PROTEIN POLYMERIZING ACTIVITY OF PAPAIN

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Received April 8, 1962

Since both thrombin and papain can promote the formation of a gel from fibrinogen, it has been assumed that these two proteolytic enzymes act to produce clots in a similar manner (Eagle and Harris, 1937; Laki, 1951 a; Steiner and Laki, 1951). Thrombin induces fibrinogen to aggregate by a process of limited hydrolysis in which the fibrinopeptide fragments are removed from the parent molecule (Lorand,1951). There is a concomitant change in the N-terminal residues of fibrinogen, e.g. from glutamic acid and tyrosine to those of glycine and tyrosine in the bovine species (Lorand and Middlebrook, 1952). Papain also seems to liberate glycine endgroups when clotting bovine fibrinogen (Blomback and Yamashina, 1958). The gel produced by thrombin can be readily dissolved in 1% monochloroacetic acid (Lorand, 1950), and the clot obtained by the action of papain has also been thought to be similarly dispersible. However, we can now demonstrate marked differences between the solubilities of these clots which, no doubt, reflect differences in the nature of the gel structures themselves. The difference is most noticeable during the early part of papain action, and is especially apparent when smaller amounts of papain are used to clot fibrinogen (Figs. 1 and 2). Over a considerable period of its existence, such a gel cannot be dissolved in 1% monochloroacetic acid. This fact is taken to signify (Lorand, 1950) that, unlike thrombin, papain introduces some covalent crosslinking between the protein units of the network.

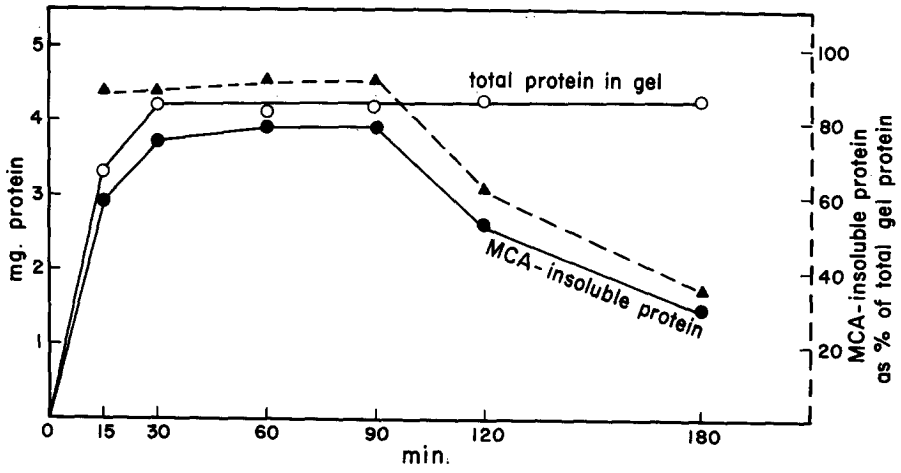

Fig. 1. Papain Induced Polymerization of Fibrinogen.

Papain (Worthington, cryst.) was allowed to react (at 20°) with fibrinogen (Laki, 1951 b) in two sets of mixtures, 2.5 ml. each, containing the following ingredients: 5 mg. of fibrinogen, dissolved in 1.9 ml. of Tris (hydroxymethylaminomethane)-HCl-NaCl buffer of pH 7.5 and μ:0.18; 0.5 ml. of 0.1 M cysteine freshly dissolved into above buffer with adjusting pH to 7.5; 2γ. of papain in 0.1 ml. of buffer.

At various times, shown on the abscissa, the "total protein in gel" was measured by taking the clots (drained and washed) of the first set of mixtures. To the other set, 2.5 ml. of 2% monochloroacetic acid (MCA) was added and the "MCA-insoluble protein" residues were estimated (Lorand and Jacobsen, 1958).

The dashed line (relating to the ordinate on the right) expresses the acid-insoluble portions as percentage of the total clot proteins.

The nature of the crosslinks produced by papain in the polymer is not known. We should like to draw attention, however, to the well known ability of papain in catalyzing the synthesis or exchange of carbonyl amide bonds. It would be necessary only that the fibrinogen-acylpapain intermediate be cleaved by an amine of a neighboring protein molecule rather than by water. Acylpapains are believed to react with amines faster than with water (see Fruton, 1957).

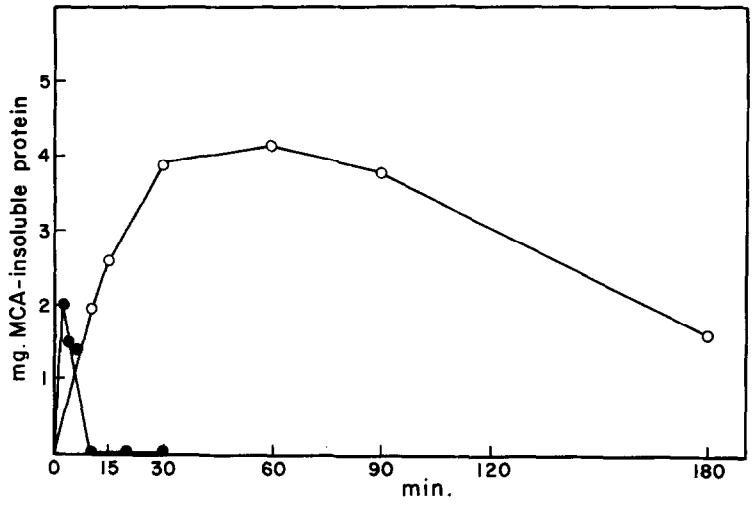

Fig. 2. Influence of Papain Concentration on the Polymerization of Fibrinogen.

Clotting mixtures contained 20γ. (full circles) and 2γ. (open circles) of papain. Other conditions were identical to those given in the legend of Fig. 1. Acid-insoluble clot residues are shown on the ordinate.

Acknowledgment

This work was aided by grant H-2212 of the National Heart Institute, National Institutes of Health.

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