Fibrinogen and Fibrinolysis

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Fibrinogen is a rod shaped beta-globulin glycoprotein produced predominantly in hepatocytes.
Schematic Model of Native Fibrinogen

Fibrinogen consists of three pairs of non-identical polypeptide chains: $\alpha$, $\beta$, and $\gamma$. 
Fibrinogen is a symmetric dimeric structure consisting of three pairs of non-identical polypeptide chains Aα, Bβ and γ.
The amino acid terminal regions of all three chains form the central E-domain. The central E-domain is 15% of the mass of native fibrinogen.
The carboxyl terminal regions of all three chains form the two D-domains. The two D-domains account for 50% of the mass of native fibrinogen.
There is a HMW and a LMW type of fibrinogen in plasma: HMW is most abundant (70%) and has high affinity calcium binding sites. LMW (30%) resulting from loss of C-terminal of one A-alpha chain, is slower to polymerize.
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<th>POSITIVE ACUTE PHASE REACTANTS</th>
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Changes in fibrinogen quantity and quality

Fibrinogen is an acute phase reactant and its production is increased by glucocorticoids and cytokine IL-6 and interferon gamma gamma INF-
Fibrinogen Gene Region
Interactions with nuclear proteins and interleukin 6 govern fibrinogen acute phase response and plasma levels.
Fibrinogen Gene Region
B-beta gene polymorphisms interactions with nuclear proteins and interleukin 6 provoke inappropriate fibrinogen acute phase response and elevated fibrinogen levels.
Factors that influence fibrinogen quality and quantity

- Beta gene polymorphisms provoke lower threshold acute phase reactions
- Acute phase reaction increases degree of phosphorylation from 24% to 60% in 24 hours and also elevates fibrinogen concentration
- Acute phase fibrinogen is cleaved at a faster rate by thrombin (old fibrinogen is less phosphorylated)
High-affinity calcium binding sites are localized to \( \gamma \)-chain residues 311-336. Calcium contributes to the structural integrity of fibrinogen and has a protective action against plasmin degradation.
Numerous normal and abnormal conditions produce fibrinogen variants by altering:

- Ratio of HMW fibrinogen (340,000 KD) to LMW fibrinogen (300,000 KD)
- Number of fibrinogen molecules with high affinity Ca$^{+}$ binding sites
- Degree of phosphorylation
- Degree of sulfation
- Sialic acid content
Acquired Altered fibrin(ogen) (variant fibrinogen)

- Acquired normal variant (fetal fibrinogen, acute phase reaction fibrinogen)
- Acquired abnormal variant fibrinogen (liver disease, hepatoma, hepatitis C, chronic and acute alcoholisms, drugs)
Acquired changes in fibrin(ogen) quality and quantity alter:

- Rate of conversion to fibrin
- Polymerization rate
- Blood viscosity
- Degradation by plasmin
Fibrinogen: Contribution to
1. Blood fluidity
2. Bleeding
3. Thrombosis

How does fibrinogen maintain blood fluidity
Section I How does fibrinogen maintain blood fluidity

fibrinogen maintains blood fluid by:

1. Fibrinogen concentration
1. Fibrinogen blood concentration maintains blood fluidity

Fibrinogen concentration (normal range 2-4 grams/Liter) is critical in maintaining blood fluid because elevated fibrinogen levels increase blood viscosity, an identified risk factor for thrombosis.
Fibrinogen blood concentration

Fibrinogen is an acute phase reactant and multiple factors influence blood fibrinogen concentrations.

Fibrinogen blood concentration is a major contributor to blood viscosity.
Blood viscosity following initiation of ancrod therapy.
Ancrod is derived from the venom of a snake.

