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Abstract:

Fibrinogen is extremely susceptible to attack by reactive oxygen species (ROS). Having been suffered an oxidative modification, the fibrinogen molecules, now with altered spatial structure and function of fibrin network, affect hemostasis differently. However, the potential effects of the oxidative stress on the early stages of the fibrin self-assembly process remain unexplored. To clarify the damaging influence of ROS on the knob 'A': hole 'a' and the D:D interactions, the both are operating on the early stages of the fibrin polymerization, we have used a novel approach based on exploration of FXIIIa-mediated self-assembly of the cross-linked fibrin oligomers dissolved in the moderately concentrated urea solutions. The oligomers were composed of monomeric desA fibrin molecules created by cleaving the fibrinopeptides A off the fibrinogen molecules with a thrombin-like enzyme, reptilase. According to the UV-absorbance and fluorescence measurements data, the employed low ozone/fibrinogen ratios have induced only a slight fibrinogen oxidative modification that was accompanied by modest chemical transformations of the aromatic amino acid residues of the protein. Else, a slight consumption of the accessible tyrosine residues has been observed due to intermolecular dityrosine cross-links formation. The set of experimental data gathered with the aid of electrophoresis, elastic light scattering and analytical centrifugation has clearly witnessed that the oxidation can serve as an effective promoter for the observed enhanced self-assembly of the covalently cross-linked oligomers. At urea concentration of 1.20 M, the pristine and oxidized fibrin oligomers were found to comprise a heterogeneous set of the double-stranded protofibrils that are cross-linked only by γ–γ dimers and the fibers consisting on average of four strands that are additionally linked by α polymers. The amounts of the oxidized protofibrils and the fibers accumulated in the system were higher than those of the non-oxidized counterparts. Moreover, the γ and α polypeptide chains of the oxidized molecules were more readily crosslinked by the FXIIIa. Upon increasing the urea solution concentration to 4.20 M, the cross-linked double-stranded desA fibrin protofibrils have dissociated into the single-stranded fibrin oligomers, whereas the fibers dissociated into the double-stranded desA fibrin oligomers, the structural integrity of the latter being maintained by means of both the intermolecular α polymers, and the single-stranded fibrin oligomers cross-linked only by γ–γ dimers. The data we have obtained in this study indicate that the FXIIIa-mediated process of assembling the cross-linked protofibrils and the fibers constructed from the oxidized monomeric fibrin molecules was facilitated due to the strengthening of D:D interactions. The findings infer that the enhanced longitudinal D:D interactions become more essential in the assembly of soluble protofibrils when the interactions knobs 'A': holes 'a' are injured by oxidation. The new experimental findings presented here could be of help for elucidating the essential adaptive molecular mechanisms capable of mitigating the detrimental action of ROS in the oxidatively damaged fibrin self-assemblage processes.

Keywords: oxidation; fibrinogen; fibrin self-assembly; cross-linked soluble double-stranded protofibrils; cross-linked fibers; protofibril and fiber dissociation; D-D interaction; γ–γ dimers; α polymers.