

The Determination of N-terminal Amino Acids during the Conversion of Fibrinogen to Fibrin

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It has been suggested by earlier workers that the conversion of fibrinogen to fibrin is the result of a proteolytic reaction. Actually, in 1951, Lorand, Bettelheim and Bailey¹ showed by means of Sanger's method, differences in the N-terminal amino acids of fibrinogen and of fibrin. They also found "fibrinopeptides" in the supernatant of a fibrin clot produced by the action of thrombin²⁻⁵. However, due to the difficulties of determining the N-terminal amino acids quantitatively, no conclusive figures as to the number of polypeptide chains in either fibrinogen or in fibrin have been obtained⁶. Moreover, the doubtful purity of the fibrinogen preparations, used by earlier workers, might result in the release of contaminating substances from the fibrinogen preparation into the supernatant during the formation of fibrin.

Recently, a highly purified preparation of bovine fibrinogen has become available in our laboratory⁷. At least 99 % of the protein in this preparation is coagulable with thrombin, indicating that the fibrinopeptides make up only a very small part of fibrinogen. By applying Edman's method to this preparation, we reinvestigated the N-terminal amino acids both in fibrinogen and in fibrin. Determinations were also performed at different times dur-

ing the conversion of fibrinogen to fibrin by thrombin.

The results show the presence of glutamine, glutamic acid and tyrosine as N-terminal amino acids in fibrinogen and the presence of glycine and tyrosine in fibrin. One glutamyl residue (amide plus acid) and one tyrosyl residue are present in about 1.7×10^5 g of fibrinogen and two glycylyl residues and one tyrosyl residue in the corresponding amount of fibrin. Since the molecular weight of fibrinogen has been estimated to be $3.4 \times 100\,000$ ^{8,9}, two glutamyl residues in the fibrinogen molecule seem to be replaced by four glycylyl residues by the action of thrombin.

We have found that fibrin formed by means of papain or an enzyme extracted from snake venom has the same N-terminal amino acids as the normal fibrin. However, only two glycylyl residues were found in those fibrins instead of four in the normal, thus indicating that there are differences in the mode of action among these three enzymes.

We have followed the proteolysis caused by thrombin in low concentration. The reaction was followed also by determining light-scattering¹⁰. During the action of thrombin on fibrinogen there is a time lag before polymerization and the subsequent coagulation occur. Glycine appeared as N-terminal amino acid immediately after the addition of thrombin to the fibrinogen solution, showing that there is a formation of an intermediary product, soluble fibrin, during this lag (Fig. 1). The amount of glycine released reaches a maximum value, about 40 % of the total, before any coagulation occurs.

During the coagulation the glycine in the supernatant decreases as shown in Fig. 2.

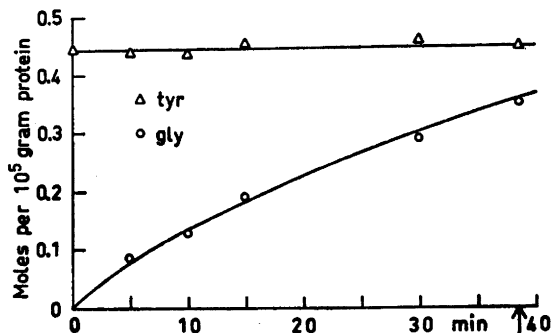


Fig. 1. The appearance of N-terminal glycine in the fibrinogen solution after addition of thrombin. The arrow indicates the coagulation-time.

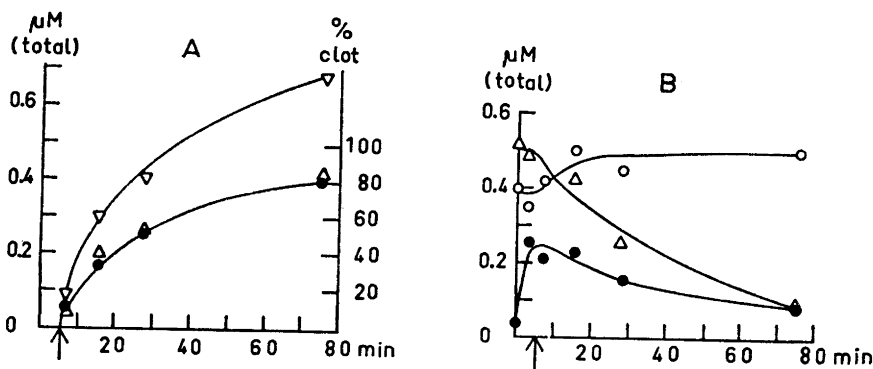


Fig. 2. A. N-Terminal amino acids and protein content of clots collected at different times. The arrow indicates the coagulation-time. ∇ gly; Δ tyr; \bullet coagulated protein in per cent of total.

B. N-Terminal amino acids in the supernatant after removal of the clot. The arrow indicates the coagulation-time. Δ tyr; \circ glu; \bullet gly.

Glutamic acid present originally as an N-terminal amino acid of fibrinogen remained in the supernatant throughout the reaction. The low figure found for glutamic acid in the early stage of the transformation is due to the lower yield of the phenylthiohydantoin amino acid in the presence of high concentrations of protein.

The total amount of tyrosine in fibrinogen and in fibrin was constant.

Judging from these experimental results, it is quite evident that the conversion of fibrinogen to fibrin includes the formation of soluble fibrin, and the subsequent polymerization of soluble fibrin to insoluble fibrin.

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Corrections to "Structure of the 1:1 Compound Pyridine — Iodo Monochloride"

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In the paper¹ two corrections should be inserted: (1) The sign of the y parameter of the chlorine atom has to be changed from + to -, and (2) the distance quoted for the N-Cl separation (2.26) refers to the N-I bond distance.

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