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Impact of fibrinogen post-translational modifications on its structure and function

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Introduction:

Post-translational modifications (PTMs) can influence structure and/or function of proteins by changing structure or charge of the original amino acid. PTMs of fibrinogen are associated with thrombosis, vascular diseases and other (patho)physiological stages.

The aim of this study is to verify if molecular dynamic simulations are able to describe effect of PTMs on protein secondary structure and to characterize impact of diseases and oxidizing agents onto fibrin clot.

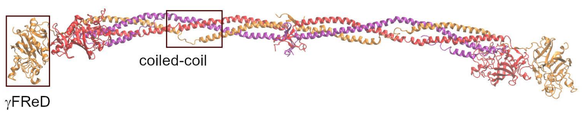

Fig. 1: Crystal structure of fibrinogen (PDB: 3GHG); incomplete. Parts used for molecular dynamic simulations are highlighted by black boxes. Aα chain of fibrinogen is shown in purple, Bβ is red and γ is orange.

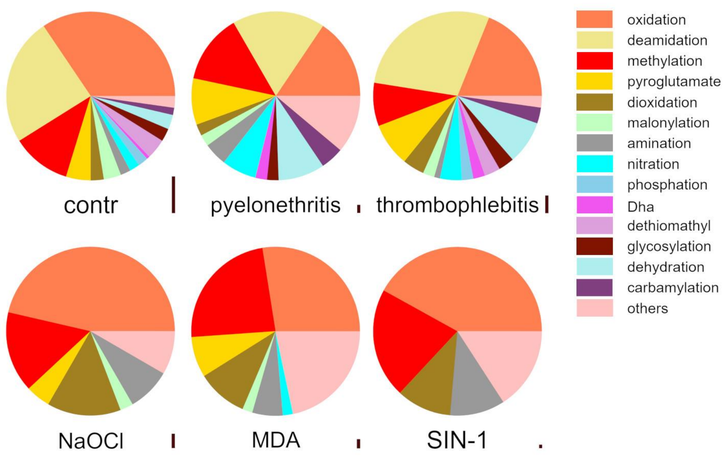

Fig. 2: Comparison of number of PTMs induced into fibrinogen in diseases and *in vitro* by oxidizing agents. The bar next to the label represents number of PTMs detected in the given sample.

Methods:

In patient samples, fibrinogen was purified from citrated plasma. Fibrinogen solution for *in vitro* modifications was prepared from lyophilized human fibrinogen. It was modified by 20min exposure to NaOCl or 120min exposure to malonaldehyde (MDA) in dark or to 60min exposure to linsidomine (SIN-1). PTMs were detected by LC-MS/MS. Fibrin networks were visualized by scanning electron microscopy. Molecular dynamics simulations of fully hydrated parts of crystal structure 3GHG were performed in Gromacs with Gromos 54a7 force field for 250 ns (FReD; amino acids γ148 – γ394) or for 100 ns (coiled-coil region; amino acids Aα70 – Aα126, Bβ101 – Bβ157, γ47 – γ97).

Conclusions:

Pathophysiological state resp. oxidizing agent influences number and nature of detected PTMs.

Impact of the given PTM on fibrinogen secondary structure and function varies from negligible alternations to serious changes.

Prolines (or pyroglutamic acids) γP70 and γP76 are necessary for preserving the surrounding loop, whose approaches to α helices of the others fibrinogen chains lead to their destabilization. This makes this region more prone to enzymatic cleavage and introduces a kink into the coiled-coil region of fibrinogen.

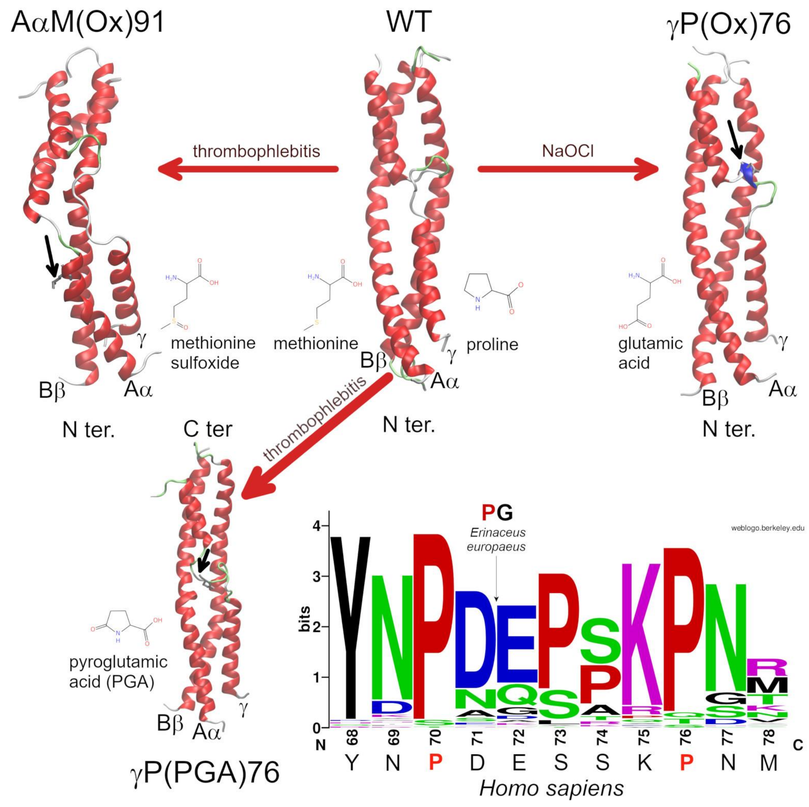

Fig. 3: Structure of part of the coiled-coil region of oxidized AαM91, wild type (WT) and γP76 oxidized either to glutamic or pyroglutamic (PGA) acid after 100ns molecular dynamic simulation. Sequence analysis shows the importance of prolines in the loop made by amino acids γY68 – γM76. There are 1 (in 2 cases) to 4 prolines (modus 3) conserved in this region among 87 mammals.

