Identification of Fibrin Clot-Bound Plasma Proteins

Simone Talens1, Frank W. G. Leebeek1, Jeroen A. A. Demmers2, Dingeman C. Rijken1*

1 Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands, 2 Proteomics Center, Erasmus University Medical Center, Rotterdam, The Netherlands

Abstract
Several proteins are known to bind to a fibrin network and to change clot properties or function. In this study we aimed to get an overview of fibrin clot-bound plasma proteins. A plasma clot was formed by adding thrombin, CaCl₂ and aprotinin to citrated platelet-poor plasma and unbound proteins were washed away with Tris-buffered saline. Non-covalently bound proteins were extracted, separated with 2D gel electrophoresis and visualized with Sypro Ruby. Excised protein spots were analyzed with mass spectrometry. The identity of the proteins was verified by checking the mass of the protein, and, if necessary, by Western blot analysis. Next to established fibrin-binding proteins we identified several novel fibrin clot-bound plasma proteins, including α₂-macroglobulin, carboxypeptidase N, α₁-antitrypsin, haptoglobin, serum amyloid P, and the apolipoproteins A-I, E, J, and A-IV. The latter six proteins are associated with high-density lipoprotein particles. In addition we showed that high-density lipoprotein associated proteins were also present in fibrinogen preparations purified from plasma. Most plasma proteins in a fibrin clot can be classified into three groups according to either blood coagulation, protease inhibition or high-density lipoprotein metabolism. The presence of high-density lipoprotein in clots might point to a role in hemostasis.

Introduction
Arterial and venous thrombosis are major causes of morbidity and mortality in the Western world. These thrombotic disorders are considered as separate diseases, with different pathology, pathophysiology, epidemiology and treatments. However, there is evidence that suggests that there is an association between venous and arterial thrombosis [1]. Multiple genetic and acquired risk factors contribute to the development of thrombosis. Some risk factors for arterial thrombosis may also play a role in venous thrombosis and the other way around [2,3]. Although there are several risk factors known, there are still a number of patients without a known thrombotic risk factor [4,5]. Moreover, the site specificity of thrombosis is poorly understood [6]. Identification of novel players in hemostasis can help in determining new risk factors and additionally in understanding the pathogenesis of thrombotic disorders.

Elevated fibrinogen is a risk factor for both arterial and venous thrombosis [4,7]. Several proteins are known to bind to fibrin and to change clot properties or clot function via effects on fibrin formation and degradation [8]. For example, the main enzyme in fibrinolysis, plasmin, is formed by activation of the zymogen plasminogen by tissue plasminogen activator (t-PA). The interactions of t-PA and plasminogen with fibrin accelerating plasminogen activation [9]. Lipoprotein(a) (Lp(a)) has structural similarities to fibrin, which is seen in patients with fibrinogen Naples I, is associated with thrombosis [13]. There are several other proteins that are known to bind to fibrin including α₂-antiplasmin, plasminogen activator inhibitor-2 (PAI-2), hepatocyte-derived fibrinogen-related protein-1 (HFREP-1), albumin, fibroblast growth factor-2, vascular endothelial growth factor, interleukin-1β, activated factor X, tissue factor pathway inhibitor, thrombin-activatable fibrinolysis inhibitor (TAFI), von Willebrand factor, thrombopsonadin, actin, factor V and factor XIII (FXIII) [14–21]. Some of these proteins are cross-linked to fibrin by FXIIIa, e.g. α₂-antiplasmin, fibronectin, PAI-2, TAFI, von Willebrand factor, thrombopsonadin, actin and factor V.

In this study we aim to establish the protein composition of fibrin clots made from plasma. Changes in the protein composition can influence clot formation and breakdown and may therefore play a role in arterial and venous thrombosis. We identified 18 fibrin clot-bound plasma proteins by 2D gel electrophoresis followed by mass spectrometry. Nine of them were novel plasma clot components of which six proteins are associated with high-density lipoprotein (HDL).

