Z. Ernährungswiss. 19, 21–23 (1980)

ISSN 0044-264 X

Biochemistry Laboratory, National Research Centre, Dokki, Cairo (Egypt)

Fibrinolytic activity of an enzyme produced by Bacillus subtilis

K. I. Fayek and *Sanaa T. El-Sayed*

With 2 figures

(Received July 3, 1979)

The fibrin plate method for assay of fibrinolytic activity proved to be a successful way. Many modifications were introduced to this method since *Astrup* and his coworkers developed this method (1, 2, 8). The presence of contaminating plasminogen usually found in bovine fibrinogen preparations used in plate, cause difficulties in distinguishing between plasminogen activators (3) and other proteolytic enzymes.

Streptokinase and urokinase are plasminogen activators which are now used as thrombolytic agents. Applying streptokinase to a fibrin plate plasminogen-free, lysis does not appear unless plasmin is added (7). Thus it was thought advisable to use fibrin plate plasminogen-free to test the fibrinolytic activity of the enzyme prepared from *B. subtilis,* produced in our laboratory.

Material and methods

Fibrin plate plasminogen-free: Human fibrinogen (from plasma substitutes laboratories, Agouza, Cairo, Egypt) was treated by bentonite as described by *Lynn Deogny* et al. (7). Thrombin 20 units/ml (Parke-Davis) was used.

Preparation of plates: Fibrinogen treated with bentonite was dissolved in gelatine buffer, coagulated by 0.2 ml thrombin before addition of agar, to give plasminogen-free plate. The other type of plates fibrinogen was used without treatment. Fibrinolytic activity: Different dilutions of the enzyme preparation was carried out using gelatin buffer used in the plate, and applied in a final volume of 0.07 ml in each well. Diluted serum or plasma were used ranging from 100 to 300 mg protein. The mixture of plasma or serum and the enzyme were incubated at 37 °C for 30 minutes before applying to the plate. The lysis diameter was measured after incubating the plate at 37 °C for 18 hours.

Results and discussion

The proteolytic activity of any protease on plasminogen-free and plasminogen-rich substrates in vitro before considering it as thrombolytic agent is recommended (11). Results presented in figure 1 show that the fibrinolytic activity of this enzyme is the same in the presence or absence of plasminogen; thus one can say that its fibrinolytic activity is not through plasminogen activation but has a direct action on fibrin. A linearity between the logarithm of the dose and the lysis diameter is clearly demonstrated.

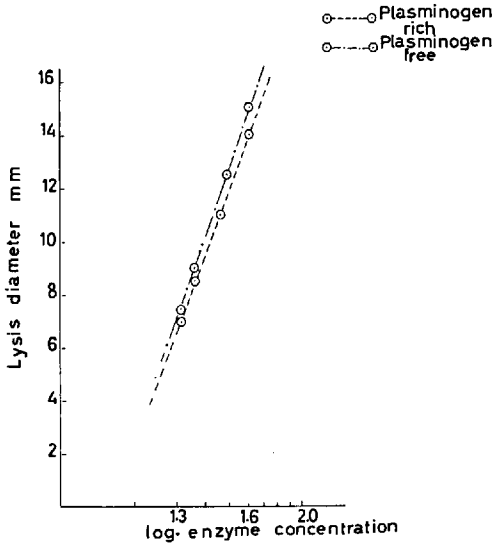

Figure 1: Enzyme activity in fibrin plates plasminogen-free and plasminogen-rich.

