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Some chemical changes associated with prothrombin activation

In the activation of purified prothrombin preparations several stages in the development of thrombin activity have been recognized[1]. First, prothrombin loses the capacity to become thrombin in the presence of lung thromboplastin, Ac-globulin and Ca^{++} (2-stage analytical reagents). Then the protein regains its sensitivity to these substances, and lastly thrombin activity arises. The sequence which accounts for experiments completed thus far may be described as follows: Prothrombin (sensitive to Ca^{++} + Ac-globulin + brain thromboplastin) → Prothrombin-derivative I (not sensitive to Ca^{++} + Ac-globulin + brain thromboplastin) → Prothrombin-derivative II (sensitive to Ca^{++} + Ac-globulin + brain thromboplastin) → thrombin and other reaction products.

This unique capacity of the zymogen to undergo modifications implies an intermediate(s) in the activation process. For instance intermediates have been found in the autocatalytic activation of prothrombin in 25 % sodium citrate[2]. We have now found that the first step in prothrombin activation is associated with the appearance of N-terminal proline, and this is true with the use of three different materials; namely, thrombin, purified platelet factor 3, and sedimentable lung thromboplastin.

In the activation with sedimentable lung extract thromboplastin (1 mg/ml) and Ca^{++} (0.023 *M*) about 50 % of the prothrombin (2000 units/ml) was converted to thrombin in 5 h, and the remaining prothrombin became a derivative (not convertable to thrombin in 2-stage analytical reagents). We then removed the thromboplastin by high-speed centrifugation and precipitated the protein by adding acetone to a concentration of 50 % at 0°. The precipitated protein was dried from the frozen state and was found to contain N-terminal proline and glutamic acid. From other experiments it is known that the N-terminal amino acid of bovine prothrombin is alanine[3] and for thrombin it is glutamic acid[1]. A non-thrombin derivative of prothrombin thus has proline as the N-terminal amino acid.

Another activation was studied with the use of purified platelet factor 3 (50 units/ml), prothrombin (2,000 units/ml), and Ca^{++} (0.023 *M*). In 5 h half of the prothrombin activity was lost (not convertable to thrombin in 2-stage analytical reagents) while the rest remained as prothrombin activity. There was no thrombin. The protein was precipitated by adding cold acetone to a concentration of 50 % and then was dried from the frozen state. The material was only partially soluble in physiological saline solution, but the soluble portion contained N-terminal proline.

In a third way of activation, purified prothrombin (2,000 units/ml) was mixed with purified resin thrombin (1.7 units/ml) at pH 8.5, in water, and without added Ca^{++}. Within 2 h 80 % of the prothrombin had lost its activity while the rest remained as prothrombin. No thrombin titre developed. The protein was precipitated (50 % acetone) and dried from the frozen state. All the material was soluble and contained proline as N-terminal amino acid.

Our analysis identifies a first stage in the activation of prothrombin with the appearance of N-terminal proline on the protein. This is so whether an enzyme such as thrombin is used for the activation or whether platelet factor 3 or sedimentable lung thromboplastin are used. In previous work from this laboratory it was found that prothrombin may lose its activity (capacity to become thrombin by 2-state analysis) and acquire accelerator properties[5,6]. The changes described in terms of bioassay are now supported in terms of the appearance of proline as N-terminal amino acid on the protein.

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