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

Ž. Sovová¹, J. Štikarová¹, J. Suttner¹, J. Kaufmanová², P. Mária³, P. Šácha³, J. Loužil¹, M. Malý⁴, O. Kučera⁴, J.E. Dyr¹

¹Institute of Hematology and Blood Transfusion, Czech Republic; ²University of Chemistry and Technology, Prague, Czech Republic; ³Institute of Organic Chemistry and Biochemistry ASCR, Prague, Czech Republic; ⁴Charles University in Prague, Military University Hospital, Prague, Czech Republic; e-mail: sovova@uhkt.cz

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 on fibrin clot.

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

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 lensidomine (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 Ax α70 – Ax α126, Bβ[110] – Bβ[157], 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. 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.

Results:
PTMs affect architecture of fibrin networks.
Citralization of γR375 leads to release of Ca²⁺ ion that is necessary for fibrin polymerization. Oxidation of γR227 stabilizes P domain of FReD via β bridges. Oxidation of AxM91 destabilizes coiled-coil region of fibrinogen in its proximity.

Fig. 3: Structure of part of the coiled-coil region of oxidized AxM91, wild type (WT) and γP76 oxidized either to glutamic or pyroglutamic (PGA) acid system. After 100ns molecular dynamic simulation. Sequence analysis shows the importance of prolines in the loop made by amino acids γ168 – γ394. 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Fiber thickness (nm)</th>
<th>Average no. of fibers per field (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>contr</td>
<td>156.1 ± 44.1</td>
<td>33.0 ± 15.6</td>
</tr>
<tr>
<td>NaOCl</td>
<td>107.4 ± 23.7</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>prefl</td>
<td>188.89 ± 38.9</td>
<td>29.9 ± 15.6</td>
</tr>
<tr>
<td>fibrin</td>
<td>142.1 ± 42.1</td>
<td>32.4 ± 10.4</td>
</tr>
<tr>
<td>SIN-1</td>
<td>102.1 ± 34.0</td>
<td>20.4 ± 2.2</td>
</tr>
</tbody>
</table>

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

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 lensidomine (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 Ax α70 – Ax α126, Bβ[110] – Bβ[157], 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. 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.

Results:
PTMs affect architecture of fibrin networks.
Citralization of γR375 leads to release of Ca²⁺ ion that is necessary for fibrin polymerization. Oxidation of γR227 stabilizes P domain of FReD via β bridges. Oxidation of AxM91 destabilizes coiled-coil region of fibrinogen in its proximity.

Fig. 3: Structure of part of the coiled-coil region of oxidized AxM91, wild type (WT) and γP76 oxidized either to glutamic or pyroglutamic (PGA) acid system. After 100ns molecular dynamic simulation. Sequence analysis shows the importance of prolines in the loop made by amino acids γ168 – γ394. 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Fiber thickness (nm)</th>
<th>Average no. of fibers per field (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>contr</td>
<td>156.1 ± 44.1</td>
<td>33.0 ± 15.6</td>
</tr>
<tr>
<td>NaOCl</td>
<td>107.4 ± 23.7</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>prefl</td>
<td>188.8 ± 38.9</td>
<td>29.9 ± 15.6</td>
</tr>
<tr>
<td>fibrin</td>
<td>142.1 ± 42.1</td>
<td>32.4 ± 10.4</td>
</tr>
<tr>
<td>SIN-1</td>
<td>102.1 ± 34.0</td>
<td>20.4 ± 2.2</td>
</tr>
</tbody>
</table>

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