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EVOLUTIONARY CONSERVATION OF THE TISSUE FACTOR DISULFIDE BONDS AND IDENTIFICATION OF A POSSIBLE OXIDOREDUCTASE BINDING MOTIF

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Tissue Factor (TF), a transmembrane glycoprotein, initiates the extrinsic coagulation cascade. Most TF resides on the cell surface in an inactive or cryptic state and can be decrypted upon cellular stimulation; however, the underlying mechanism for decryption remains a matter of debate. Increased exposure of phosphatidylserine at the cell surface has been suggested as a potential decrypting event, whereas others have hypothesized a role for TF disulfide switching[1-3]. The extracellular part of TF is composed of two fibronectin type III domains, each containing a disulfide bond: the N-terminal Cys49-Cys57 disulfide and C-terminal Cys186-Cys209 disulfide. The latter disulfide exists in an –RHStaple configuration, which is a common hallmark of allosteric disulfides facilitating the cysteine link between two adjacent strands within a β-sheet[4]. Allosteric disulfides are generally considered to control protein function in a redox-dependent manner, and, accordingly, the Cys186-Cys209 disulfide has been proposed to be involved in TF decryption[3;4]. TF mutagenesis studies have confirmed that the TF procoagulant activity is dependent on the presence of the Cys186-Cys209 disulfide bond, and modulation of this disulfide is suggested to be regulated by the oxidoreductase Protein Disulfide Isomerase (PDI)[2;6].

In order to investigate whether disulfide switching of allosteric disulfides is a common regulatory element in TF, we examined the evolutionary conservation of the cysteine residues involved. To this end, we performed a search in genomic databases and aligned the derived TF amino acid sequences from species classified in evolutionary taxa using the CLUSTAL W algorithm (AlignXModule; Invitrogen Corporation, Carlsbad, CA, USA). We observed strong sequence homology between the extracellular TF domains of various species (Fig. 1A). This is in accordance with an earlier *in silico* analysis, demonstrating that the genetic sequence of the extracellular TF domain is highly conserved, whereas the transmembrane and intracellular domains showed minor sequence similarity[7]. Alignment of the various TF sequences demonstrated that the cysteines involved in the allosteric disulfide Cys186-Cys209 are fully preserved throughout evolution (Fig. 1A).

Further comparison of human TF amino acid sequence 176-219 with sequences of two proteins relevant to human pathophysiology that are known to contain an established -RHStaple allosteric disulfide (HIV-1 glycoprotein 120 and human CD4) revealed a common motif positioned C-terminal to Cys186 (Fig. 1B). This motif consists of the polar and hydrophilic residues Pro, Ser/Thr, and Arg, followed by an Asn (PSR-N), which have a propensity to be present in or near loop structures. Close examination of the available crystal structures confirmed that these residues are indeed part of a surface-exposed loop connecting those anti-parallel β-sheet strands that are linked by the Cys-Cys bond[8-10]. Interestingly, based on the data available, only disulfides with a –RHStaple configuration exist in conjunction with the PSR-N motif (TF, T-cell surface glycoprotein CD4 , and HIV-1 glycoprotein 120), while a typical example of an -RHHook allosteric disulfide that does not connect anti-parallel β-sheets

(β2-glycoprotein 1) is not associated with this motif (Fig. 1B)[5]. Strikingly, a similar combination of these residues is well preserved in most TF species (Fig. 1A), with the exception of bovine, rat and mouse TF that lack a proline, possibly reflecting species-specific differences in allosteric disulfide modulation. Although its exact role remains to be identified, we speculate that the PSR-N motif not only plays an important role in formation of the secondary β-sheet structure, but, considering its polar and hydrophilic nature, may also be involved in recognition and binding by oxidoreductases. Even though our current analysis is hampered by the limited number of allosteric disulfides characterized to date, it will prove interesting to test this hypothesis as more allosteric disulfides regulating protein function will be identified.

