Characterization of a Reduced Form of Plasma Plasminogen as the Precursor for Angiostatin Formation

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Introduction: Angiostatin, an internal fragment of plasminogen, is a potent inhibitor of angiogenesis, halting tumor growth and maintaining metastatic and primary tumors in a dormant state. Generation of angiostatin in blood involves activation of plasminogen to the serine protease plasmin and facilitated cleavage of two disulfide bonds and up to three peptide bonds in the kringle 5 domain of the protein. The mechanism of reduction of the two allosteric disulfides is poorly understood and has been explored in this study.

Material and methods: For this, we used purified human plasminogen, thiol alkylating agents, mass spectrometry and an assay for angiostatin formation. Results and discussion: We show that the Cys462-Cys541 disulfide bond is already cleaved in a fraction of plasma plasminogen and that this reduced plasminogen is the precursor for angiostatin formation. From the crystal structure of plasminogen, we propose that plasmin ligands such as phosphoglycerate kinase induce a conformational change in reduced kringle 5 that leads to attack by the Cys541 thiolate anion on the Cys536 sulfur atom of the Cys512-Cys536 disulfide bond, resulting in reduction of the bond by thiol/disulfide exchange. Cleavage of the Cys512-Cys536 allosteric disulfide allows further conformational change and exposure of the peptide backbone to proteolysis and angiostatin release. Conclusion: Understanding these events and the factors involved in angiostatin generation is fundamental for the rational design of angiogenesis inhibitors that can keep the tumor in a quiescent state, maintaining a balance between proliferation and apoptosis.

Key words: Angiogenesis, Angiostatin, Cysteines, Disulfide bonds, Plasminogen

Supported by NHMRC Australia