Materials and Methods

Materials
Urea, thiourea, CHAPS, dithiothreitol (DTT) and iodoacetic acid were obtained from Fluka (St. Louis, MO, USA). Aprotinin (TrasyloL) was obtained from Bayer (Leverkusen, Germany). Tris
Ruby was obtained from Invitrogen (Paisley, UK) and 0.45 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trypsin apparatus were from Bruker Daltonics (Bremen, Germany). The goat polyclonal IgG to human nitrocellulose transfer membrane from Whatman (Dassel, Germany). Human fibrinogen (plasminogen, von Willebrand factor and 2-macroglobulin; the goat polyclonal IgG to human apolipoprotein J was from Abgent (Cambridge, UK). The rabbit polyclonal IgG to human apolipoprotein A-I was from Calbiochem (Darmstadt, Germany) and the goat polyclonal IgG to human apolipoprotein J was from Abgent (San Diego, CA, USA). The Odyssey apparatus and IRDye® 800 CW secondary donkey-anti-goat and goat-anti-rabbit antibodies were obtained from Li-Cor Bioscience (Lincoln, NE, USA). Human fibrinogen (plasminogen, von Willebrand factor and fibronectin depleted) was obtained from Enzyme Research Laboratories (South Bend, IN, USA).

Plasma Clot Preparation

In vitro clots of 500 µl citrated platelet-poor plasma (pool from 10 healthy volunteers, Sanquin, location Leiden, the Netherlands) were prepared by adding calcium chloride (20 mM), thrombin (1 NIH U/ml) and aprotinin (100 KIU/ml) [15]. After 2 hours of incubation at room temperature, the clots were extensively washed by perfusing them with 10 ml Tris-buffered saline (50 mM Tris-HCl, 100 mM NaCl, pH 7.4) containing aprotinin (100 KIU/ml) at 4°C. Where indicated, the NaCl concentration was increased to 0.5 M. The clots were compacted by centrifugation, washed with deionized water and non-covalently clot-bound proteins were extracted with 150 µl rehydration buffer (7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.5% (v/v) IPG 3–10 buffer) for 1 hour at room temperature. For optimal 2D gel electrophoresis 1% (v/v) DeStreak was added to the extract.

2D Gel Electrophoresis

Plasma clot extract was separated with 2D gel electrophoresis. The proteins in the 150 µl extract were separated in the first dimension with a 11 cm immobiline drystrip with a 3–10 NL pH range by isoelectric focusing on the IPGphor with the following running protocol: 30 V for 12 hours (rehydration), 1000 V for 4 hours (gradient), 8000 V for 5 hours (step-n-hold), with a 50 µA limit per gel. After isoelectric focusing the gel strip was equilibrated in buffer (6 M urea, 50 mM Tris-HCl pH 8.8, 20% (v/v) glycerol, 2% (w/v) SDS) with 1% (v/v) DTT for 15 minutes followed by a second equilibration step with equilibration buffer with 1% (w/v) iodoacetamide for 15 minutes. For the second dimension the gel strip was laid on a 12% Bis-Tris gel and run for 1 h at 200 V constant, using the XT MOPS buffer as running buffer. The proteins in the gel were visualized by Sypro Ruby staining according to manufacturer’s instructions and scanned on a Typhoon Trio at an excitation wavelength of 532 nm and an emission wavelength of 610 nm.

Mass Spectrometry Analysis

The highly abundant proteins were analyzed with Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-ToF). Therefore proteins spots were excised with Spot Picker using a 2 mm picker head and destained in 30% (v/v) acetonitrile (ACN)/30 mM NH₄HCO₃. Destained gel pieces were vacuum-dried and rehydrated in 4 µl trypsin digest solution (75 µg/ml Trypsin Gold in 20 mM NH₄HCO₃, pH 8.0) for digestion overnight at room temperature. Peptide extraction was performed with 5 µl of 50% ACN/0.1% trifluoroacetic acid. The extracted sample was spotted on an anchoring plate with saturated α-cyano-4-hydroxycinnamic acid matrix solution in 100% ACN (1:1). Digested peptide fragments were analyzed in a MALDI-ToF mass spectrometer using an Ultraflex-II apparatus. Flexanalysis 2.4 and BioTools 3.1 software were used for data processing. The mass spectra obtained were analyzed using peptide mass fingerprint spectra with the online Matrix Science Database with MASCOT software (www.matrixscience.com). The NCBI nr database 20100624 (11299630 sequences; 3855426203 residues) was searched with the Mascot parameters set as follows: Taxonomy, Homo sapiens; mass tolerance, 100 ppm; maximally one missed cleavage per peptide; fixed modification of carboxymethylation of cysteine residues; variable modification of partial oxidation of methionine residues. Mowse scores above NCBI nr database threshold of 66 were considered significant (p<0.05).