Malaysian Pit Viper
Calloselasma rhodostoma
Ancrod mode of action

- Ancrod → FPA
- Plasminogen
- Soluble fibrin complexes → t-PA
- PAI-1
- Decrease blood viscosity → Plasmin
Polymorphisms in B-beta gene promoter region

- Cause chronically elevated fibrinogen levels that are an exaggerated response to IL-6 and nuclear proteins.
- Indirectly increase blood viscosity
- Increased blood viscosity contributes to intra vascular clotting by altering blood rheology.
Section I How does fibrinogen maintain blood fluidity

fibrinogen maintains blood fluid by:

2. High negative charge on Fibrinopeptides A and B keeps fibrinogen molecules apart and prevents intramolecular interactions (polymerization)
2. Fibrinopeptides A and B maintain blood fluidity by:

- High negative charge on Fibrinopeptides A and B.
- Negative charge keeps fibrinogen molecules apart by preventing intra-molecular interactions (polymerization)
Degree of sulfation and sialination change from adult to fetal life and may vary with disease

• **Sulfation**: Sulfate groups provide the acidic properties of the Fibrinopeptides and confer a protective effect against polymerization.

• Sulfate groups and sialic acid provide the acidic properties of fibrinogen
Non-inherited regional fibrinogen variants

• **Phosphorylation:** In the fetus and during acute phase reactions 70% of the human Aα-chain is phosphorylated at 2 serine residues. This is a marker for acute phase reactions.
Three types of non-inherited fibrinogen variants related to a degree of sulfate groups provide the acidic properties of FPA and FPB. Sialic acid is increased in liver disease. Acidic charge of fibrin results in a delay in the rate of fibrinogen and fibrin polymerization.
Some disease states alter negative charge

- Altered fibrinogen molecules stick together (polymerize) and occlude blood vessels slowing or preventing the flow of blood. This is not thrombosis. It is similar to vascular occlusion by sickle cells. Thrombosis invariably follows untreated vascular occlusion.
Fibrinogen: Contribution to

- Fibrinogen and bleeding
Section II: Fibrinogen and Bleeding

Inherited diseases:

- Afibrinogenemia or total absence of fibrinogen, is a homozygous recessive disorder (1/1,000,000)
- Severe hypofibrinogenemia
- Dysfibrinogenemia
Fibrinogen is the fibrin precursor

- Thrombin proteolysis generates fibrin by removal of fibrinopeptides A and B from fibrinogen
Fibrinopeptides A and B account for 1% of the mass of native fibrinogen.
Impaired Cleavage of FPA and FPB by Thrombin

Mutation $\alpha$-Arg 16 is highly specific for thrombin cleavage of FPA. Mutation at this site results in delayed FPA release and an inherited bleeding disorder.
Inherited Bleeding Disorders Associated with Delayed Thrombin Binding

Mutation of $\alpha$-Glu$\Downarrow$Gly and $\alpha$-Gly$\Downarrow$Val is associated with defective thrombin binding and delayed FPA release.
Proteolytic Degradation

• Affecting Aα- and γ-chains: This occurs in blood of normal and healthy individuals, in liver disease, and some infections. Fibrin clots derived from degraded fibrinogen are less stable.

• Affecting γ-chains: C-terminally degraded γ-chains are shorter and are unable to crosslink or to interact with platelets. Affected individuals have prolonged bleeding times and normal platelet function tests. Bleeding phenotype manifests with trauma or surgery.
Fibrinogen mediates platelet aggregation
Korean child with congenital vascular malformation. Attempt at surgical removal triggered in low grade DIC.
Fibrin Assembly and cross linking

- Fibrin assembly and factors that influence fibrin assembly
Thrombin cleaves Fibrinopeptides A and B from fibrinogen to form fibrin.
Fibrin I: non-covalent interaction between E- and D-domains

This type of fibrin is extremely susceptible to plasmin degradation.
Fibrin Assembly: Influence of Sialic Acid

The amount of sialic acid influences the rate of fibrin polymerization; an increase in acidic charge delays polymerization.
Non-inherited regional fibrinogen variants that delay the rate of fibrin polymerization

• **Phosphorylation**: In the fetus and during acute phase reactions 70% of the human Aα-chain is phosphorylated at 2 serine residues.