The PSR-N motif was not observed in sequences surrounding the N-terminal disulfide Cys49-Cys57 in TF (Fig. 1A), which is consistent with a non-procoagulant role for this cysteine pair as previously described[6]. However, recent data suggested that the Cys49–Cys57 disulfide modulates the redox potential of Cys186–Cys209[11]. In line with the latter study, the TF sequence alignment analysis showed a strong conservation of Cys49-Cys57 (Fig. 1A), thereby substantiating a role for the N-terminal disulfide in regulating TF coagulant function.

In conclusion, the strong evolutionary conservation of the disulfide bonds in the human TF extracellular domain supports, but does not prove, a pivotal role for disulfide bonding in regulating TF-dependent coagulation.

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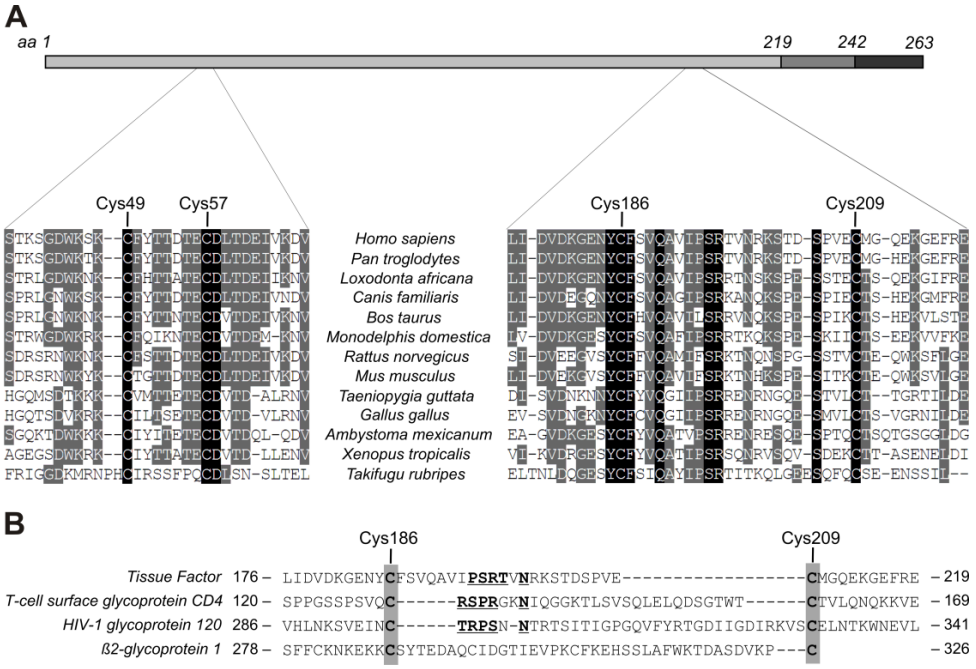

Figure 1. (A) Alignment of extracellular TF domain amino acid sequences from species of different phylogenetic groups. Schematic overview of TF consisting of extracellular (1-219 aa), transmembrane (220-242 aa), and cytoplasmic (243-263 aa) domains. Alignments of regions surrounding cysteine pairs 49-57 and 186-209 with corresponding TF sequences from species of different phylogenetic groups are shown in detail. Residues highlighted in black indicate identical amino acids, whereas grey residues are highly conserved. (B) Alignment of TF sequence surrounding Cys186-Cys209 with sequences comprising an allosteric disulfide. The extracellular region 176-219 comprising disulfide bond Cys186-Cys209 derived from the human TF sequence was aligned using a modified CLUSTAL W algorithm (AlignXModule; Invitrogen Corporation, Carlsbad, CA, USA) to corresponding regions from proteins with established –RHStaple-type allosteric disulfides[7] or an –RHHook conformation. Cysteines involved in an allosteric disulfide bond are indicated in bold and highlighted in grey, the numbers indicated above refer to the position of the cysteine residues in human TF; a conserved sequence motif consisting of Pro, Ser/Thr, and Arg, followed by Asn is indicated in bold and underlined.