For the less abundant proteins mass spectrometry analysis was done with nanoflow LC-MS/MS. Picked gel spots were subjected to in-gel reduction with DTT, alkylation with iodoacetamide and digestion with Trypsin Gold, essentially as described by Wilm et al. [22]. Nanoflow LC-MS/MS was performed on an 1100 series capillary LC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) operating in positive mode and equipped with a nanospray source. Peptide mixtures were trapped on a ReproSil C18 reversed phase column (Dr Maisch GmbH, Ammerbuch-Entringen, Germany; column dimensions 1.5 cm×100 µm, packed in-house) at a flow rate of 8 µl/min. Peptide separation was performed on ReproSil C18 reversed phase column (Dr Maisch GmbH, Ammerbuch-Entringen, Germany; column dimensions 15 cm×500 µm, packed in-house) using a linear gradient from 0 to 80% B (A = 0.1% formic acid; B = 80% (v/v) acetonitrile, 0.1% formic acid) in 70 min and at a constant flow rate of 200 nl/min using a splitter. The column eluent was directly sprayed into the ESI source of the mass spectrometer. Mass spectra were acquired in continuum mode; fragmentation of the peptides was performed in data-dependent mode. Peak lists were automatically created from raw data files using the Mascot Distiller software (version 2.1; MatrixScience). The Mascot search algorithm (version 2.2, MatrixScience) was used for searching against the NCBI nr database (release NCBI nr_20090808.fasta; taxonomy: Homo sapiens). The peptide tolerance was typically set to 2 Da and the fragment ion tolerance was set to 0.8 Da. A maximum number of 2 missed cleavages by trypsin were allowed and carbamidomethylated cysteine and oxidized methionine were set as fixed and variable modifications, respectively. The Mowse score cut-off value for a positive protein hit was set to 60. For identification as fibrin clot-binding protein the cut-off value for the emPAI score [23] was set at 0.15. Individual peptide MS/MS spectra with Mascot scores below 40 were checked manually and either interpreted as valid identifications or discarded.

Fibrinogen Purification from Plasma

Fibrinogen was purified from barium-adsorbed citrated plasma with immunoaffinity chromatography according to Takebe et al. [24] with some changes. In short, IF-1 antibody was conjugated to CNBr-activated Sepharose 4B according to manufacturer’s...
antitrypsin (A), apolipoprotein J (B) and apolipoprotein A-I (C) are indicated by white ellipses. gel and analyzed with mass spectrometry. B) The trains of spots that resemble the same protein are indicated by white ellipses. They include: fibronectin (I), \( \gamma_2 \)-macroglobulin (II, III and VIII), plasminogen (IV), FXIII A chain (V), albumin (VI), \( \gamma_1 \)-antitrypsin (VII), apolipoprotein J (IX), apolipoprotein E, HFREP-1 (X) and apolipoprotein A-I (XI). C) A zoomed image of the 2D gel with a lower fluorescent signal. The isoforms of \( \gamma_1 \)-antitrypsin (A), apolipoprotein J (B) and apolipoprotein A-I (C) are indicated by white ellipses.

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Western Blot Analysis

To detect \( \gamma_2 \)-macroglobulin, a clot extract was made and separated by 2D gel electrophoresis as described above. The proteins were transferred from the 12% Bis-Tris precast gel to a nitrocellulose membrane by semi-dry blotting at 0.33 mA constant for 1 hour. The membrane was incubated with block buffer (PBS, 1% BSA, pH 7.4). After the blocking step the membrane was first incubated with the \( \gamma_2 \)-macroglobulin antibody (20 \( \mu \)g/ml) diluted in block buffer containing 0.1% Tween 20 and then with the secondary antibody IRDye\textsuperscript{800} CW donkey-anti-goat diluted 10,000 times in 5% milk and 0.1% Tween 20 in PBS, pH 7.4. All incubation steps were performed for 1 hour at room temperature. To visualize the protein, the membrane was scanned on an Odyssey scanner.

To detect fibrinogen-bound proteins, fibrinogen in the fractions of the IF-I column experiment described above and fibrinogen from Enzyme Research Laboratories were analyzed with SDS-PAGE. Reduced samples of 30 \( \mu \)g fibrinogen were run on a 12% Bis-Tris precast gel with XT MES buffer for 1 h at 200 V constant and analyzed with Western blotting as described above. The different apolipoproteins were detected by using the specific apolipoprotein A-I antibody (1000 times diluted), apolipoprotein J antibody (0.5 \( \mu \)g/ml) and apolipoprotein A-II antibody (1 \( \mu \)g/ml).

