Structural organization of C-terminal parts of fibrinogen Aα-chains

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Calorimetric studies of fibrinogen melting and of its early degradation products have shown that the C-terminal parts of both the Aα-chains form structural domains which strongly interact with each other in the native fibrinogen molecule.

Fibrinogen   *X-fragment*   *Aα-chain*   *Domain*   *Unfolding*   *Calorimetry*

1. INTRODUCTION

There is a widespread opinion that about 400 residue-long C-terminal parts of both the Aα-chains in fibrinogen do not form a compact ordered structure since they are hydrolyzed too fast at treatment of the molecule by proteolytic enzymes [1]. However, our calorimetric study of fibrinogen melting and of its fragments have shown that parts of this molecule which are removed on the early stage of its degradation at proteolysis have a cooperative structure which melts at heating with a slight, but quite noticeable heat effect [2]. This heat effect is largely screened by a much more pronounced effect of melting of the other parts of the molecule. Thus, in our first study we did not succeed in analyzing it quantitatively to get more definite information on the structure formed by the easily removable parts of fibrinogen. This has been done now and we here report the obtained results showing that two domains formed by the C-terminal part of both Aα-chains strongly interact with each other as they are in direct contact between themselves in the native fibrinogen molecule.

2. MATERIALS AND METHODS

Bovine fibrinogen was prepared from oxalate plasma by salting out with sodium sulphate [3] and consequent purification [4]. Fibrinogen was hydrolyzed by human plasmin in 0.06 M sodium-phosphate buffer (pH 7.0), 0.1 M NaCl, at 25°C and at a plasmin activity of 0.005 caseinolytic units/ml. Hydrolysis was stopped by adding *p*-nitrophenyl *p'*-guanidinobenzoate [5]. Then plasmin was removed by passing the solution through a Lys–Sepharose column. The X_1-fragment has been obtained from a 20-min hydrolysate (fig.1a) by a step-wise salting out with ammonium sulphate at 4°C as in [4]. The X_2-fragment was obtained from a 90-min hydrolysate by gel filtration on Sephadex G-200 as in [6].

Clottability of the obtained preparation was 98% for intact fibrinogen, 92% for the X_1-fragment and 7% for the X_2-fragment. Purity of preparations was checked by SDS-electrophoresis (fig.1b). No detectable impurities were found in the intact fibrinogen and X_2-fragment. The amount of impurities in the X_1-fragment was less than 10%.

The concentration of proteins in the solution was determined by the nitrogen content measured on a Perkin-Elmer 240 B elemental analyzer.

Calorimetric studies were carried out on a scann-

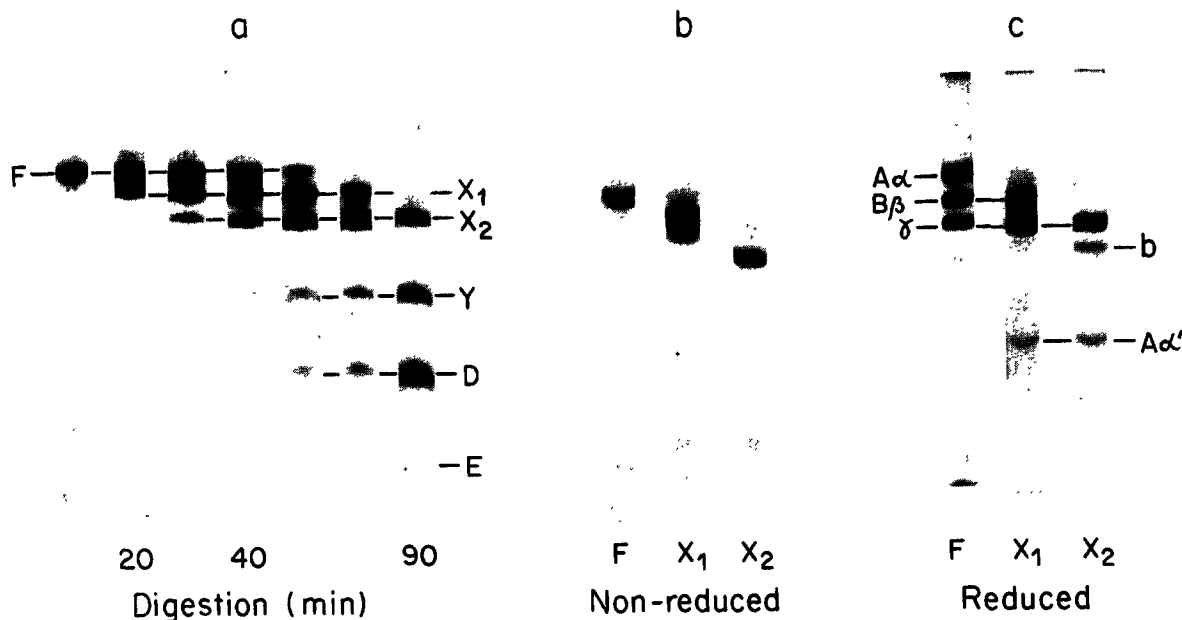* To whom correspondence should be addressed

