CALCIUM-BINDING PROPERTIES OF HUMAN FIBRIN(OGEN) AND DEGRADATION PRODUCTS

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Received 4 December 1978

1. Introduction

Calcium ions play an important role in fibrin monomer aggregation [1,2] and fibrin stabilization by factor XIII [3]. They also partially protect fibrinogen against heat denaturation [4,5]. In [6] we described qualitatively the protecting effect of Ca^{2+} in the plasmin degradation of human and bovine fibrinogen. We based a preparation method on this observation [7] in order to reduce the well known size heterogeneity of D-fragments. Upon exhaustive digestion with plasmin in the presence of Ca^{2+} ions only D-fragments with mol. wt 93 000 (D(cate)) are formed, and in the presence of EGTA only D-fragments with mol. wt 80 000 (D(EGTA)). Calcium appeared to protect the C-terminal part of the γ-chain remnant in D(cate) against further plasmic attack [6,7].

In direct Ca^{2+}-binding studies [8] we determined the number and affinity of Ca^{2+}-binding sites in rat fibrinogen and its degradation products.

Here we report the results obtained with human fibrinogen and its degradation products. Our results differ from those obtained in [9] but strongly support the model suggested earlier by us for Ca^{2+}-binding by rat fibrinogen.

2. Materials and methods

Fibrin(ogen) and degradation products were prepared as in [7,10]. Equilibrium dialysis experiments were carried out as in [8] for the rat.

3. Results and discussion

Figures 1–3 and table 1 summarize the results of the experiments. From these results it is clear that human fibrinogen differs from rat [8] and bovine [11] fibrinogen in its Ca^{2+}-binding properties. In rat and bovine fibrinogen, three apparently identical high-affinity binding sites were deduced from the

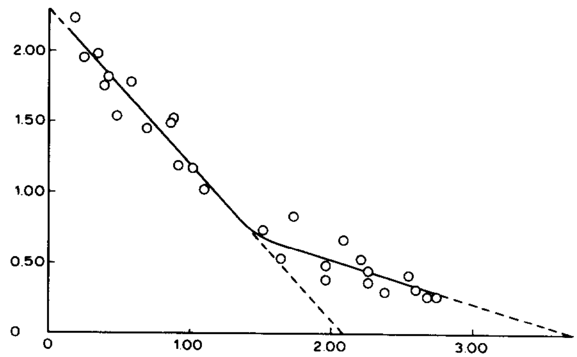

Fig.1. Scatchard plot of no. Ca^{2+} bound/mol human fibrinogen and free [Ca^{2+}]. Details in [8].

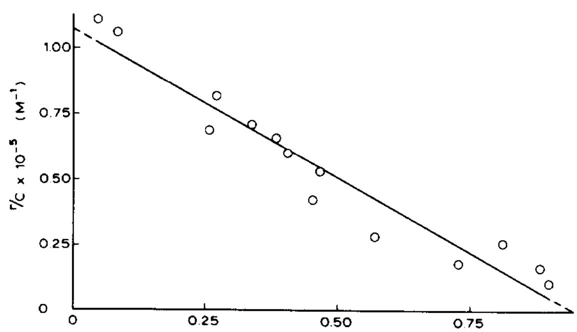

Fig.2. Scatchard plot of no. Ca^{2+} bound/mol human D(cate) and free [Ca^{2+}]. Details in [8].

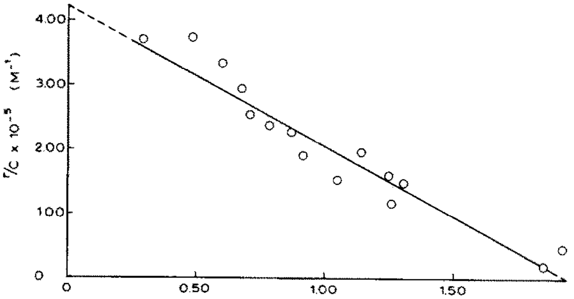

Fig.3. Scatchard plot of no. Ca^{2+} bound/mol human D-dimer and free [Ca^{2+}]. Details in [8].

Scatchard plots. From pH–Ca^{2+}-binding studies for bovine fibrinogen [11] and by summation of sites in the degradation products of rat fibrinogen [8] it could be concluded that two of the three Ca^{2+} were bound at identical sites in the symmetrical fibrinogen molecules.

In human fibrinogen the two identical binding sites (K_d 9.0 × 10^{-6}) are discriminated from the third, directly in the Scatchard plot. It must be concluded from our data that the binding site with K_d 32 × 10^{-6} corresponds to one of the three apparently identical sites found in rat and bovine fibrinogen. Apparently the model suggested by us for Ca^{2+}-binding by rat fibrinogen is also consistent with the Ca^{2+}-binding by human fibrinogen.

Our results for human fibrinogen are at variance with results in [9] with human fibrinogen. They found three high-affinity binding sites with K_d 8.7 × 10^{-6}. These authors probably did not measure Ca^{2+}-binding at low enough [Ca^{2+}] to be able to discriminate between the K_d 9 × 10^{-6} and K_d 32 × 10^{-6} sites. Also our results with D-dimer differ by a factor two from those obtained in [9] (K_d 9.5 × 10^{-6} versus our K_d 4.6 × 10^{-6}). On the other hand our results for D(cate) are in fairly good agreement with the results obtained [9] with fragments D_1 (K_d 10.6 × 10^{-6} versus our result 8.9 × 10^{-6}). Although no detailed information is available of the preparation in [9] it is likely that their D_1-fragment is identical with our D(cate).

Table 1
Number of Ca^{2+}-binding sites and K_d values in human fibrinogen and fibrin(ogen) degradation products

Class:	I		II	
Protein	Sites	K_d (M)	Sites	K_d (M)
Fibrinogen	2	9.0 × 10^{-6}	1–2	32 × 10^{-6}
D(Cate)	1	8.9 × 10^{-6}	0	–
D-Dimer	2	4.6 × 10^{-6}	0	–

Like rat, human D-dimer binds two Ca^{2+}. Their K_d values are lower than in the corresponding fibrinogens. This could indicate that also human fibrin (like in rat) Ca^{2+} bind tighter than in fibrinogen. Human D(EGTA) fragments do not bind Ca^{2+}, nor did E-fragments isolated from digests prepared in the presence of Ca^{2+} or EGTA as in [7]. This is also in agreement with the corresponding rat products [8].

Furthermore, our studies on the anticlotting properties of D-fragments [12,13] have demonstrated a strong anticlotting potency of D(cate) and D-dimer fragments but none of D(EGTA) and E-fragments.

In conclusion, our results show considerable differences with those in [9] and are consistent with our results obtained with the rat [8] and those obtained with bovine fibrinogen [11] with respect to two of the three high-affinity binding sites.

These two Ca^{2+}-binding sites protecting fibrinogen against further plasmic attack and the anticlotting properties of the D- and D-dimer fragments are related to the C-terminal parts of the γ-chains.

Direct evidence is given that the third binding site in human fibrinogen is different. This could only be deduced from circumstantial evidence in rat and bovine fibrinogen. Our present and previous observations strongly suggest that in fibrinogens in general three Ca^{2+} are bound with a high-affinity and that two of these are each bound identicaly to one of the two D(cate) fragments. The third site is located elsewhere in the molecules.

It would be of interest to know the role of this third binding site. It might play a role in the first steps of fibrin polymerization.

Further studies along these lines are in progress.

Acknowledgements

The authors are indebted to Mr W. Termorshuizen for his cooperation in the experiments, to Mr Nieboer for drawing the figures and to Mrs Horsting for typing the manuscript.

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