The amounts of apolipoprotein A-I and apolipoprotein B present in plasma clot extracts were estimated using Western blot analysis. For quantification of apolipoprotein A-I a clot extract and different concentrations of purified apolipoprotein A-I were run on a Tris-HCl gel (15%) and for apolipoprotein B quantification a clot extract and different concentrations of purified low-density lipoprotein (LDL) were run on a Tris-HCl gel (5%). LDL was purified according to Redgrave et al. [25]. Calibration curves were made of the different concentrations of apolipoprotein A-I and apolipoprotein B which were used to estimate the amount of apolipoprotein in the plasma clot extract. Western blot analysis was done as described above using a specific apolipoprotein A-I antibody (1000 times diluted) and a specific apolipoprotein B antibody (1 \( \mu \)g/ml).

Results

To investigate the protein composition of a fibrin clot, in vitro plasma clots were made by adding CaCl\textsubscript{2}, thrombin and aprotinin to platelet-poor citrated plasma. Unbound proteins were washed away and non-covalently bound proteins were extracted, separated with 2D gel electrophoresis and visualized with Sypro Ruby (Figure 1A). Spots that were identified using mass spectrometry were reproducibly detected in at least 7 out of 10 2D gels. The high-abundant protein spots (spots 7, 8 and 15) were analyzed with MALDI-ToF mass spectrometry and the other protein spots were analyzed with nanoflow LC-MS/MS. This resulted in the identification of 18 different proteins that were present in a plasma clot. Detailed information from Mascot analysis is shown in table 1. Several of the proteins identified were not previously described as plasma clot proteins including \( \gamma_2 \)-macroglobulin, carboxypeptidase N (CPN), \( \gamma_1 \)-antitrypsin, haptoglobin, serum amyloid P and the apolipoproteins A-I, A-IV, E and J. The latter six proteins are associated with the HDL particle. In addition we identified proteins that have previously been described as fibrin clot-bound proteins, including fibronectin, plasminogen, factor XIII, HFREP-1, actin and thrombin.

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# Table 1. Mascot analysis of fibrin clot-bound proteins.

| Spot | Accession # | Description | Mowse score | Seq. cov. (%) | Mw | Tot. pept. | Uniq. pept. | pI |
|------|-------------|-------------|-------------|--------------|----|-----------|------------|----|
| 1    | gi|16933542 | Fibronectin | 2025         | 20   | 262656    | 30         | 5.49 |
| 2    | gi|46812315  | α₂-macroglobulin | 2206       | 29   | 167505    | 33         | 6.06 |
| 3    | gi|46812315  | α₂-macroglobulin | 1996       | 24   | 167505    | 29         | 6.06 |
| 4    | gi|190026    | Plasminogen | 2240        | 49   | 93233     | 34         | 7.04 |
| 5    | gi|51173528  | carboxypeptidase N, polypeptide 2 | 864        | 29   | 61433     | 13         | 5.72 |
| 6    | gi|119395709 | coagulation factor XIII, A1 polypeptide | 864        | 21   | 83267     | 15         | 5.75 |
| 7    | gi|33451     | immunoglobulin heavy constant mu | 555        | 21   | 51506     | 9          | 5.92 |
| 8    | gi|4502027   | albumin (*) | 280         | 54   | 71317     | 25         | 5.92 |
| 9    | gi|15080499  | α₁-antitrypsin (*) | 162        | 45   | 46864     | 13         | 5.36 |
| 10   | gi|113584    | immunoglobulin heavy constant alpha 1 | 282        | 13   | 38486     | 4          | 6.08 |
| 11   | gi|45030111  | carboxypeptidase N, polypeptide 1 | 210        | 7    | 52538     | 3          | 6.86 |
| 12   | gi|4501887   | actin, gamma 1 | 693        | 33   | 42108     | 10         | 5.31 |
| 13   | gi|178759    | apolipoprotein A-IV | 424        | 14   | 45307     | 6          | 5.23 |
| 14   | gi|306882    | haptoglobin | 209         | 8    | 45860     | 3          | 6.24 |
| 15   | gi|177827    | α₁-antitrypsin | 373        | 12   | 46787     | 7          | 5.42 |
| 16   | gi|338305    | apolipoprotein J | 344        | 16   | 36997     | 7          | 5.74 |
| 17   | gi|178849    | apolipoprotein E | 1124       | 53   | 36302     | 17         | 5.65 |
| 18   | gi|22023090  | HFREP-1     | 448        | 26   | 36640     | 7          | 5.58 |
| 19   | gi|38018090  | thrombin    | 599         | 23   | 34072     | 8          | 8.52 |
| 20   | gi|4557321   | apolipoprotein A-I (*) | 153        | 49   | 30759     | 18         | 5.56 |
| 21   | gi|178775    | apolipoprotein A-I | 723        | 44   | 28944     | 15         | 5.45 |
| 22   | gi|149673887 | immunoglobulin light chain | 442        | 40   | 23665     | 5          | 6.97 |
| 23   | gi|337758    | serum amyloid P | 306        | 18   | 25495     | 4          | 6.10 |