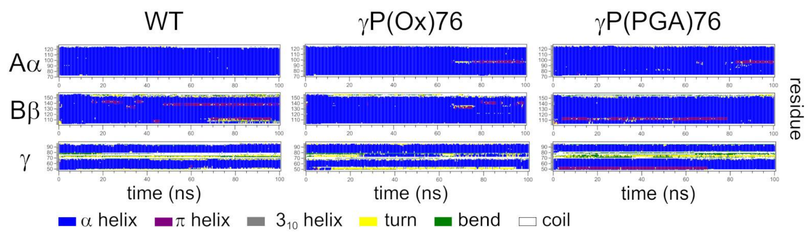

Fig. 4: Development of secondary structure (DSSP) in time for selected systems.

Results:

PTMs affect architecture of fibrin networks.

Citrullinization of γR375 leads to release of Ca^{2+} ion that is necessary for fibrin polymerization.

Oxidation of γW227 stabilizes P domain of FReD via β bridges.

Oxidation of AαM91 destabilizes coiled-coil region of fibrinogen in its proximity.

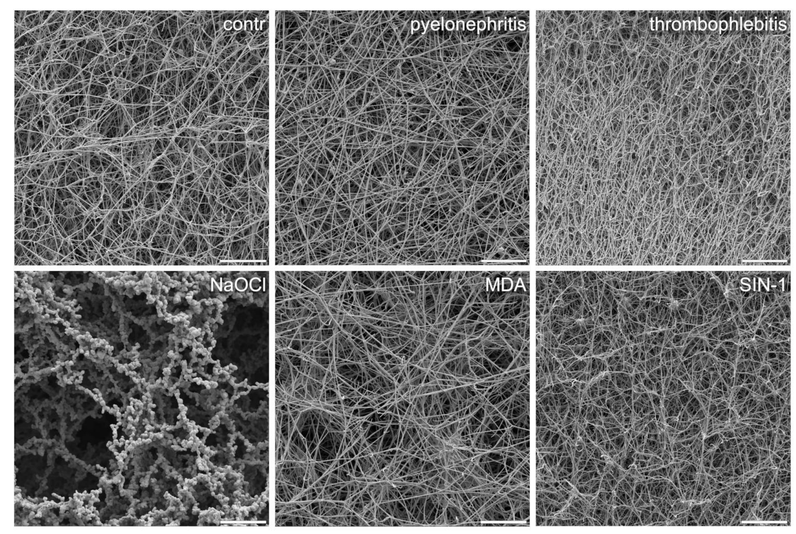

Fig. 5: Fibrin networks obtained under different conditions. The scale represents 10 μm.

	fiber thickness (nm)	average no. of fibers per field (1μm²)
contr	156.1 ± 44.1	33.0 ± 15.6
pyelo.	188.6 ± 68.9	29.9 ± 15.6
throm.	142.1 ± 25.2	32.4 ± 10.2

	fiber thickness (nm)	average no. of fibers per field (1μm²)
NaOCl	107.4 ± 23.7	2.0 ± 1.2
MDA	102.0 ± 21.8	20.5 ± 3.3
SIN-1	102.1 ± 34.0	20.4 ± 2.2

Table 1: Properties of fibrin clots obtained in different environment. “Pyelo” stands for pyelonephritis and “throm” represents thrombophlebitis.

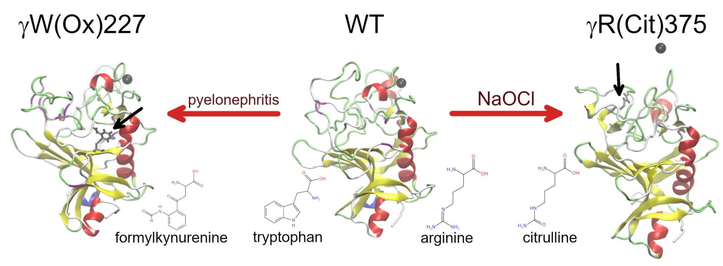

Fig. 6: Structure of γ FReD domain of oxidized γW227, wild type (WT) and citrullinized γR375 after 250ns molecular dynamics simulation.

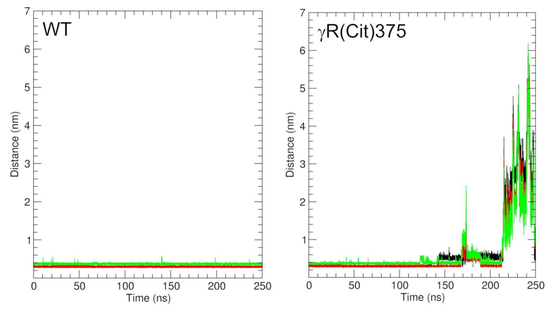

Fig. 7: Distance of Ca^{2+} ion from C_γ carbon of γD318 (black), and γD320 (red) and from C carbon of γF322 (green).

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