Reports

Thrombin-E as a Fibrinolytic Enzyme

Abstract. Thrombin-E has been produced in adequate quantity for pharmacological studies. After intravenous infusion in dogs there were no significant changes in blood pressure, temperature, pulse rate, and respiratory movements. There were drops in platelet and white cell count, and there was an increase in blood-sugar level. Powerful fibrinolytic phenomena were observed.

The recent discovery of thrombin-E followed from observations of the properties of thrombin and experiments on the activation of prothrombin (*1, 2*). First, the observation that thrombin preparations have both the power to clot fibrinogen and also the power to dissolve a clot (*3*) needed further clarification, since the lytic power might reside with a contaminating enzyme and not with the thrombin (*4*). This objection was removed when thrombin was produced as a purified enzyme and found to contain lytic and clotting properties (*5*). However, when certain thrombin solutions were allowed to stand, the clotting power could be seen to disappear while much of the lytic activity remained (*1*). Such thrombin was called esterase thrombin or thrombin-E, to distinguish it from thrombin-C (clotting thrombin), which is the classical thrombin.

By studying the activation of prothrombin, conditions were found wherein practically only thrombin-E activity developed. In all activations studied, thrombin-E arose in greater concentration than thrombin-C (*6*). In other words, thrombin-E activity develops first, and thrombin-E is an enzyme distinctly different from thrombin-C. With regard to substrates, thrombin-E uses fibrin and *p*-toluenesulfonyl - L - arginine - methyl ester (TAMe) but not fibrinogen. Thrombin-C, on the other hand, uses fibrin, *p*-toluenesulfonyl-L-arginine-methyl ester, and fibrinogen as a substrate. To state this another way, thrombin-C clots fibrinogen and subsequently dissolves a fibrin clot. Thrombin-E does not clot fibrinogen but dissolves fibrin clots.

One of the problems with which we were confronted was the need for producing thrombin-E free of any significant amounts of thrombin-C. This was eventually accomplished by acetylating thrombin-C with acetic anhydride (*7*). This blocked, among others, the amino groups of the N-terminal glutamic acid residues, and the thrombin-C lost its clotting power. One of the gratifying observations was the almost doubling of thrombin-E activity as the thrombin-C activity was destroyed. The acetylated thrombin was obtained as a single component, which can readily be dried from the frozen state and generally seems to be quite stable.

Thrombin itself has been found useful in certain instances where anticoagulant drugs were ineffective in the treatment of thrombosis (*8*). This is all the more remarkable in view of the clotting potential of the enzyme. Evidently this was kept under control by using only small amounts and by using them slowly. Since we had thrombin-E as acetylated thrombin there was no risk of producing intravascular clots, but the question was whether any lytic power would be noticed upon intravenous infusion. It was at once evident that this property is exhibited to a very substantial degree by acetylated thrombin when given to dogs.

In our work bovine thrombin was purified (*5*) and acetylated with acetic anhydride. The acetylated thrombin was separated and assayed with *p*-toluenesulfonyl-L-arginine-methyl ester as a substrate. It was practically devoid of clotting power, and could be given intravenously without promoting the formation of clots. Many experiments have been performed, and the following one is typical. About 15 mg of thrombin-E, dissolved in 100 ml of physiological saline solution, was infused (through the femoral vein of a dog under pentobarbital anesthesia) slowly over a period of 1 hour. The dog tolerated this very well. The blood pressure, temperature, pulse rate, and respiratory movements all remained unaltered.

Blood samples were repeatedly withdrawn and analyzed. At the conclusion of the infusion the blood sample clotted, but it subsequently lysed within an hour. The leukocyte count dropped from 9000 to 1500 per microliter and the platelet level was also down, but no changes in red cell count were found. Of the several chemical analyses performed on the plasma the one which showed a rise in blood-sugar level was the most unusual: the original concentration was approximately doubled. All these manifestations persisted for some time, but in 24 hours all values were again at normal (preinfusion level), the return to normal being already noticeable in 6 hours. Occasionally the lytic phenomenon was so pronounced that the blood would not clot spontaneously upon withdrawal or even after thrombin was added.

In test tubes, thrombin-E, in the form of acetylated thrombin, does not have especially rapid action as a fibrinolytic agent. When given intravenously, however, a spectacular result is obtained. It may be that the thrombin-E functions by activating physiological processes concerned with fibrinolysis. This opens possibilities for the useful application of thrombin-E, which can probably be made from bovine sources owing to the low precipitinogenic qualities of bovine thrombin (*9*). An interesting theoretical consideration relates to the view that thrombin-E might be derived from prothrombin in our normal physiology to help maintain the fluidity of the blood—a view based on the possibility that thrombin-E is derived from prothrombin before thrombin-C in the prothrombin activation sequence (*10*).

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References and Notes

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Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

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For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].