Protein spots shown in Fig. 1A were analyzed by mass spectrometry. Proteins with an asterisk were analyzed with MALDI-ToF and the other protein spots were analyzed with nanoflow LC-MS/MS. Accession number of the NCBInr database, protein description, Mowse score, sequence coverage (%), calculated molecular weight (Mw), total identified peptides, unique identified peptides and the calculated pI are given.

HFREP-1; hepatocyte-derived fibrinogen related protein-1.

![Figure 2. Western blot analysis with specific α₂-macroglobulin antibodies.](image_url)

Fibrin clot-bound plasma proteins were separated with 2D gel electrophoresis and analyzed with Western blot analysis using specific α₂-macroglobulin antibodies. The arrows indicate the three different α₂-macroglobulin trains that were also identified as α₂-macroglobulin with mass spectrometry (protein spots 2, 3 and 9 in figure 1A and table 1). The molecular mass of the protein marker is indicated in kDa.

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Discussion

In this study, using 2D gel electrophoresis and mass spectrometry, we identified 18 different fibrin clot-bound proteins, which are not cross-linked to fibrin by FXIIIa. Several of these proteins have not been described before as plasma clot components.

Eleven out of the 18 fibrin clot-bound proteins can be classified into three groups related to their function: blood coagulation, protease inhibition and HDL metabolism. Plasminogen, factor XIII and thrombin are involved in blood coagulation while \( \alpha_2 \)-macroglobulin and \( \alpha_1 \)-antitrypsin are protease inhibitors [26,27]. Plasma proteins that are associated with HDL play a role in its metabolism are haptoglobin, serum amyloid P and the apolipoproteins A-I, A-IV, J and E [28–31]. The presence of actin as a plasma clot component could be due to small amounts of platelets present in the platelet-poor plasma. However, actin can also be released into the bloodstream by dying cells or tissue damage [32].

The intensity of the stained spots suggests that the 18 identified plasma proteins represent nearly the entire protein material non-covalently bound to a fibrin clot. However, with this approach, we do not visualize the very low-abundant proteins. For example t-PA, a known fibrin-binding protein, was not observed. A second limitation of 2D gel electrophoresis is that high molecular weight proteins are underrepresented [33]. However, we did observe the high molecular weight proteins fibronectin and \( \alpha_2 \)-macroglobulin with 2D gel electrophoresis. In addition, with 1D gel electrophoresis (SDS-PAGE) and protein staining we did not observe any proteins that were related to coagulation, protease inhibition and HDL metabolism. There are some clear differences between the two proteomic approaches. The most important difference is that Howes et al. [35] described the total protein composition of the whole clot, thereby also identifying proteins that are cross-linked via FXIIIa, while we focused on non-covalently plasma clot-bound proteins. Identifying proteins by examining the whole clot is technically more challenging because of the high abundance of fibrin compared to the other plasma clot components.

