions, somewhat neglected. This lack of interest could be explained by several statements in literature suggesting that posttranslational modifications are not important for the function of this protein. Unfortunately, no solid (if any) data were provided to support these statements. Additionally, most of the experiments were done using recombinant tissue factor proteins, which were different with respect to posttranslational modifications from the natural protein. These differences are translated into differences in physiologically-relevant activities of tissue factor. Lately, the interest in tissue factor structure-activity relationship has been rekindled, primarily by the controversies related to the role of disulfides of the extracellular domain and that of glycosylation. Additionally, there has been an increasing number of studies accomplished using natural human tissue factor or that present on the cell surface instead of recombinant proteins. This increased interest in the tissue factor protein leads us to believe that existing gap in the knowledge related to the structure-activity relationship will be filled with new research data.

5. ACKNOWLEDGEMENT

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TF structure and activity

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