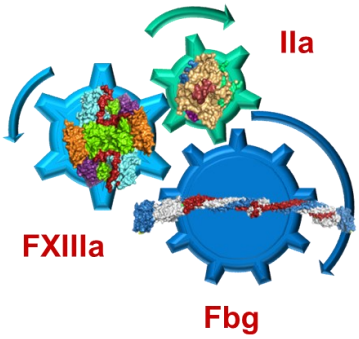
Relaxing With the Maurer Research Group in Louisville: 2011-2013

...While Three Key Players in the Clotting Cascade Continue to Function Along...



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STATEMENT ABOUT RESEARCH ACTIVITIES

(Dr. Muriel C. Maurer, University of Louisville)

Our research has focused on enzymes involved in blood coagulation and related processes. Members of this complex cascade have far reaching effects on wound healing, heart disease, stroke, and arteriosclerosis. Further knowledge, however, is still needed on the activation and substrate binding properties of the individual enzymes participating in the cascade. In our laboratory, emphasis has been placed on examining the transglutaminase Factor XIII (FXIII) and the serine protease thrombin. Activated FXIII (FXIIIa) catalyzes the formation of covalent crosslinks (γ-glutamyl-ε-lysyl bonds) within the fibrin blood clot network. Thrombin (IIa) is involved in converting fibrinogen into fibrin and in activating proteins including FXIII and the protease activated receptors (PARs). A greater understanding of the molecular details associated with FXIIIa and thrombin may be used to develop new therapeutic agents to control the functions of these enzymes.

One of our research themes has been to address **the kinetic and structural impact of amino acid substitutions to the FXIII activation peptide (AP).** This segment obstructs access to the FXIII catalytic site and is cleaved by thrombin (IIa). An important model system for our studies is FXIII V34L, a common polymorphism correlated with cardioprotective effects. Our first kinetic and NMR work with peptides based on FXIII AP (28-41) helped to explain why the medically interesting FXIII V34L is more easily activated than the V34 enzyme *(1, 2)*. The V34L peptide binds more effectively to the thrombin surface and the conversion to cleaved product is more efficient. Additional peptides with V34X substitutions have been examined to assess which sequences generate activation peptides that can be cleaved more or less readily by IIa. There may be instances where a quick release of activated FXIII is desired whereas, in other cases, there may be a need to develop an extensive clot and then bring in activated FXIII. Structural studies have revealed that FXIII peptides containing the amino acids L34 or F34 exhibit critical interactions with P36 thus promoting more extensive binding to the thrombin active site surface. By contrast, I34, A34, and P34 are not conducive for such properties *(3, 4)*. Further studies indicate that thrombin activity is unaltered by N-terminal truncation of the FXIII AP segment *(5)*. Additional kinetic studies and surface plasmon resonance measurements have been used to characterize interactions among the ternary players thrombin, FXIII, and fibrin(ogen) *(6)*. We have also evaluated the influences of introducing selective features of fibrinogen Aα into the FXIII AP sequence *(7)*. In another series of projects, we have examined the roles that several thrombin residues play in binding and promoting cleavage of the FXIII activation peptides. Of particular note are the thrombin mutants W215A and W215A/E217A that are being screened in anticoagulation therapies by other researchers. Distinct effects on binding and/or hydrolysis rates have been observed for FXIII AP V34, L34, P34, and F34 *(8,9)*. One of these mutants FXIII AP F34 is an intriguing candidate for generating a transglutaminase that could function in the presence of anticoagulant thrombin W215A/E217A. The new knowledge gained may be used to design FXIII enzymes whose ability to be activated can be controlled. Such potentially therapeutic FXIII species could function in particular clot environments.

We have also been monitoring **the conformational changes that occur to FXIII under different activation conditions using amide proton hydrogen/deuterium exchange (HDX), chemical modifications, and limited proteolysis.** Results from all three solution-based measurements are detected by MALDI-TOF mass spectrometry. Segments of the β-sandwich domain, the catalytic core domain, and β-barrels 1 and 2 have been observed to undergo changes in exposure to solvent upon activation *(10, 11)*. The subtle local and long range conformational effects are proposed to occur in preparation for substrate binding. This research was extended to examine the additional structural changes that occur when FXIIIa is inhibited at the active site with either the small molecule iodoacetamide or the K9 DON peptide *(12)*. Most recently, we have probed the conformational influences of different cations *(13)* and examined FXIII A_2 to B_2 interactions. With the increased use of FXIII for therapeutic purposes, there is a need to better understand the

conformational changes that this transglutaminase undergoes in the presence of different solution environments. Moreover, there are no reported X-ray crystal structures yet for a FXIII-inhibitor complex.

