Journal of Physical Chemistry B 2017 vol.121 N33, pages 7833-7843

# Conformational Flexibility and Self-Association of Fibrinogen in Concentrated Solutions

Zuev Y., Litvinov R., Sitnitsky A., Idiyatullin B., Bakirova D., Galanakis D., Zhmurov A., Barsegov V., Weisel J.

*Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia*

## Abstract

© 2017 American Chemical Society. We studied the hydrodynamic behavior of fibrinogen, a blood plasma protein involved in blood clotting, in a broad 0.3-60 mg/mL range of concentration and 5-42 °C temperature using pulsed-field gradient 1 H NMR-diffusometry. Arrhenius plots revealed the activation energy for fibrinogen diffusion E d = 21.3 kJ/mol at 1.4 mg/mL and 28.4 kJ/mol at 38 mg/mL. We found a dramatic slowdown in fibrinogen self-diffusion with concentration beginning at 1.7-3.4 mg/mL, which deviated from the standard hard-particle behavior, suggesting a remarkable intermolecular entanglement. This concentration dependence was observed regardless of the absence or presence of the GPRP peptide (inhibitor of fibrin polymerization), and also in samples free of fibrin oligomers. By contrast, diffusivity of fibrinogen variant I-9 with truncated C-terminal portions of the Aα chains was much less concentration-dependent, indicating the importance of intermolecular linkages formed by the αC regions. Theoretical models combined with all-atom molecular dynamics simulations revealed partially bent fibrinogen solution conformations that interpolate between a flexible chain and a rigid rod observed in the crystal. The results obtained illuminate the important role of the αC regions in modulating the fibrinogen molecular shape through formation of weak intermolecular linkages that control the bulk properties of fibrinogen solutions.

http://dx.doi.org/10.1021/acs.jpcb.7b05654

## References

[1] Weisel, J. W.; Litvinov, R. I. Fibrin Formation, Structure and Properties. In Fibrous Proteins: Structures and Mechanisms; Parry, D. A. D., Squire, J. M., Eds.; Springer International Publishing, 2017; Vol. 82, pp 405-456.

[2] Corral, M.; Ferko, N.; Hollmann, S.; Hogan, A.; Jamous, N.; Batiller, J.; Shen, J. Clinician Reported Ease of Use for a Novel Fibrin Sealant Patch for Hemostasis: Results from Four Randomized Controlled Trials Curr. Med. Res. Opin. 2016, 32, 367-375 10.1185/03007995.2015.1128405

[3] Mosesson, M. W.; Siebenlist, K. R.; Hainfeld, J. F.; Wall, J. S. The Covalent Structure of Factor XIIIa Crosslinked Fibrinogen Fibrils J. Struct. Biol. 1995, 115, 88-101 10.1006/jsbi.1995.1033

[4] Fowler, W. E.; Erickson, H. P. Trinodular Structure of Fibrinogen: Confirmation by Both Shadowing and Negative Stain Electron Microscopy J. Mol. Biol. 1979, 134, 241-249 10.1016/0022-2836(79)90034-2

[5] Williams, R. C. Morphology of Bovine Fibrinogen Monomers and Fibrin Oligomers J. Mol. Biol. 1981, 150, 399-408 10.1016/0022-2836(81)90555-6

[6] Weisel, J. W.; Stauffacher, C. V.; Bullitt, E.; Cohen, C. A Model for Fibrinogen: Domains and Sequence Science 1985, 230, 1388-1391 10.1126/science.4071058

[7] Kollman, J. M.; Pandi, L.; Sawaya, M. R.; Riley, M.; Doolittle, R. F. Crystal Structure of Human Fibrinogen Biochemistry 2009, 48, 3877-3886 10.1021/bi802205g

[8] Yee, V. C.; Pratt, K. P.; Côté, H. C.; Le Trong, I.; Chung, D. W.; Davie, E. W.; Stenkamp, R. E.; Teller, D. C. Crystal Structure of a 30 kDa C-terminal Fragment From the γ-Chain of Human Fibrinogen Structure 1997, 5, 125-138 10.1016/S0969-2126(97)00171-8

[9] Spraggon, G.; Everse, S. J.; Doolittle, R. F. Crystal Structures of Fragment D From Human Fibrinogen and its Crosslinked Counterpart From Fibrin Nature 1997, 389, 455-462 10.1038/38947

[10] Brown, J. H.; Volkmann, N.; Jun, G.; Henschen-Edman, A. H.; Cohen, C. The Crystal Structure of Modified Bovine Fibrinogen Proc. Natl. Acad. Sci. U. S. A. 2000, 97, 85-90 10.1073/pnas.97.1.85