Two-thirds of the newly identified fibrin clot-bound proteins are associated with HDL suggesting that HDL particles have affinity for fibrin, which is specific for HDL and not for LDL because only low amounts of apolipoprotein B were present in a fibrin clot. In addition, this apolipoprotein B most likely comes from bound lipoprotein(a), which can bind with its apolipoprotein(a) to fibrin [10]. The presence of HDL-proteins in purified fibrinogen suggested affinity of HDL to fibrinogen as well. These findings are in line with the detection of fibrinogen in purified HDL preparations [29,36,37]. What the role is of the binding of HDL to a fibrin clot is not known. However, recent studies have shown that HDL levels are negatively associated with both arterial and venous thrombosis [38–40], for which the exact mechanism is not known. HDL is a reverse cholesterol transporter, which is considered to be the most important property of HDL in preventing atherosclerosis. Several other properties can contribute to the atheroprotective effect of HDL including antioxidant, anti-inflammatory, antiproliferative, antithrombotic and vasodilatory properties [41]. HDL consists of a heterogeneous population of particles containing different types and amounts of (apolipo)proteins and lipids. The existence of different subpopulations in HDL is consistent with the fact that HDL has multiple biological activities [42]. It is possible that the HDL particle present on a fibrin clot as identified in this study represents a distinct subfraction of HDL.

A direct role of HDL in coagulation or fibrinolysis is not yet clear. It was suggested that HDL enhances the activated protein C pathway [43], but this may be due to the contamination of negatively charged phospholipid membranes [44]. Another possible mechanism that can play a role in the anticoagulant effect of HDL is that anionic phospholipids lose their procoagulant properties when incorporated into HDL [45].

In conclusion, we have identified several novel plasma clot components of which two-thirds was associated with HDL particles. This suggests that the presence of HDL on a fibrin clot may be of importance in clot formation or fibrinolysis and may play a role in the hemostasis and thrombosis.

Author Contributions
Conceived and designed the experiments: ST FWGL DCR. Performed the experiments: ST JAAD. Analyzed the data: ST JAAD DCR. Wrote the paper: ST DCR.
References