In a third research project, we have focused on elucidating **the kinetic and structural features associated with productive binding of glutamine substrates to FXIIIa.** Our studies have revealed that reactive glutamine-containing peptides encounter a distinctive FXIIIa active site region and that these peptides bind in an extended conformation *(14)*. Further characterization of individual substrate amino acids has revealed a site beyond the FXIIIa catalytic site that may be important for specificity *(15)*. In addition, solutions conditions can be manipulated to either promote the cross-linking reaction or promote conversion of the glutamine to a non-reactive glutamic acid *(15)*. We have also been probing the role of the Q4 residue of α_2-antiplasmin, an enzyme that becomes cross-linked into fibrin by FXIIIa and limits blood clot breakdown. Kinetic studies based on α_2 antiplasmin (1-15, Q4X) reveal that the Q4 position influences binding to FXIIIa and turnover into product *(16)*. To further define the basis behind FXIII specificity, research continues with sequences derived from TAFI, Fibrinogen, and the *S. Aureus* fibronectin binding A protein.

In addition to examining the role of thrombin in activating FXIII, we have also been exploring other functions for this serine protease. **By cleaving the protease-activated receptors (PAR), thrombin plays a role in initiating platelet aggregation/secretion and inflammation**. Kinetic and NMR measurements with peptide models have demonstrated that PAR4 optimizes its interactions with the thrombin active site surface and does not take advantage of the distant thrombin anion binding exosite-I *(17)*. Our solution NMR data on the thrombin-bound structure of PAR4 (38-47) have been confirmed by X-ray crystallography. Interestingly, **bradykinin has been reported to inhibit thrombin** functions associated with platelet activation and cellular permeability effects. Our studies have proven that bradykinin serves as a competitive inhibitor of this serine protease *(18)* and functions at high local levels or requires the presence of a cofactor.

A final project has involved characterizing **the interactions between thrombin and the ligands fibrinogen γ′ chain, GpIbα, hirudin, PAR3, and PAR1.** Previous studies suggested that the γ′ chain targets thrombin anion binding exosite-II (ABE-II), the same site utilized by the anticoagulant heparin. NMR studies have revealed that a β-turn exists between γ′ residues (422-425) in the presence of thrombin *(19)*. HDX results have then revealed that the C-terminal γ′ peptide (410-427) targets thrombin residues at or near ABE-II. γ′ peptide binding also affects the deuterium exchange properties of distant thrombin sites thus establishing potential lines of communication across the thrombin surface *(19)*. A similar series of NMR and HDX mass spectrometry studies has been carried out with segments of GpIbα, a glycoprotein found on platelet surfaces *(20)*. GpIbα (269-286) has been shown to bind to thrombin in an extended conformation. The GpIbα peptide (269-286) and the larger domain (1-290) alter the HDX dynamics of thrombin *(20)* in a manner analogous to the γ′ (410-427). Efforts have continued with peptides based on hirudin (leech anticoagulant), PAR3, and PAR1 binding to thrombin ABE-I. Interestingly, the ability to promote local and long-range effects appears to be ligand dependent. Each ligand exerts its predominant influences on thrombin and also allows interexosite communication *(21)*. The results obtained support the proposal that thrombin is a highly dynamic protein. The knowledge gained from these NMR and HDX mass spectrometry approaches may be used to design better drug inhibitors of thrombin.

Our kinetic measurements, solution NMR studies, and MALDI-TOF mass spectrometry projects form a series of complementary approaches for elucidating the activation and substrate/ligand binding properties of Factor XIII(a) and thrombin. Research efforts are moving toward examining more intact physiological proteins and their selective mutants. While very important for wound healing, blood clots can also increase risk for heart disease, stroke, and arteriosclerosis. A greater understanding is needed on how the coagulation related enzymes are activated and how they select their targets. Further knowledge may lead to novel medical strategies to control the actions of these critical proteins and the resultant fibrin clot architecture.

