Chapter 8 - Disulfide switching: a conserved mechanism in Tissue Factor decryption?

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Dear Sirs,

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, thereby linking two adjacent strands within a β-sheet. The -RHStaple structure is a common hallmark of known allosteric disulfides recognized by the relatively short distance between the alpha-carbon atoms of both Cys residues[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 or absence of Cys186-Cys209 disulfide bond, and modulation of this disulfide is suggested to be regulated by the oxidoreductase Protein Disulfide Isomerase (PDI)[2;5].

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 in these disulfide bonds. 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[6]. Furthermore, the cysteines involved in the allosteric disulfide Cys186-Cys209 are fully preserved throughout evolution (Fig. 1A).

Further comparison of human TF amino acid sequence 174-219 with sequences of various proteins that hold an established allosteric disulfide revealed a common motif positioned in the C-terminal direction from Cys186 (Fig. 1B). This motif consists of the polar and hydrophilic residues Pro, Ser, and/or Arg, followed by an Asn, which have a propensity to be present in or near loop structures. Strikingly, a similar combination of these residues is preserved in TF throughout evolution (Fig. 1A). Moreover, the majority of residues surrounding Cys186-Cys209 have polar and hydrophilic properties, which we speculate to

facilitate localization of this disulfide at the protein surface. Being solvent exposed would allow PDI to interact and subsequent catalyze the redox modulation of these cysteines. In support of this, PDI has been shown to mediate the reduction of HIV-1 envelope glycoprotein 120 from which it is known to contain an established allosteric disulfide[7].

No similar motif was observed in sequences surrounding the N-terminal disulfide Cys49-Cys57, which is consistent with a non-procoagulant role for this cysteine pair as previously described[5]. However, more recent data suggested that the Cys49–Cys57 disulfide modulates the redox potential of Cys186–Cys209 disulfide8. In line with the latter study, our TF sequence alignment analysis shows a strong conservation of the Cys49-Cys57 disulfide (Fig. 1), thereby suggesting 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 of disulfide bonding in regulating TF-dependent coagulation.

Reference List

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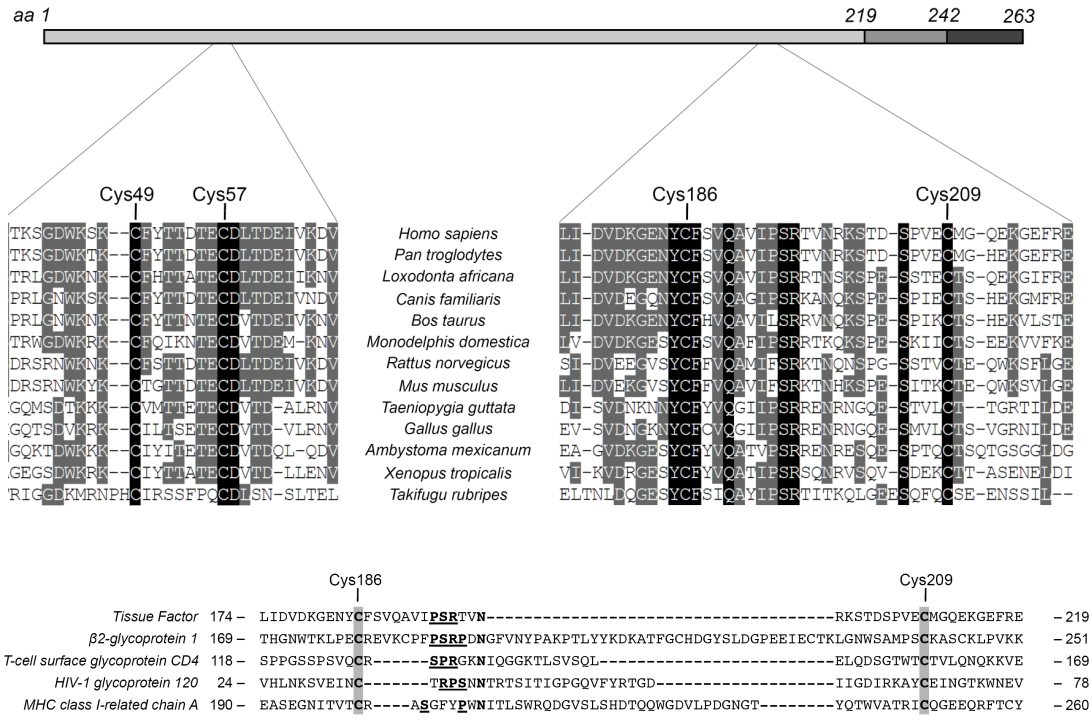

Fig. 1. (A) Alignment of extracellular TF domain amino acid sequences from species of different phylogenetic groups. Schematic overview of TF consisting of extracellular (1-219 aa), transmembrane (220-242 aa), and cytoplasmic (243-263 aa) domains. Alignments of regions surrounding cysteine pairs 49-57 and 186-209 (indicated with asterisks) with corresponding TF sequences from species of different phylogenetic groups are shown in detail. Residues highlighted in black indicate identical amino acids, whereas amino acids indicated in grey are highly conserved. (B) Alignment of the TF sequence surrounding Cys186-Cys209 with sequences comprising an allosteric disulfide. The extracellular region 174-219 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 several proteins with established allosteric disulfides[7]. Cysteines involved in an allosteric disulfide bond are indicated in bold and highlighted in grey; a conserved sequence motif consisting of Pro, Ser, and/or Arg, followed by Asn is indicated in bold and underlined.