Fig.1. SDS–polyacrylamide gel electrophoretic patterns of timed plasmic digest of bovine fibrinogen (a) and patterns of fibrinogen (F), the X_1- and X_2-fragments, non-reduced (b) and reduced (by 0.2% β-mercaptoethanol) (c). Aα, Bβ, γ are the bands of the corresponding polypeptide chains of fibrinogen [4]. The Aα-chain degraded to $M_r \simeq 26000$ is denoted as Aα'. Electrophoresis was performed in 4% (a,b) and 7.5% (c) polyacrylamide gels as in [7].

ing microcalorimeter DASM-1M as in [2].

3. RESULTS AND DISCUSSION

A detailed study of the early stages of fibrinogen proteolysis (fig.1a) has shown that degradation of this molecule proceeds through the formation of two discrete forms: the early product, the X_1-fragment, and the later one, the X_2-fragment [8,9]. In the X_1-fragment, judging from the SDS electrophoregrams at reducing conditions (fig.1c), only the Aα-chain is modified which lacks a large piece from the C-terminal end [10]; in the X_2-fragment the Bβ-chain is also reduced which, according to [9], lacks a 6000-M_r piece from the N-terminal end and some of the chains have single cuts in the region which is between the central (fragment E) and terminal (fragment D) parts of the molecule (band b, fig.1c) [11].

Fig.2. presents the calorimetrically obtained melting profiles of the intact fibrinogen, the X_1-fragment and the X_2-fragment. As seen, the only difference between the profiles for the intact fibrinogen and the X_1-fragment is in the second
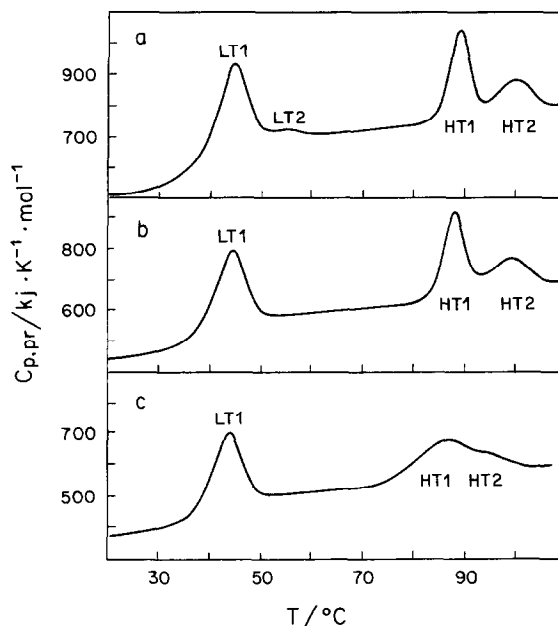

Fig.2. Temperature-dependence of the partial molar heat capacity of fibrinogen (a), the X_1-fragment (b) and the X_2-fragment (c), in 0.05 M glycine, pH 3.5.

low temperature heat absorption peak LT2. Since these two samples differ only by the C-terminal part of the Aα-chains it is clear that the LT2 peak corresponds to melting of a structure formed by these regions. Thus, one can conclude that the C-terminal part of the Aα-chain is able to form a cooperative structure which melts in the temperature range of the peak LT2 at heating.