[11] Madrazo, J.; Brown, J. H.; Litvinovich, S.; Dominguez, R.; Yakovlev, S.; Medved, L.; Cohen, C. Crystal Structure of the Central Region of Bovine Fibrinogen (E5 Fragment) at 1.4-Å Resolution Proc. Natl. Acad. Sci. U. S. A. 2001, 98, 11967-11972 10.1073/pnas.211439798

[12] Tsurupa, G.; Tsonev, L.; Medved, L. Structural Organization of the Fibrin(ogen) αC-Domain Biochemistry 2002, 41, 6449-6459 10.1021/bi025584r

[13] Veklich, Y. I.; Gorkun, O. V.; Medved, L. V.; Nieuwenhuizen, W.; Weisel, J. W. Carboxyl-terminal Portions of the Alpha Chains of Fibrinogen and Fibrin. Localization by Electron Microscopy and the Effects of Isolated Alpha C Fragments on Polymerization J. Biol. Chem. 1993, 268, 13577-13585

[14] Protopopova, A. D.; Barinov, N. A.; Zavyalova, E. G.; Kopylov, A. M.; Sergienko, V. I.; Klinov, D. V. Visualization of Fibrinogen αC Regions and Their Arrangement During Fibrin Network Formation by High-resolution AFM J. Thromb. Haemostasis 2015, 13, 570-579 10.1111/jth.12785

[15] Weisel, J. W.; Medved, L. V. The Structure and Function of the αC Domains of Fibrinogen Ann. N. Y. Acad. Sci. 2001, 936, 312-327 10.1111/j.1749-6632.2001.tb03517.x

[16] Litvinov, R. I.; Yakovlev, S.; Tsurupa, G.; Gorkun, O. V.; Medved, L.; Weisel, J. W. Direct Evidence for Specific Interactions of the Fibrinogen αC-Domains with the Central E Region and with Each Other Biochemistry 2007, 46, 9133-9142 10.1021/bi700944j

[17] Raynal, B.; Cardinali, B.; Grimbergen, J.; Profumo, A.; Lord, S. T.; England, P.; Rocco, M. Hydrodynamic Characterization of Recombinant Human Fibrinogen Species Thromb. Res. 2013, 132, e48-e53 10.1016/j.thromres.2013.04.005

[18] Martinez, M. C. L.; Rodes, V.; de la Torre, J. G. Estimation of the Shape and Size of Fibrinogen in Solution from its Hydrodynamic Properties Using Theories for Bead Models and Cylinders Int. J. Biol. Macromol. 1984, 6, 261-265 10.1016/0141-8130(84)90005-9

[19] Cardinali, B.; Profumo, A.; Aprile, A.; Byron, O.; Morris, G.; Harding, S. E.; Stafford, W. F.; Rocco, M. Hydrodynamic and Mass Spectrometry Analysis of Nearly-intact Human Fibrinogen, Chicken Fibrinogen, and of a Substantially Monodisperse Human Fibrinogen Fragment X Arch. Biochem. Biophys. 2010, 493, 157-168 10.1016/j.abb.2009.10.008

[20] Wasilewska, M.; Adamczyk, Z. Fibrinogen Adsorption on Mica Studied by AFM and in Situ Streaming Potential Measurements Langmuir 2011, 27, 686-696 10.1021/la102931a

[21] Adamczyk, Z.; Cichocki, D.; Ekiel-Jeżewska, M. L.; Słowicka, A.; Wajnryb, E.; Wasilewska, M. Fibrinogen Conformations and Charge in Electrolyte Solutions Derived from DLS and Dynamic Viscosity Measurements J. Colloid Interface Sci. 2012, 385, 244-257 10.1016/j.jcis.2012.07.010

[22] Price, W. S. NMR Studies of Translational Motion. Principles and Application; Cambridge University Press: Cambridge, 2009.

[23] Price, W. S. NMR Gradient Methods in the Study of Proteins Annu. Rep. Prog. Chem., Sect. C: Phys. Chem. 2000, 96, 3-53 10.1039/b000773k

[24] Nesmelova, I. V.; Skirda, V. D.; Fedotov, V. D. Generalized Concentration Dependence of Globular Protein Self-diffusion Coefficient in Aqueous Solutions Biopolymers 2002, 63, 132-140 10.1002/bip.10023

[25] Mutina, A. R.; Skirda, V. D. Rodlike Polymer Gelatination as Studied by PFG NMR: Fibrin Polymerization in Human Plasma Appl. Magn. Reson. 2004, 27, 527-538 10.1007/BF03166748

[26] Mosesson, M. W.; Galanakis, D. K.; Finlayson, J. S. Comparison of Human Plasma Fibrinogen Subfractions and Early Plasmic Fibrinogen Derivatives J. Biol. Chem. 1974, 249, 4656-4664