• **Glycosylation**: In liver disease, an increase in sialic acid increases the acidic charge of fibrin and delays the rate of fibrin polymerization.
Fibrin Assembly and cross linking

- Factor XIII
Factor XIII

- Thrombin releases the fibrin cross-linking activity by cleavage of a peptide bond in the presence of fibrinogen,
  - Fibrinogen lowers the concentration of thrombin required for cleavage of Factor XIII in vitro.
- Much of FXIII circulates in blood in association with fibrinogen. Concentration in plasma is 70nM. It has a half-life of 9-14 days.
Factor XIII, the precursor of a transglutaminase enzyme is activated by thrombin.
Factor XIII

- Factor XIII is a blood coagulation protein distributed both extracellularly (in plasma) and intracellularly (in megakaryocytes, platelets and placenta).
- It is a heterotetramer consisting of 2 identical proenzyme subunits \( (A_2) \) and 2 identical carrier protein subunits \( (B_2) \).
Fibrin Assembly and cross linking

- Fibrin cross linking by factor XIIIa
Cross-linking Mechanisms

Fibrin undergoes factor XIII-mediated crosslinking by formation of epsilon gamma glutamyl-lysyl bonds mainly between $\gamma$ and $\alpha$ chains. Crosslinked fibrin clots are slowly degraded by plasmin. It takes one to three months for complete breakdown by plasmin of intravascular clots.
Factor XIII Contribution to bleeding

- Fibrin that is not cross linked is highly susceptible to plasmin degradation
- Individuals deficient of factor XIII bleed because of rapid clot dissolution
- Treatment of bleeding in factor XIII deficiency is directed at preventing clot lysis
Fibrin I: non-covalent interaction between E- and D-domains
This type of fibrin is extremely susceptible to plasmin degradation.
Severe factor XIII deficiency

- Factor XIII concentrates are used to treat most severely deficient patients.
Factor XIII and thrombosis

- Newly recognized factor XIII polymorphisms very close to the thrombin cleavage site on the A- subunit enhance the rate of factor XIII activation by thrombin, resulting in the rapid cross-linking of a fibrin that is highly resistant to plasmin.
Fibrinolysis: Contribution to
1. Blood fluidity 2. Bleeding
3. Thrombosis

- How does fibrinolysis maintain blood fluidity
- Fibrinolysis association with bleeding
- Fibrinolysis and thrombosis
Fibrinolysis Mechanisms

Plasminogen, t-PA, and α-2-antiplasmin attach to fibrin by molecular interaction at lysine residues in the fibrinolysis proteins. Activation of t-PA by fibrin occurs in the α-chain. Activated t-PA cleaves plasminogen to plasmin on fibrin surfaces.
Fibrinolysis Mechanisms: Plasmin Inhibition by $\alpha$-2-antiplasmin

In blood, plasmin is rapidly inactivated by $\alpha$-2-AP with a plasmin half-life of 0.1 sec. Plasmin bound to fibrin is inactivated 50 times more slowly.
**Fibrinolysis Mechanisms**

When crosslinked fibrin undergoes fibrinolysis, the peptides joining D- and E- domains are cleaved, leading to fibrin split products.
Fibrin Split Products: Fragments D-dimer, D-trimer, and D-tetramer 

Of interest is whether D-dimer antibody-based assays also measure D-trimer and D-tetramer.
Calcium contributes to the integrity of fibrinogen and has a protective action against plasminogen degradation. Fibrinogen variants lacking in calcium binding sites have altered fibrin polymerization and manifest in excessive bleeding phenotype.
Fibrinolysis and bleeding

- Fibrinogen variants lacking high-affinity calcium binding sites have altered fibrin polymerization and manifest in excessive bleeding phenotypes.
Molecular Model of the Ternary Complex Between Plasmin, Staphylokinase, and Plasminogen
Schematic Model of Fibrin

Thrombin is incorporated into fibrin clots. Small amounts of thrombin (10-20nM) are present at the moment of clotting. Ten-fold concentrations of thrombin within the clot result from factor XI activation.
Fibrinolysis and thrombosis

One of the mechanisms by which thrombin is inactivated is by becoming incorporated into clots and thrombi.

Thrombolytics release the entrapped thrombin back into blood resulting in re-occlusion.

All thrombolytic protocols include concomitant heparin or a comparable anticoagulant to be administered.
Lipoprotein(a) Inhibits Proteolytic Degradation by Plasmin

• Lipoprotein(a) is an LDL-like lipoprotein that contains an additional protein apo(a). The apo(a) gene is highly homologous to that of plaminogen.

