

Tissue factor activation involves disulfide switching

Vivien Chen¹, Michael Berndt², Wolfram Ruf³, Philip Hogg¹

1 University of New South Wales, Sydney NSW, Australia.

2 Monash University, Clayton, Victoria, Australia

3 The Scripps Research Institute, La Jolla, California, USA

Tissue factor (TF), the essential cofactor for FVIIa, is required for activation of FX and FIX to generate thrombin. Transmembrane TF resides in a cryptic configuration on the cell surface with low procoagulant activity, however TF can be rapidly switched to an active configuration on exposure to certain stimuli. The nature of this switch is unknown. The extracellular part of TF consists of 2 fibronectin type III domains. The disulphide-bond in the membrane proximal domain (Cys186-Cys209) is atypical for fibronectin domains in that it links adjacent strands in the same 1/2-sheet, a cross-strand bond. The Cys186-Cys209 TF bond has the same unusual configuration as the disulphide-bond in the second domain of CD4, which controls CD4 function by switching between oxidized (disulphide) and reduced (dithiol) states (Matthias et al. *Nature Immunol.* 3,727, 2002). Ablation of the cross-strand bond severely impairs procoagulant activity (Rehemtulla et al. *J. Biol. Chem.* 266, 10294, 1991). Labeling with a biotinylated maleimide, we demonstrate that cryptic tissue factor is reduced at the domain 2 disulfide and oxidised on activation. In HL60 cells, membrane based tissue factor procoagulant activity is blocked by the mono-thiol alkylator N-ethylmaleimide but increased by formation of the disulfide via the thiol oxidiser, HgCl₂ or thiol cross-linkers, bismaleimidohexane and bismaleimidoethane. Using the VIC7 anti-TF antibody which recognises an epitope between aa181-214 in TF (Magdolen et al *Biol Chem* 1998) we demonstrate that activation of cryptic TF on HL60 cells correlates with a change in the conformation of the TF region that is constrained by the cys186-cys209 disulfide. These results indicate that the activation of TF involves a change of conformation of the domain 2 of extracellular TF caused by formation of the cross strand cys186-cys209 disulfide bond. This is likely to be the physiological change that facilitates productive binding of FIX and FX in coagulation.