Protein Science (1992), *1*, 1719–1720. Cambridge University Press. Printed in the USA.

FOR THE RECORD

The synthetic peptide Gly-Pro-Arg-Pro-amide limits the plasmic digestion of fibrinogen in the same fashion as calcium ion

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(RECEIVED November 10, 1992; REVISED MANUSCRIPT RECEIVED November 12, 1992)

It has long been known that bound calcium ions limit the vulnerability of mammalian fibrinogens to proteases like plasmin. Thus, in the presence of calcium, plasmin breaks fibrinogen down into a set of core fragments, the largest of which is a high-molecular-weight fragment D. In the absence of calcium, or in the presence of EGTA, the final fragment D is significantly smaller. The difference resides mainly in the sizes of the constituent γ-chains (Haverkate & Timan, 1977), the additional degradation reducing the original 37-kDa chain first to 33 kDa and then to 26 kDa.

A number of observations have tied the calcium-binding site to the polymerization site, including the fact that the smaller fragment D formed in the absence of calcium ion is not able to bind synthetic peptides beginning with the sequence Gly-Pro-Arg, which are known to inhibit the early steps of fibrin polymerization (Doolittle & Laudano, 1980; Laudano & Doolittle, 1980). Moreover, both sites reside in the carboxyl-terminal halves of the γ-chain (Varadi & Scheraga, 1986). Additionally, several variant human fibrinogens that exhibit defective polymerization also bind calcium poorly, and sometimes the defective polymerization can be remedied by excess amounts of calcium (Matsuda, 1991).

For all these reasons, we wondered whether Gly-Pro-Arg peptides might have a protective effect similar to that of calcium ions. To this end, we set up a series of digestions of human fibrinogen and fragment D under various conditions in which calcium ions were either added or removed and the peptides Gly-Pro-Arg-Pro-amide and Gly-His-Arg-Pro-amide were either present or absent. The proteins were purified as in our earlier work (Yamazumi & Doolittle, 1992); the substrate concentrations were either 5 mg/mL (fibrinogen) or 2 mg/mL (fragment D). In all cases the added plasmin (human plasmin obtained from Kabi) amounted to 0.24 casein units.

We now report that the peptide Gly-Pro-Arg-Pro-amide protects both fibrinogen and fragment D from further breakdown by plasmin, even in the presence of EGTA that removes any contaminating calcium. Thus, the principal form of fragment D formed from the digestion of fibrinogen is clearly smaller when the digestion is carried out in the absence of calcium (Fig. 1A, lane 3), but the action is reversed when Gly-Pro-Arg-Pro-amide is present (Fig. 1A, lane 5). Similar results were obtained when affinity-purified fragment D was redigested with plasmin (Fig. 1B, lane 10). The peptide Gly-His-Arg-Pro-amide, which corresponds to the amino-terminus of the fibrin β-chain, had only a very slight effect (Fig. 1, lanes 4 and 9), perhaps due to secondary binding to the Gly-Pro-Arg site (Laudano & Doolittle, 1980, 1981).

These results emphasize the close proximity of the calcium-binding and peptide-binding sites in the fragment D part of fibrinogen. It may even be that some side chains contribute to the binding of both ligands. The results are fully concordant with the model for the terminal domains of fibrinogen presented in Doolittle (1992 [this issue]).

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Acknowledgment

This investigation was supported by NIH grant HL26873.

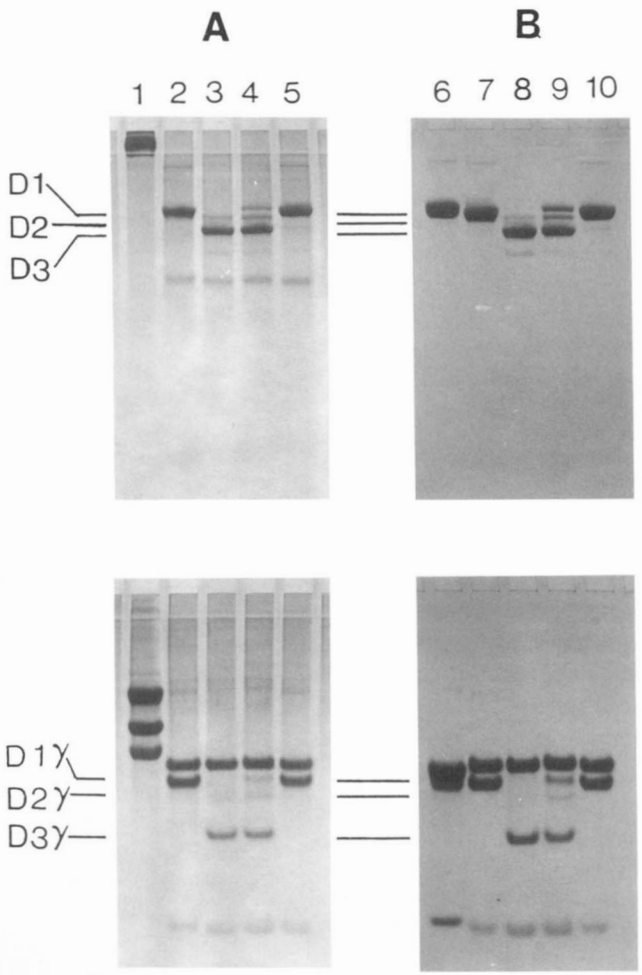

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of human fibrinogen (**A**) or fragment D (**B**) digested with plasmin (Kabi) for 12 h under various conditions. All the digestions were carried out in 0.15 M NaCl–0.05 M imidazole, pH 7.5, at 22 °C. Upper panels, non-reduced samples; lower panels, reduced samples. Lane 1, native fibrinogen; lanes 2 and 7, 5 mM $CaCl_2$; lanes 3 and 8, 0.5 mM EGTA; lanes 4 and 9, 0.5 mM EGTA and 5 mM GHRP-amide; lanes 5 and 10, 0.5 mM EGTA and 5 mM GPRP-amide; lane 6, native fragment D. The three different forms of fragment D and their three corresponding γ-chains are designated by horizontal lines.

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