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Hydrodynamic and mass spectrometry analysis of nearly-intact human fibrinogen, chicken fibrinogen, and of a substantially monodisperse human fibrinogen fragment X

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ABSTRACT

The shape and solution properties of fibrinogen are affected by the location of the C-terminal portion of the Aα chains, which is presently still controversial. We have measured the hydrodynamic properties of a human fibrinogen fraction with these appendages mostly intact, of chicken fibrinogen, where they lack 11 characteristic 13-amino acids repeats, and of human fragment X, a plasmin early degradation product in which they have been removed. The human fibrinogen/fragment X samples were extensively characterized by SDS–PAGE/Western blotting and mass spectrometry, allowing their composition to be precisely determined. The solution properties of all samples were then investigated by analytical ultracentrifugation and size-exclusion HPLC coupled with multi-angle light scattering and differential pressure viscometry detectors. The measured parameters suggest that the extra repeats have little influence on the overall fibrinogen conformation, while a significant change is brought about by the removal of the C-terminal portion of the Aα chains beyond residue Aα200.

Introduction

Fibrinogen (FG)[1] is a high molecular weight (~340,000), centrosymmetric, dimeric glycoprotein found in the blood of vertebrates, where physiologically plays a critical role in the coagulation system [1,2]. Each half of the molecule consists of three polypeptide chains, called Aα, Bβ and γ, whose six N-termini are contained in a central, symmetric globular region ("E-region") [2–4]. Two triple coiled-coil connectors join this region to two outer globular regions ("D-regions"), formed by the C-terminal parts of the Bβ and γ chains, each folding into independent domains [2–4]. The triple coiled-coil connectors are held in register at their beginning and end by two disulfide bridge rings [3–7]. At the end of each coiled-coil, after the second disulfide ring, the Aα chain reverses direction, forming a fourth coiled helix continuing for about half of the connector's length [6,7]. Thereafter, the location of the C-terminal parts of the Aα chains (αC regions) is instead still controversial, ranging from partially free-swimming appendages [3,7], to forming a fourth globular region positioned on top of the central one [8–13]. Interestingly, there is a wide variation in the length of this portion of the Aα chain among vertebrate species [14–20], mostly depending on the number of several 13-amino acids imperfect tandem repeats. In particular, ten such repeats were originally identified in the human species' Aα chain [14], which has 610 residues in the mature form, but at least another repeat was later reported [21]. Instead, the repeats are completely absent in the chicken Aα chain, which has only 476 residues [18]. In Fig. 1, an alignment of the human and chicken sequences for the Aα chain residues after the second disulfide ring is presented, where the definition of a possible additional, non-canonical repeat is also suggested. In contrast, the Bβ and γ chains have pretty much the same length in humans and chickens, containing 461/463 and 411/409 residues, respectively [7,24,25]. The αC regions are very susceptible to proteolytic attack, and their complete removal by

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[1] *Abbreviations used:* FG, fibrinogen; hFG, human fibrinogen; hFrX, human fibrinogen fragment X; hHMW-FG, human high molecular weight fibrinogen; hLMW-FG and hLMW′-FG, human low molecular weight (prime) fibrinogen; AUC, analytical ultracentrifugation; SEC, size-exclusion chromatography; SE-HPLC, size-exclusion high-performance liquid chromatography; MALLS, multi-angle laser light scattering; DPV, differential pressure viscometry; TIC, total ion chromatogram.

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H166  S R A L A R E V D L K D Y E D Q Q K Q L E Q V I A          H190 4th helix on coiled coil
C167  A R S F D Y Q V D K E G Y D N I Q K H L T Q A S S          C191

H191  K D L L P S R D R Q H L P L I K M K P V P D L - V P G      H216
C192  I D M H P D F Q T T T L S T L K M R P L K D S N V P E      C218 4th helix on coiled coil

H217  N F K S Q L Q K V P P E W K A L T D M P Q                  H237 Predicted mostly helix
C219  H F K L K P S P E M Q A M S A F N N I K Q                  C239

H238  M R M E L E R P G G N E I T                               H251 Predicted mostly sheet
C240  M Q V V L E R P E T D H V A                               C253