[27] Mosesson, M. W.; Alkjaersig, N.; Sweet, B.; Sherry, S. Human Fibrinogen of Relatively High Solubility. Comparative Biophysical, Biochemical, and Biological Studies with Fibrinogen of Lower Solubility Biochemistry 1967, 6, 3279-3287 10.1021/bi00862a038

[28] Wu, D. H.; Chen, A. D.; Johnson, C. S. An Improved Diffusion-Ordered Spectroscopy Experiment Incorporating Bipolar-Gradient Pulses J. Magn. Reson., Ser. A 1995, 115, 260-264 10.1006/jmra.1995.1176

[29] Hoye, T. R.; Eklov, B. M.; Ryba, T. D.; Voloshin, M.; Yao, L. J. No-D NMR (No-Deuterium Proton NMR) Spectroscopy: A Simple Yet Powerful Method for Analyzing Reaction and Reagent Solutions Org. Lett. 2004, 6, 953-956 10.1021/ol049979+

[30] Humphrey, W.; Dalke, A.; Schulten, K. VMD-Visual Molecular Dynamics J. Mol. Graphics 1996, 14, 33-38 10.1016/0263-7855(96)00018-5

[31] Šali, A.; Blundell, T. L. Comparative Protein Modelling by Satisfaction of Spatial Restraints J. Mol. Biol. 1993, 234, 779-815 10.1006/jmbi.1993.1626

[32] Burton, R. A.; Tsurupa, G.; Hantgan, R. R.; Tjandra, N.; Medved, L. NMR Solution Structure, Stability, and Interaction of the Recombinant Bovine Fibrinogen αC-Domain Fragment Biochemistry 2007, 46, 8550-8560 10.1021/bi700606v

[33] Ferrara, P.; Apostolakis, J.; Caflisch, A. Evaluation of a Fast-Implicit Solvent Model for Molecular Dynamics Simulations Proteins: Struct., Funct., Genet. 2002, 46, 24-33 10.1002/prot.10001

[34] Zhmurov, A.; Kononova, O.; Litvinov, R. I.; Dima, R. I.; Barsegov, V.; Weisel, J. W. Mechanical Transition From α-helical Coiled coils to β-sheets in Fibrin(ogen) J. Am. Chem. Soc. 2012, 134, 20396-20402 10.1021/ja3076428

[35] Zhmurov, A.; Brown, A. E.; Litvinov, R. I.; Dima, R. I.; Weisel, J. W.; Barsegov, V. Mechanism of Fibrin (ogen) Forced Unfolding Structure 2011, 19, 1615-1624 10.1016/j.str.2011.08.013

[36] Monkos, K. A. Comparison of the Activation Energy of Viscous Flow for Hen Egg-white Lysozyme Obtained on the Basis of Different Models of Viscousity for Glass-forming Liquids Curr. Top. Biophys 2011, 34, 1-9 10.2478/v10214-011-0001-6

[37] Tanaka, K. Measurements of Self-diffusion Coefficient of Water in Pure Water and in Aqueous Electrolyte Solutions J. Chem. Soc., Faraday Trans. 1 1975, 71, 1127-1131 10.1039/f19757101127

[38] Medved, L. V.; Weisel, J. W. Recommendations for Nomenclature on Fibrinogen and Fibrin J. Thromb. Haemostasis 2009, 7, 355-359 10.1111/j.1538-7836.2008.03242.x

[39] Laudano, A. P.; Cottrell, B. A.; Doolittle, R. F. Synthetic Peptides Modeled on Fibrin Polymerization Sites Ann. N. Y. Acad. Sci. 1983, 408, 315-329 10.1111/j.1749-6632.1983.tb23254.x

[40] Chernysh, I. N.; Nagaswami, C.; Purohit, P. K.; Weisel, J. W. Fibrin Clots Are Equilibrium Polymers That Can Be Remodeled Without Proteolytic Digestion Sci. Rep. 2012, 2, 879-884 10.1038/srep00879

[41] Tokuyama, M.; Oppenheim, I. Dynamics of Hard-sphere Suspensions Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top. 1994, 50, R16-R19 10.1103/PhysRevE.50.R16

[42] Asadi, M. Beet-Sugar Hand Book; John Wiley and Sons Ltd: UK, 2007.

[43] Rubinstein, M.; Colby, R. Polymer Physics; Oxford University Press: Oxford, 2012.

[44] Weast, R. C.; Astle, M. J.; Beyer, W. H. CRC Handbook of Chemistry and Physics; CRC press: Boca Raton, FL, 1988.