As for the melting profile of the X$_2$-fragment, it differs from that of the X$_1$-fragment by the high temperature peaks HT1 and HT2. This is not surprising since, as has been shown in [2], peak HT1 represents the heat effect of melting of the central part of the fibrinogen molecule corresponding to the E-fragment containing the N-terminal part of the Bβ-chains which are cleaved in the X$_2$-fragment. The peak HT2 corresponds to melting of the connector between the central and terminal parts, and the cut of one of the chains observed in the X$_2$-fragment should influence its stability.

The quantitative analysis of the LT2 peak is complicated by the much more pronounced preceding peak LT1 which represents the heat effect of melting of the terminal parts of the fibrinogen molecule corresponding to the D-fragment [2]. Fortunately it has been found that the LT1 peak, in contrast to the LT2 peak, is irreproducible at repeated heating of the same sample; i.e., the process of melting the D-fragment is irreversible, while the process of melting the structure formed by the C-terminal part of the Aα-chains is reversible. This circumstance enables us to isolate peak LT2 just by eliminating peak LT1 by preincubation of a sample at 43°C (fig.3).

The most notable feature of peak LT2 is that it is accompanied by a distinct heat capacity change. The heat capacity increase at melting of the molecular structure is an indication that the process proceeds with an unfolding of a structure and the exposure of buried non-polar groups on water [12]. It then follows that the structure formed by the C-terminal part of the Aα-chains is compact and has a hydrophobic non-polar core; i.e., it is a typical structural domain.

The enthalpy Δ_mH (the peak area) and the heat capacity change of the peak LT2 are equal to (440 ± 30) kJ.mol^{-1} and (25 ± 3) kJ.K^{-1}.mol^{-1}, respectively, calculated per mole of the fibrinogen molecule ($M_r \simeq 340000$). Taking into account that fibrinogen includes two Aα-chains and that the
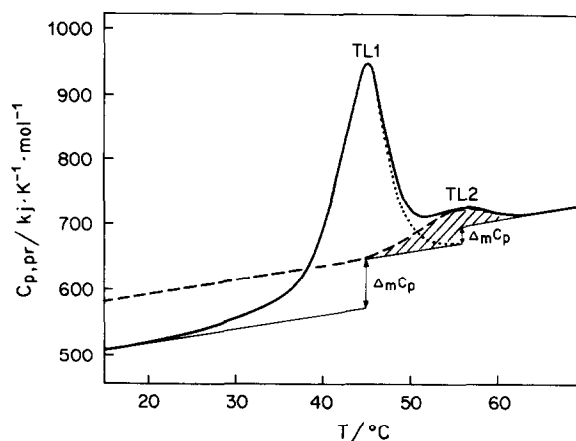

Fig.3. Temperature-dependence of the partial molar heat capacity of fibrinogen (solid line) and fibrinogen preliminarily incubated for 60 min at 43°C (dashed line) in 0.05 M glycine, pH 3.5.

molecular mass of each cleaved C-terminal part is about 45000 [8,9], for the specific enthalpy and the heat capacity change at melting of these parts, we get (5.2 ± 0.4) J.g^{-1} and (0.25 ± 0.04) J.K^{-1}.g^{-1}, respectively. These values are somewhat lower than observed for compact globular proteins [7]. The difference between the considered structure of fibrinogen and compact globular proteins becomes especially evident at comparison of the specific melting enthalpy values extrapolated to 110°C by the equation:

$$\Delta_mH(T) = \Delta_mH(T_m) + \int_{T_m}^{T} \Delta_mC_p dT$$

As has been shown [12], this extrapolated to 110°C value for compact globular proteins is close to 50 J.g^{-1}. For the structure formed by the C-terminal part of the Aα-chains we get 20 J.g^{-1} at extrapolation, which is only 40% of the value characteristic for compact globular structures.

The observed deficit in the enthalpy value cannot be explained by degradation of the studied fibrinogen preparation. As indicated in section 2, the studied intact fibrinogen was practically devoid of lower M_r fragments (fig.1b). It is therefore likely that the relatively small melting enthalpy value observed for the C-terminal part of the Aα-chain

reflects some specificity of its structure, namely that it is not as compact as that of usual globular proteins and is stabilized by the smaller amount of secondary bonds. One can consider the following alternatives: either this structure is looser as a whole, or it has a compact and a non-compact part; e.g., as in histone H_1 [13].