• Apoprotein(a) functions as an inhibitor of plasminogen activation, preventing plasmin from occurring. Linus Pauling postulates that by preventing plasmin from occurring, apoprotein(a) is an important determinant for the evolutionary advantage of lipoprotein(a).
Fibrin Assembly: Inherited Genetically Abnormal Variants

• Several abnormal fibrinogen variants that cannot bind t-PA are characterized by persistence of venous or arterial occlusion by thrombi.
• Several abnormal fibrinogen variants produce fibrin fibers that are thinner and highly branched and more compact than normal fibrin fibers. The compactness with reduced permeability of the fibrin clot makes it resistant to plasmin degradation.
Fibrin Assembly: Inherited Genetically Abnormal Variants

• There are 20 abnormal variants identified in the primary, complementary polymerization region in the C-Terminal part of the $\gamma$-chain. Delayed fibrin polymerization in such variants is associated with persistence of venous or arterial occlusion by thrombi.

• Several abnormal fibrinogen variants that cannot bind plasminogen are characterized by persistence of venous or arterial occlusion by thrombi.
**Exogenous Inhibitors of Plasmin-Induced Proteolysis are Synthetic Lysine Analogs that Bind Plasminogen Kringle 4.**

- Essential amino acid L-lysine
- Tranexamic acid and ε-aminocaproyc acid
Hemostasis is the clotting of blood to prevent exsanguination from accidental or surgical injury to a blood vessel.

Hemostasis is always a localized process.

Hemostasis produces a *hemostatic clot*.

Hemostatic clots do not form in circulating blood.
Changes in Blood Fluidity

Clotting reactions are triggered

- When blood fluidity is changed by endothelial injury or by
- Intravascular introduction of a foreign substance
The Clotting of Blood
Hemostatic clots and thrombosis develop by identical mechanisms that include:

1. Initiation of clotting by Tissue Factor and factor VII
2. Prothombin conversion to thrombin on phospholipid surfaces
3. Platelet activation
4. Fibrinopeptide A and B release from fibrinogen by thrombin
The Clotting of Blood
Hemostatic clots and thrombosis develop by identical mechanisms that include:

5. Polymerization of fibrin monomer
6. Cross-linking of fibrin by activated factor XIII
7. Entrapment of blood cells within the fibrin network
The Clotting of Blood
Step I Pre-clotting Reactions

Pre-clotting reactions:
1. Are initiated by tissue-factor+factor VII
2. Include removal of profragment FI+2 from prothrombin by factor Xa to produce thrombin
3. Include the conversion of fibrinogen to fibrin I by thrombin clipping fibrinopeptides A from the two symmetric A-alpha fibrinogen chains
The Clotting of Blood
Step II: Soluble Fibrin Processes

1. Fibrin II forms by thrombin removal of two Fibrinopeptides B from symmetric B beta chains of fibrinogen.

2. Polymerization sites in fibrin II are unmasked.

3. Fibrin II fibrils assemble (network) to produce a soluble clot that is extremely susceptible to plasmin degradation.
The Clotting of Blood
Step III: Formation of an Insoluble Clot

1. Fibrin II fibrils assemble at polymerization sites.
2. Factor XIII is activated by thrombin.
3. Fibrin II is cross linked at polymerization sites by activated factor XIII.
4. Cross-linked fibrin becomes resistant to plasmin.
The Clotting of Blood
Step IV: Tissue Repair and Wound Healing

1. Plasminogen is converted by tPA to plasmin within the clot or thrombus
2. Plasmin slowly degrades the cross linked fibrin network
3. Platelets aid tissue repair by release of platelet derived growth factors, serotonin
4. Entrapped thrombin stimulates fibroblast differentiation
Proteolytic Degradation by Plasmin is a Universal Mechanism
Proteolytic Degradation by Plasmin is a Universal Mechanism. Plasmin Proteolysis is Involved in:

• Degradation of fibrinogen (fibrinogenolysis) and fibrin (fibrinolysis)
• Activation of metalloproteinases
• Implantation of fertilized ova
• Beneficial physiological processes such as: cell migration and tissue remodeling
Proteolytic Degradation by Plasmin is a Universal Mechanism. Plasmin Proteolysis is Involved in:

- Pathological processes of vascular injury, atherosclerosis, graft arterial disease, myocardial ischemia, angiogenesis, tumor growth and dissemination of infection

- Chronic inflammatory disorders of the kidney, lung, gastrointestinal tract, skin, joints, or cornea