[45] Tsao, T. C.; Bailey, K.; Adair, G. S. The Size, Shape and Aggregation of Tropomyosin Particles Biochem. J. 1951, 49, 27 10.1042/bj0490027

[46] Shulman, S. The Size and Shape of Bovine Fibrinogen. Studies of Sedimentation, Diffusion and Viscosity J. Am. Chem. Soc. 1953, 75, 5846-5852 10.1021/ja01119a021

[47] Lederer, K.; Schurz, J. High Shear Rate Viscosity Measurements with Dilute Solutions of Bovine Fibrinogen Biopolymers 1972, 11, 1989-1993 10.1002/bip.1972.360110918

[48] Nemoto, N.; Nestler, F. H. M.; Schrag, J. L.; Ferry, J. D. Infinite-dilution Viscoelastic Properties of Fibrinogen and Intermediate Fibrin Polymer Biopolymers 1977, 16, 1957-1969 10.1002/bip.1977.360160910

[49] Armstrong, J. K.; Wenby, R. B.; Meiselman, H. J.; Fisher, T. C. The Hydrodynamic Radii of Macromolecules and their Effect on Red Blood Cell Aggregation Biophys. J. 2004, 87, 4259-4270 10.1529/biophysj.104.047746

[50] Gaigalas, A. K.; Hubbard, J. B.; McCurley, M.; Woo, S. Diffusion of Bovine Serum Albumin in Aqueous Solutions J. Phys. Chem. 1992, 96, 2355-2359 10.1021/j100184a063

[51] Köhler, S.; Schmid, F.; Settanni, G. The Internal Dynamics of Fibrinogen and Its Implications for Coagulation and Adsorption PLoS Comput. Biol. 2015, 11, e1004346 10.1371/journal.pcbi.1004346

[52] Tsurupa, G.; Hantgan, R. R.; Burton, R. A.; Pechik, I.; Tjandra, N.; Medved, L. Structure, Stability, and Interaction of the Fibrin(ogen) αC-domains Biochemistry 2009, 48, 12191-12201 10.1021/bi901640e

[53] Burton, R. A.; Tsurupa, G.; Medved, L.; Tjandra, N. Identification of an Ordered Compact Structure within the Recombinant Bovine Fibrinogen αC-Domain Fragment by NMR Biochemistry 2006, 45, 2257-2266 10.1021/bi052380c

[54] Pamies, R.; Cifre, J. G. H.; Martínez, M. D. C. L.; de la Torre, J. G. Determination of Intrinsic Viscosities of Macromolecules and Nanoparticles. Comparison of Single-point and Dilution Procedures Colloid Polym. Sci. 2008, 286, 1223-1231 10.1007/s00396-008-1902-2

[55] Erickson, H. P. Size and Shape of Protein Molecules at the Nanometer Level Determined by Sedimentation, Gel Filtration, and Electron Microscopy Biol. Proced. Online 2009, 11, 32-51 10.1007/s12575-009-9008-x

[56] Stryer, L.; Cohen, C.; Langridge, R. Axial Period of Fibrinogen and Fibrin Nature 1963, 197, 793-794 10.1038/197793a0

[57] Cohen, C.; Slayter, H.; Goldstein, L.; Kucera, J.; Hall, C. Polymorphism in Fibrinogen Aggregates J. Mol. Biol. 1966, 22, 385-386 10.1016/0022-2836(66)90144-6

[58] Gollwitzer, R.; Bode, W.; Schramm, H.-J.; Typke, D.; Guckenberger, R. Laser Diffraction of Oriented Fibrinogen Molecules Ann. N. Y. Acad. Sci. 1983, 408, 214-225 10.1111/j.1749-6632.1983.tb23246.x

[59] Stewart, G. J.; Niewiarowski, S. Nonenzymatic Polymerization of Fibrinogen by Protamine Sulfate An Electron Microscope Study Biochim. Biophys. Acta, Protein Struct. 1969, 194, 462-469 10.1016/0005-2795(69)90106-8

[60] Zhmurov, A.; Protopopova, A. D.; Litvinov, R. I.; Zhukov, P.; Mukhitov, A. R.; Weisel, J. W.; Barsegov, V. Structural Basis of Interfacial Flexibility in Fibrin Oligomers Structure 2016, 24, 1907-1917 10.1016/j.str.2016.08.009

[61] Brown, A. E. X.; Litvinov, R. I.; Discher, D. E.; Weisel, J. W. Forced Unfolding of Coiled-Coils in Fibrinogen by Single-Molecule AFM Biophys. J. 2007, 92, L39-L41 10.1529/biophysj.106.101261

[62] Gorkun, O. V.; Litvinov, R. I.; Veklich, Yu. I.; Weisel, J. W. Interactions Mediated by the N-Terminus of Fibrinogen's Bβ Chain Biochemistry 2006, 45, 14843-14852 10.1021/bi061430q