Articles Cited

1. T.A. Trumbo, M.C. Maurer (2000) "Examining Thrombin Hydrolysis of the Factor XIII Activation Peptide Segment Leads to a Proposal for Explaining the Cardioprotective Effects Observed With the FactorXIII V34L Mutation" *J. Biol. Chem. 275*, 20627-20631.
2. T.A. Trumbo, M.C. Maurer (2002) "Thrombin Hydrolysis of V29F and V34L Mutants of Factor XIII (28 41) Reveals Roles of the P_9 and P_4 Positions in Factor XIII Activation" *Biochemistry 41*, 2859-68.
3. T.A. Trumbo, M.C. Maurer (2003) "V34I and V34A Substitutions Within the Factor XIII Activation Peptide Segment (28-41) Affect Interactions With the Thrombin Active Site." *Thrombosis and Haemostasis. 89*, 647-53.
4. G. Isetti, M.C. Maurer (2004) "Probing Thrombin's Ability to Accommodate a V34F Substitution Within the Factor XIII Activation Peptide Segment (28-41)" *J. Peptide Research 63*, 241-252.
5. G. Isetti, M.C. Maurer (2004) "Thrombin Activity is Unaltered by N-terminal Truncation of Factor XIII Activation Peptides" *Biochemistry 43*, 4150-4159.
6. M.C. Maurer, T.A. Trumbo, G. Isetti, B.T. Turner Jr. (2006) "Probing Interactions Between the Coagulants Thrombin, Factor XIII, and Fibrin(ogen)" *Archives of Biochemistry and Biophysics 445*, 36 45.
7. M.A. Jadhav, G. Isetti, T.A. Trumbo, M.C. Maurer (2010) "Effects of Introducing Fibrinogen Aα Character into the Factor XIII Activation Peptide Segment" *Biochemistry 49*, 2918-24.
8. G. Isetti, M.C. Maurer (2007) "Employing Mutants to Study Thrombin Residues Responsible for Factor XIII Activation Peptide Recognition: A Kinetic Study" *Biochemistry 46*, 2444-2452.
9. M.A. Jadhav, R.C. Lucas, W.N. Goldsberry, M.C. Maurer (2011) "Design of Factor XIII V34X Activation Peptides to Control Ability to Interact with Thrombin Mutants" *BBA Proteins and Proteomics 1814*, 1955-1963..
10. B.T. Turner, M.C. Maurer (2002) "Evaluating the Roles of Thrombin and Calcium in Activation of Coagulation Factor XIII Using H/D Exchange and MALDI-TOF MS" *Biochemistry 41*, 7947 7954.
11. B.T. Turner Jr., T.M. Sabo, D. Wilding, M.C. Maurer (2004) "Mapping of Factor XIII Solvent Accessibility as a Function of Activation State Using Chemical Modification Methods" *Biochemistry 43*, 9755-65.
12. T.M. Sabo, P.B. Brasher, M.C. Maurer (2007) "Perturbations in Factor XIII Resulting From Activation and Inhibition Conditions Examined by Solution Based Methods and Detected by MALDI-TOF MS" *Biochemistry 46*, 10089-10101.
13. R.T. Woofter, Maurer, M.C. (2011) "Role of Calcium in the Conformational Dynamics of Factor XIII Activation Examined by Hydrogen-Deuterium Exchange Coupled with MALDI-TOF MS" *Arch. Biophys. Biochem. 512*, 87-95.
14. A. Marinescu, D.B. Cleary, T.R. Littlefield, M.C. Maurer (2002) "Structural Features Associated Withthe Binding of Glutamine-Containing Peptides to Factor XIII" *Archives of Biochemistry and Biophysics 406*, 9-20.
15. D.B. Cleary, M.C. Maurer (2006) "Characterizing the Specificity of Activated Factor XIII for Glutamine Containing Substrate Peptides" *Biochim. Biophys. Acta 1764*, 1207-1217
16. D.B. Cleary, P. Doiphode, M.C. Maurer (2009) "A Non-Reactive Glutamine Residue of α_2-Antiplasmin Promotes Interactions With the Factor XIIIa Active Site Region" *J. Thromb. Haemost, 7*, 1947-1949.
17. D.B. Cleary, T.A. Trumbo, M.C. Maurer (2002) "PAR4-like Peptides Bind to Thrombin Through an Optimized Interaction With the Enzyme Active Site Surface" *Archives of Biochemistry and Biophysics 403*, 179-188.
18. D.B. Cleary, W.D. Erhinger**,** M.C. Maurer (2003) "Establishing the Inhibitory Effects of Bradykinin on Thrombin" *Archives of Biochemistry and. Biophysics. 410*, 96-106.
19. T.M. Sabo, D.H. Farrell, M.C. Maurer (2006) "Conformational Analysis of γ′ Peptide (410-427) Interactions With Thrombin Anion Binding Exosite II" *Biochemistry 45*, 7434-45.
20. T.M. Sabo, M.C. Maurer (2009) "Biophysical Investigation of GpIbα Binding to Thrombin Anion Binding Exosite II" *Biochemistry 48*, 7110-7122.
21. M.V. Malovichko, T.M. Sabo, M.C. Maurer (2013) "Ligand Binding to Anion-Binding Exosites Regulates Conformational Properties of Thrombin" *J. Biol. Chem 288*, 8667-78.