H252  R G G S T S Y G - T G S E     H263 Repeat I

H264  T E S P R N P S S A G S W     H276 Repeat II

H277  N S G S S G P G S T G N R     H289 Repeat III

H290  N P G S S G T G G T A T W     H302 Repeat IV

H303  K P G S S G P G S A G S W     H315 Repeat V

H316  N S G S S G T G S T G N Q     H328 Repeat VI

H329  N P G S P R P G S T G T W     H341 Repeat VII

H342  N P G S S E R G S A G H W     H354 Repeat VIII

H355  T S E S S V S G S T G Q W     H367 Repeat IX

H368  H S E S - G S F R P D S P     H379 Repeat X

H380  G S G N A R P N N P D - W     H391 Repeat XI

H392  G T F E E V S G N V - S P     H403 Repeat XII?
C254  - - - - E A R G D - S S P     C261


H404  G T R R E Y H T E K L V T S K G D K E L R                 H424 Predicted mostly sheet
C262  S - - - - - H T G K L I T S S H R R E S P                 C277

H425  T G K E K V T S G S T T T T R R S C S K T V T K T V I - G  H452 Start globular region
C278  S L V D K T S S A S S V - - - H R C T R T V T K K V I S G  C303 (bovine NMR)

H453  P D G H K E V T K E V V T S E D G S D C P E A M D - - - -  H477
C304  P D G P R E E I V E K M V S S D G S D C S H L Q G G R E G  C332

H478  - L G T L S G I G T L D G F R H R H P D E A A F F D↓T A S  H505 (↓ = end bovine NMR)
C333  S T Y H F S G T G D F H K L D R L L P D L E S F F T H D S  C361

H506  T G K T F P G F F S P - - - - - M L G E F V S E T E S R G  H529
C362  V S T S S R H S I G S S T S S H V T G A G S S H L G T G G  C390

H530  S E S G I F T N T K E S S S H H P G I A E F P S - - R G K  H556
C391  K D K - - F T D L G E E E E D D F G G L Q - P S G F A A G  C416

H557  S S S Y S K Q F - - T S S T S Y N R G D S T F E S K S Y K  H583 End globular region
C417  S A S H S K T V L T S S S S S F N K G G S T F E T K S L K  C445

H584  - - - M A D E A G S E A D H E G T H S T K R G H A K S - R P V    H610 C-terminal
C446  T R E T S E Q L G G V Q H D Q S A E D T P D F K A R S F R P A    C476
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Fig. 1. Alignment of the human (H) and chicken (C) Aα chain C-terminal region after the second disulfide ring, as produced by T-COFFEE version 5.05 (http://www.tcoffee.org) [22]. Some features are indicated on the right side; the secondary structure predictions were made with Jpred3 (http://www.compbio.dundee.ac.uk/www-jpred/) [23]. Mature proteins numbering.

plasmin generates a FG species known as "fragment X" (FrX) [26], which also lacks the first ~54 residues of the β chain [27] (the N-terminus contains a thrombin-cleavable sequence called fibrinopeptide B, hence the name change from Bβ to β; a similar sequence, fibrinopeptide A, is at the N-terminus of the Aα chain). A similar species, but with an uncleaved Bβ N-terminus, is found in normal plasma together with nearly-intact FG; they are often referred to as "fraction I-8" and "fraction I-4", respectively [28].

Over the past ~10 years, great advancements have been made in determining the structure of fibrinogen, mainly by X-ray crystallography, e.g. [6,7,29–35]. In particular, the structure of intact chicken fibrinogen at 2.7 Å resolution was determined, but the Aα chain could not be traced beyond residue Glu218 [7]. However, two nearly symmetrical electron density blobs were defined between the elongated, staggered FG molecules in the crystals, sideways to the βC-domains, above the second half of the coiled-coil of a molecule, and below the central region of the preceding molecule (see Fig. 2 in [7]). Likewise, the αC regions could not be resolved in the recent crystal structure of intact human FG [35]. Structural studies have also been performed on the last ~200 residues of the Aα chain (αC-domain), identified as a likely globular region by calorimetry [36,37]. In particular, a recent NMR study has presented the structure of a bovine αC-domain recombinant fragment corresponding to the human 425–505 and chicken 278–361 residues [38].

The location of such a large portion of the Aα chain could clearly affect the shape and the solution behavior of FG. In order to determine their contribution, we have measured the hydrodynamic