1. Prandoni P (2007) Links between arterial and venous disease. J Intern Med 262: 341–350.
2. Mahmoodi BK, Brouwer JL, Veeger NJ, van der Meer J (2008) Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study. Circulation 118: 1659–1667.
3. Holst AG, Jensen G, Prescott E (2011) Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. Circulation 121: 1896–1903.
4. Reiner AP, Siscovick DS, Rosendaal FR (2001) Hemostatic risk factors and arterial thrombotic disease. Thromb Haemost 85: 384–395.
5. White RH (2003) The epidemiology of venous thromboembolism. Circulation 107: 14–18.
6. Smallberg JH, Kruip MJ, Janssen HL, Rijken DC, Leebek FV, et al. (2011) Hypercoagulability and hyperfibrinolysis and risk of deep vein thrombosis and splanchic vein thrombosis: similarities and differences. Arterioscler Thromb Vasc Biol 31: 483–489.
7. Flinterman LE, van Hylckama Vlieg A, Rosendaal FR, Doggen CJ (2010) Venous thrombosis of the upper extremity: effect of blood group and coagulation factor levels on risk. Br J Haematol 149: 118–123.
8. Wolberg AS (2010) Plasma and cellular contributions to fibrin network formation, structure and stability. Haemophilia 16 Suppl 3: 7–12.
9. Hoyleaerts M, Rijken DC, Lijnen HR, Collen D (1982) Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J Biol Chem 257: 2912–2919.
10. Angles-Cano E, de la Pena Diaz A, Loyau S (2001) Inhibition of fibrinolysis by lipoprotein(a). Ann N Y Acad Sci 936: 261–273.
11. Nair CH, Dhall DP (1991) Studies on fibrin network structure: the effect of some plasma proteins. Thromb Res 61: 315–325.
12. Weisel JW (2007) Structure of fibrin: impact on clot stability. J Thromb Haemost 5 Suppl 1: 116–124.
13. Koopman J, Haverkate F, Lord ST, Grimbergen J, Mannucci PM (1992) Molecular basis of fibrinogen Naples-associated with defective thrombin binding and thrombophilia. Homozygous substitution of B beta 68 Ala-Thr. J Clin Invest 90: 233–244.
14. Weisel JW (2005) Fibrinogen and fibrin. Adv Protein Chem 70: 247–299.
15. Rijken DC, Dirks SP, Luider TM, Leebek FV (2006) Hepatocyte-derived fibrinogen-related protein-1 is associated with the fibrin matrix of a plasma clot. Biochem Biophys Res Commun 339: 191–194.
16. Sahni A, Guo M, Sahni SK, Francis CW (2004) Interleukin-1beta but not IL-1alpha binds to fibrinogen and fibrin and has enhanced activity in the bound form. Blood 104: 409–414.
17. Inoue M, Takeya H, Takemitsu T, Nakagaki T, Gabazza EC, et al. (1995) A novel degradation of plasminogen by human tissue plasminogen activator, is a substrate for transglutaminases. Evidence for a sensor for proteolysis. Ann N Y Acad Sci 737: 291–307.
18. Takebe M, Soe G, Kohno I, Sugo T, Matsuda M (1995) Calcium ion-dependent monoclonal antibody against human tissue plasminogen: preparation, characterization, and application to fibrinogen purification. Thromb Haemost 73: 662–667.
19. Redgrave TG, Roberts DC, West CE (1975) Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal Biochem 63: 42–49.
20. Chiu CF, Howard GC, Maes UK, Pizzo SV (1994) Alpha 2-macroglobulin: a sensor for proteolysis. Ann N Y Acad Sci 737: 291–307.
21. Potempa J, Korzun E, Travis J (1994) The serpin superfamily of protease inhibitors: structure, function, and regulation. J Biol Chem 269: 15957–15960.
22. Rezaee F, Casatta B, Levl JH, Speijer D, Meijers JC (2006) Proteomic analysis of high-density lipoprotein. Proteomics 6: 721–730.
23. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, et al. (2007) Shotgun proteomics implicates protease inhibition and complement activation in male participants of the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 24: 2181–2187.
24. de Haas CJ, Poppeller MJ, van Kessel KP, van Strijp JA (2000) Serum amyloid P component prevents high-density lipoprotein-mediated neutralization of lipopolysaccharide. Infect Immun 68: 4954–4960.
25. Henderson RJ, Wassen KM, Leon CG (2009) Haptoglobin inhibits phospholipid transfer protein activity in hyperlipidemic human plasma. Lipids Health Dis 8: 27.
26. Lee WM, Galbraith RM (1992) The extracellular actin-scavenger system and actin toxicity. N Engl J Med 326: 1335–1341.
27. Gorg A, Weiss W, Dunn MJ (2004) Current two-dimensional electrophoresis technology for proteomics. Proteomics 4: 3665–3685.
28. Harpel PC, Hayes MB, Hugi TE (1979) Heat-induced fragmentation of human alpha 2-macroglobulin. J Biol Chem 254: 8609–8617.
29. Howes J, Richardson VR, Smith KA, Schroeder V, Sonnai R, et al. (2012) Complement C3 is a novel plasma clot component with anti-fibrinolytic properties. Dia Vasc Dis Res 9: 216–223.
30. Konatke ST, Cardill CT, Liu K, Proster AA, Naya-Viguer J, et al. (1994) Identification of proteins associated with apolipoprotein A-I-containing lipoproteins purified by selected-affinity immunosorption. Biochemistry 33: 1988–1993.
31. Park CT, Wright SD (2000) Fibrinogen is a component of a novel lipoprotein particle: factor H-related protein (FHRP)-associated lipoprotein particle (FALP). Blood 95: 190–204.
32. Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, et al. (2004) High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 24: 2181–2187.
33. Eichinger S, Pechennik NM, Hrom G, Deguchi H, Schremmer M, et al. (2007) High-density lipoprotein and the risk of recurrent venous thromboembolism. Circulation 115: 1609–1614.
34. Talens S, Hoekstra J, Dirks SP, Murad SD, Trebicka J, et al. (2011) Proteomic analysis reveals that apolipoprotein A1 levels are increased in patients with Budd-Chiari syndrome. J Hepatol 54: 908–914.
35. Florence M, Liberopoulos EN, Wierzbicki AS, Mikalidis IP (2008) Multiple actions of high-density lipoprotein. Curr Opin Cardiol 23: 370–378.
36. Rosenson RS, Brewer HB Jr, Chapman MJ, Fazio S, Hussain MM, et al. (2011) HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clin Chem 57: 392–410.
37. Gröfius M, Kojima K, Banka CL, Curtis JK, Fernandez JA (1999) High-density lipoprotein enhances the anticoagulant activities of plasma protein S and activated protein C. J Clin Invest 103: 219–227.
38. Olaskovic C, Norstrom E, Dahlback B (2010) Reevaluation of the role of HDL in the anticoagulant activated protein C system in humans. J Clin Invest 120: 1396–1399.
39. Olaskovic C, Krisinger MJ, Andersson A, Jauhiainen M, Ehholm C, et al. (2009) Anionic phospholipids lose their procoagulant properties when incorporated into high density lipoproteins. J Biol Chem 284: 3596–3598.