The choice between these alternatives can be made if one examines the sequence of the considered Aα-chain fragment [14] (fig.4). The polar and non-polar amino acids are very unequally distributed along this fragment: its first half distinctly lacks non-polar residues and has an excess of serine, glycine, threonine and proline which makes it very improbable that this half could form any compact structure. As for the second half, it has a quite common (for globular proteins) distribution of amino acid residues. A computer analysis of probability of formation of regular conformations kindly made by Dr Finkelstein according to the algorithm in [15], shows also that α-helix and β-conformation are quite probable in the 390–550 residue section of the considered polypeptide. Thus, one can suggest that only this part of the Aα-chain (i.e., about 40% of its size removable at hydrolysis) forms a compact cooperative structure in the fibrinogen molecule, while the 250–390 residue section is likely to be in a random conformation.

Other important information on the structure formed by the C-terminal parts of Aα-chain can be obtained by analyzing the shape of its melting pro-

file. From the sharpness of the heat absorption peak at its melting one can calculate the van 't Hoff enthalpy of the observed process assuming that it represents a transition between two states:

$$\Delta_m H^{VH} = \frac{4\,RT^2\,\delta\,C_p^{max}}{Q_m}$$

where δC_p^{max} is the peak height and Q_m its area in energy units [12].

For the van 't Hoff enthalpy of the peak LT2 we get (310 ± 20) kJ.mol^{-1}. This value is significantly lower than the calorimetrically measured total enthalpy of melting of the two C-terminal parts of Aα-chains in the fibrinogen molecule $((440 \pm 30)$ kJ.mol$^{-1})$ but it is higher than the calorimetric enthalpy of melting of only one Aα-chain $(220$ kJ.mol$^{-1})$. This discrepancy between the calorimetric and the van 't Hoff enthalpy values can be explained only by assuming that the structures formed by each of the Aα-chains in fibrinogen are not independent. If two C-terminal portions of the Aα-chains in fibrinogen would form a single cooperative structure, the van 't Hoff enthalpy of its melting should be equal to a total calorimetric enthalpy of 440 kJ.mol^{-1}. The observed somewhat lower van 't Hoff enthalpy value means that the two C-terminal parts of the Aα-chains do not form a single structural unit but form two domains which strongly interact with each other. It is evident that this can be the case only if both these αC-domains are in direct contact forming one structural block. This does not seem to be improbable if the αC-domains are connected with the terminal parts of fibrinogen molecules with extended flexible polypeptide junctions.

In this connection it is very interesting that authors in [16] have recently found, by means of electron microscopy, the fourth nodule in the fibrinogen molecule which is located near the central one. Since this nodule is not observed for the X-fragment they suggested that it is formed by the C-terminal parts of the two Aα-chains. Our results argue that the microscopically observed dimeric structure formed by the two Aα-chains is not an artefact induced by contrasting the sample, but is a structural block specific for a native fibrinogen molecule. This naturally raises the question as to the functional role of this dimeric block.
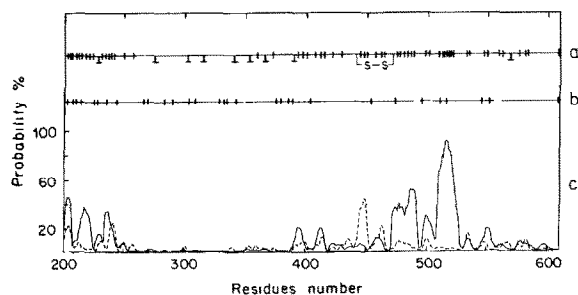

Fig.4. Distribution of non-polar residues and the probability of regular conformation for the C-terminal section of the human fibrinogen Aα-chain. (a) Non-polar residues Val, Leu, Ile, Met, Phe, Tyr (|), Trp (⊥) and Cys–Cys (-S–S-) distribution. (b) Proline residue distribution. (c) Probability of α-helices (solid line) and β-strand (dashed line).

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