Incubation of this enzyme with the same concentration from serum or plasma before testing its lysis activity showed no inhibitory effect of serum proteins in the concentrations and under the conditions described (fig. 2). Plasma proteins at concentration of 245 mg showed slight inhibitory effect. It is noteworthy to mention that results obtained for plasma and serum effect on enzyme activity were the same using plasminogen-free or plasminogen-rich fibrin plates. Applying 0.017 µg enzyme to a well in fibrin plate plasminogen-free gave a lysis diameter 5 mm. Adding

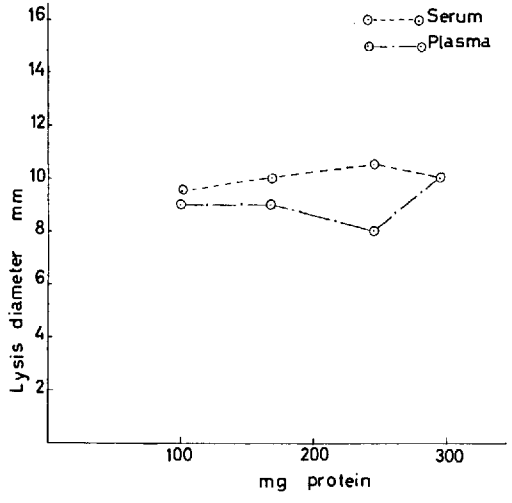

Fig. 2: Inhibitory effect of serum and plasma on enzyme activity.

$CuSO_4 \cdot 5\ H_2O$ (2.6×10^{-3}M) concentration of Cu^{++} (103 ppm) to the same enzyme concentration gave a lysis diameter of 7.5 mm.

Several antiproteolytic glycoproteins were separated from human plasma. α_1-antichymotrypsin which specifically inhibit chymotrypsin was isolated by *Heimburger* and *Haupt* (6). Antitrypsin which inhibits chymotrypsin and trypsin was isolated from human plasma (9, 10), moreover another trypsin inhibitor having an electrophoretic mobility inter-α globulin isolated by *Heide* et al. (5) and known as inter alpha trypsin inhibitor. α_2 macroglobulin is claimed to have antiprotease activity (4).

Since all globulin fractions previously mentioned are present in plasma and serum and if any of these could have the inhibitory effect on the enzyme, thus the results should have been the same in case of serum and plasma. However, there must be another factor in plasma which is destroyed or masked in blood clotting.

Summary

Proteolytic activity of a fibrinolytic enzyme isolated from *B. subtilis* was tested on fibrin plates plasminogen-free and plasminogen-rich. Results showed that the enzyme is not a plasminogen activator. Inhibitory effect of human serum and plasma was also tested. The plasma and not serum showed a slight inhibitory effect on proteolytic activity of this enzyme.

References

1. *Astrup, T., N. Alkjaersig:* Arch. Biochem. Biophys. **7,** 99 (1952). – 2. *Astrup, T., S. Mullertz:* Arch. Biochem. Biophys. **40,** 346 (1952). – 3. *Brakman, P., T. Astrup:* Thrombosis and bleeding disorders, p. 332 (New York, 1971). – 4. *Gentou, C.:* Compt. Rend. **266,** 2358 (1968). – 5. *Heide, K., N. Heimburger, H. Haupt:* Clin. Chim. Acta **11,** 82 (1965). – 6. *Heimburger, N., H. Haupt:* Clin. Chim. Acta **12,** 116 (1965). – 7. *Lynn Deogny, Annita Weidenbach, James W. Hampton:* Clin. Chim. Acta **60,** 85 (1975). – 8. *Permin, P. M.:* Nature **160,** 571 (1947). – 9. *Schultze, H. E., K. Heide, H. Haupt:* Clin. Chim. Acta **7,** 854 (1962). – 10. *Schultze, H. E., N. Heimburger, K. Heide, H. Haupt, K. Storiko, S. Schwick:* Proc. 9th Congr. European Soc. Haematol., Lisbon 1963, p. 1315 (New York, Basel, 1963). – 11. *Wijngaards, G., F. Haverkate:* Prog. Chem. Fibrinolysis Thrombolysis **3,** 379–389 (1978).

Authors' address:

Dr. *Kamal I. Fayek,* Biochemistry Laboratory, National Research Centre, Dokki, Cairo (Egypt)